UNIVERSITY OF
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TRAINING-INTENSITY DISTRIBUTION, PHYSIOLOGICAL ADAPTATION AND IMMUNE FUNCTION IN ENDURANCE ATHLETES

BY

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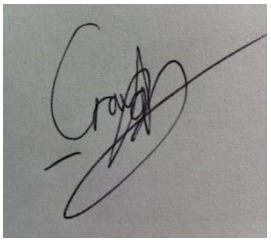
DECLARATION

I declare that this thesis was composed by myself and that all the data were collected and analysed by myself. Neither the thesis nor the original work therein has been submitted to this or any other institution for a higher degree.



Craig Neal
25/10/11

ABSTRACT

Exercise intensity and its distribution is probably the most important and most heavily debated variable of endurance training. Training induces adaptation but also induces stress responses. Controlling the training-intensity distribution may provide a mechanism for balancing these two effects. It has been reported that elite endurance athletes train with a high volume and load, relative to the sport. These athletes spend the vast majority (>80%) of training time at relatively low intensities (lower than the lactate threshold, zone one), and therefore <20% of training time above the lactate threshold (zones two and three). Experimental studies support the beneficial effects of a high training volume in zone one, and show detrimental effects of replacing zone one training with training in zone two. This is likely due to enhanced recovery from training in zone one compared with training in zone two. The acute recovery following training sessions in zones two and three has been reported to not be different, but the recovery following training in zone one has been reported to be faster. Improvements in physiological adaptation and endurance performance have been reported to be greater following training programmes with higher exercise intensities. Therefore, it has been suggested that a polarised training model, which includes ~80% of training in zone one with ~20% of training in zone three is more beneficial than a threshold training model, with the majority of training in zone two. However, research into an optimal training-intensity distribution is limited. Therefore, the aims of this thesis were to assess the effectiveness of training-intensity distribution on the improvements in physiological adaptation, endurance performance and assess if manipulating training-intensity distribution had an effect on immune function.

Study one revealed that the lactate threshold, the lactate turnpoint and maximal performance measures in swimming, cycling and running, assessed using the methods outlined in the study, are reproducible in trained endurance athletes. These tests can therefore be used by trained endurance athletes as part of a physiological testing programme to assess not only endurance performance, but also to demarcate training intensity zones for exercise intensity prescription and monitor moderate to large adaptations to training. Practitioners should take care when deciding on the duration between tests to test for adaptations from training, as adaptations need to be greater than these detected test-retest variations to be considered physiologically meaningful.

To the best of the author's knowledge, study two was the first study to have assessed training-intensity distribution in a group of multisport athletes. Training was monitored over a 6-month period, and testing took place every two months to assess the effect of the training on physiological adaptation. Although speculative due to the number of variables involved, the results suggest that a greater proportion of training time spent in zone one and a lower proportion of training time spent in zone two is beneficial to physiological adaptation. However, given the number of variables associated with assessing the training-intensity distribution in multisport athletes, it is not easy to draw conclusions as to the effectiveness of the training in the different disciplines on the key measures of adaptation in the different disciplines. Study two highlighted the need for future research to focus on experimental manipulation of training-intensity distribution and thus improve our understanding of its impact on the training-induced adaptations in endurance athletes.

Study three manipulated the training-intensity distribution in trained endurance athletes in just one discipline, to reduce the number of variables involved. A polarised training model was

compared to a threshold training model on the effectiveness to improve physiological adaptation and endurance performance. Results revealed that a polarised training model is recommended for trained cyclists wishing to maximally improve performance and physiological adaptation over a short-term (six week) training period.

The first part of study four assessed the effect of a polarised and a threshold training model on immune function markers in trained cyclists. Both endurance training programmes had similar volume, and were sufficient to induce improvements in performance and physiological adaptation. However, despite likely differences in recovery, both training programmes had no effect on the proportion of low or high differentiated or senescent CD8+ or CD4+ T-cells in blood. Therefore, training adaptation was achieved at no cost to this particular aspect of immune function. From these results and evidence from previous studies, it seems likely that athletes need to be overreached to induce any change in immune function following a period of intensified training.

The second part of study four assessed the impact of an ironman triathlon race on Epstein-Barr virus (EBV) and Varicella-Zoster virus (VZV) antibody titres and the frequency of low and high differentiated and senescent blood T-cells in trained endurance athletes. Previous work has revealed that an ironman triathlon race increases the proportion of senescent CD4+ T cells and decreases the proportion of naive CD4+ T cells, and thus induces changes the immune space which could leave an individual at a greater risk of infection. This study however, did not find any changes in the proportions of these T cell subsets following an ironman triathlon race. The mean results of this study suggest that there is no relationship between EBV and VZV-specific antibody concentrations and the proportion of senescent, low and highly differientiated T cells. However, on analysis of individual subject data, it seems possible that subjects with a high antibody titre for EBV or VZV 3 wks before a competition might be more

at risk of infection post race. A greater subject number would be needed in order to make a more conclusive statement about this relationship.

The results of this thesis suggest that future research is required in the area of training-intensity distribution. Firstly, our understanding of the physiological mechanisms responsible for the effectiveness of a polarised training model in trained endurance athletes is limited, and thus studies should attempt to address this issue. Our current knowledge on the mechanisms underlying a blunted T cell response following strenous exercise is also limited. A change in the immune space to a greater proportion of senescent T cells and a lower proportion of naive T cells might contribute to this blunted response. In the current thesis however, the proportions of these T cell markers were unchanged following the training/racing interventions. It is possible that with a higher training load, there could be changes in these markers, and thus this is an exciting area that could have potential implications on athlete health. Finally, testing for antibody titres in endurance athletes is possibly an avenue to detect individuals at the greatest risk of infection if subjected to a large physical and/or mental stress. This could have implications on maintaining athlete health and therefore, allowing athletes to train consistently.

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CHAPTER ONE

INTRODUCTION AND AIMS

1.1 INTRODUCTION

The aim of exercise training for an endurance athlete is to maximally improve endurance performance. This is achieved by maximally improving the components of endurance performance, whilst avoiding overtraining. In this thesis, endurance performance will be classified as the performance in an event that is predominantly aerobic. In well trained runners, maximal running for greater than 75 seconds is mostly aerobically driven (Duffield et al, 2004, 2005a, b). Endurance training leads to numerous adaptations to the cardiovascular, respiratory, metabolic, neuromuscular and endocrine systems. Manipulation of the frequency, intensity and duration of training can cause different rates of improvement in physiological adaptation, endurance performance, but can also lead to an increased susceptibility to overtraining and viral infections. One of the most important variables an endurance athlete can manipulate over a training period is the training intensity and its distribution.

Descriptive studies have revealed that elite endurance athletes train with a large volume, relative to the sport in which they compete. In addition, many anecdotal reports exist of World class endurance athletes utilising high volume training with great success. One such example is that of Sir Peter Snell, who broke the 800m World record in 1962 following high volume training (up to 100 miles per week). It may seem surprising that such large volumes of training were carried out for an event that lasts less than two minutes. In the descriptive studies mentioned, in most cases, of the large volume, a high proportion (~80%) is performed at a relatively low intensity, and the remaining training time (~20%) at a relatively high intensity. It can be assumed that this represents an effective training-intensity distribution, as it is adopted by a number of elite endurance athletes, although research is yet to support this. However, it has been reported that by making major adjustments to the training-intensity

distribution, large improvements in physiological adaptation and performance can be achieved. Therefore, it is possible that adjusting the training-intensity distribution may improve endurance performance, physiological adaptation and recovery, and reduce potential overtraining and the probability of illness.

There is still much to be understood regarding effective distributions in training intensity in endurance athletes. The aim of this thesis is to answer some questions in the overall area of how endurance athletes respond to different distributions in training intensity.

1.2 AIMS

The overall aim of this thesis was:

To determine the impact of training-intensity distribution on endurance performance, physiological adaptation and immune function in endurance athletes.

The lactate threshold and the lactate turnpoint demarcate training zones one, two and three; therefore it was important to assess the reproducibility of these measures. Once the training zones are determined, the amount of time an athlete spends in each training zone, the training-intensity distribution can be determined. The maximal performance measure is a good measure of endurance performance. As these measures would be used for the remaining chapters in the thesis, reproducibility was important to establish.

No study has yet assessed the training-intensity distribution in multisport athletes. With three disciplines in a triathlon, we aimed to assess how much time was spent in each training zone in each of the three disciplines (swimming, cycling and running) and whether there were any relationships between the amount of time spent in each zone and the physiological adaptation throughout the 6-month lead up to an important race.

Research in the area of manipulating training-intensity distribution in endurance athletes is limited. Previous research has suggested that a polarised training model is more effective than a threshold training model, yet no study has directly compared these two training models. In order to minimise the effect of other variables, a single discipline was chosen (cycling), and all training above the lactate threshold was carefully controlled. Endurance performance was assessed using a 40km time trial, peak power output and time to exhaustion at 95% of pre-training peak power output, and physiological adaptation was assessed by monitoring changes in the lactate threshold, the lactate turnpoint, the maximal activities of

mitochondrial enzymes, heart rate and mood was monitored to give an indication of overtraining.

Training periods with a different training-intensity distribution lead to different rates of improvement in physiological adaptation and endurance performance, and different rates of recovery in trained endurance athletes. It is therefore possible that different training-intensity distributions leave an athlete at a higher risk of infection, which may impair overall adaptation. Following intensive periods of endurance training in trained endurance athletes, the cell mediated immune response has been reported to be decreased. Although speculative, it is possible that this is due to an increase in the proportion of senescent T cells, as these are unable to proliferate in response to antigenic stimuli. It has been reported that an increase in senescent T cells can be caused by oxidative stress or reactivation of latent herpes viruses. The aim of this study was therefore to assess the changes in the proportion of low and high differentiated and senescent blood T cells following two 6wk periods of endurance training with different training-intensity distributions. It was hypothesised that as the polarised training model has been suggested to lead to enhanced recovery over the threshold training model, that the polarised training model would have less of an effect on the change in the proportion of the T cell subsets.

In a previous study (Cosgrove et al, 2011), we found that 3wks after an ironman triathlon race, there was a significant increase in the proportion of senescent T cells in resting, fasted blood, compared with 3wks before the race. In addition, there was a significant decrease in the proportion of naive T cells 3wks after the ironman triathlon race, suggesting that there were changes taking place in the immune space. However, from our previous study (Cosgrove et al, 2011), we did not establish the time course of these changes, as we only compared immune function changes from 3wks before the ironman traithlon race to 3wks following the race. An

aim of this study was therefore to confirm the findings from Cosgrove et al (2011), but also to establish when these changes in the immune function markers take place following an ironman triathlon race. We also did not establish the mechanisms responsible for these changes in the immune space. Therefore, another aim of this study was to assess whether there were changes in the Epstein-Barr virus and Varicella-Zoster virus antibody titres following training, and whether these changes were related to the changes in the immune space.

The objectives of this thesis were therefore to determine:

1. **The reproducibility of the lactate threshold, the lactate turnpoint and a maximal performance measure in swimming, cycling and running in trained endurance athletes.**
2. **The training-intensity distribution in a group of trained triathletes in the 6-month lead up to an ironman triathlon, and the effect upon physiological adaptation.**
3. **The effect of manipulating the training-intensity distribution in trained cyclists on endurance performance and physiological adaptation.**
4. **The effect of manipulating the training-intensity distribution in trained cyclists on the proportion on low and high differentiated and senescent blood T cells.**
5. **The impact of an ironman triathlon race on Epstein - Barr virus and Varicella - Zoster virus antibody titres and the frequency of highly differentiated and senescent blood T-cells**

CHAPTER TWO

REVIEW OF THE LITERATURE

2.1. ADAPTATIONS TO ENDURANCE TRAINING

2.1.1. The Training Process

A training stimulus of sufficient intensity, duration and frequency can cause a temporary decline in exercise performance (Morton 1997); Fig 2.1). After adequate recovery, the performance can be increased to above baseline values, in a process known as supercompensation. However, if recovery from the training stimulus is inadequate, performance will remain lower level than baseline. If inadequate recovery continues, overreaching and eventually, overtraining can result (Fig 2.1).

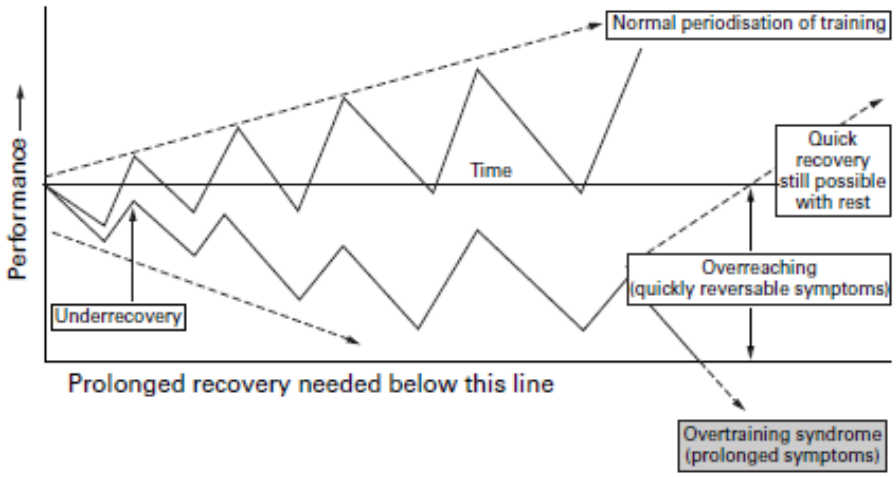

Fig 2.1 The process of positive training adaptation (top line), overreaching and overtraining (bottom line). Taken from Budgett (1998).

2.1.2. The Key Adaptations to Endurance Training

Numerous adaptations to the metabolic, cardiovascular, respiratory, neuromuscular and endocrine systems occur following endurance exercise (Jones & Carter 2000). The results of these adaptations are improvements in the key parameters of endurance performance; such as maximal oxygen uptake (VO_{2max}), the lactate threshold (LT) and exercise economy (Midgley et al., 2007); Fig 2.2).

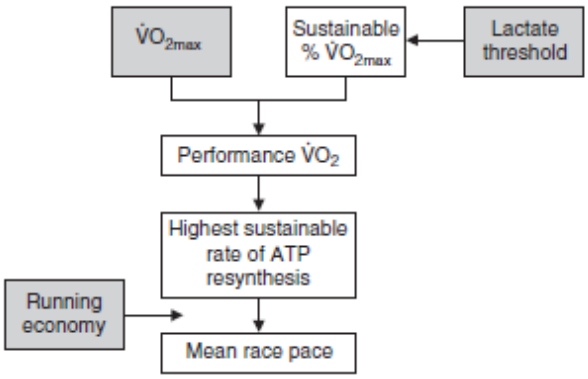

Fig. 2.2 Flow diagram illustrating that endurance performance (mean race pace) is primarily determined by the VO_{2max}, the lactate threshold and exercise economy. **ATP** = Adenosine triphosphate. Taken from Midgley et al.,(2007)

2.1.2.1. Adaptations that Enhance VO_{2max}

In the Fick equation (Eq. 1), VO_{2max} is a product of the cardiac output (Q) and the difference between the arterial oxygen content (CaO_2) and the venous oxygen content ($CavO_2$).

$VO_{2max} = Q\ (CaO_2 – CavO_2)$ (Eq. 1)

A greater VO_{2max} after endurance training has therefore been associated with an increased Q and an enhanced extraction of oxygen by the exercising muscle (Spina et al.,1996; Shephard 1992). Adaptations in the heart, blood and skeletal muscle lead to increases in Q and the arterio-venous oxygen difference (Table 2.1).

Table. 2.1 The key physiological adaptations leading to an increased VO_{2max} (Adapted from Midgley and colleagues (Midgley et al., 2007)

Site of adaptation	Increase
Heart	Left ventricular size and wall thickness
Blood	Erythrocyte mass
	Plasma volume
Skeletal muscle	Mitochondrial density
	Oxidative enzyme concentration
	Capillarity
	Myoglobin concentration

CO: Cardiac Output, **a-VO_{2diff}**: Arterio-venous oxygen difference.

However, increases in endurance performance in already well-trained endurance athletes have been shown to occur in the absence of improvements in VO_{2max} (Costill et al., 1976). Therefore, in highly trained endurance athletes, the LT and the economy are more sensitive at differentiating between performance standards (Jones & Carter 2000).

2.1.2.2. Adaptations that Enhance the Lactate Threshold

The blood lactate concentration response to increasing exercise intensities is shown in Fig. 2.3. Following endurance training, the curve shifts to the right, and therefore, the exercise intensities corresponding to the LT and the lactate turnpoint (LTP) increase.

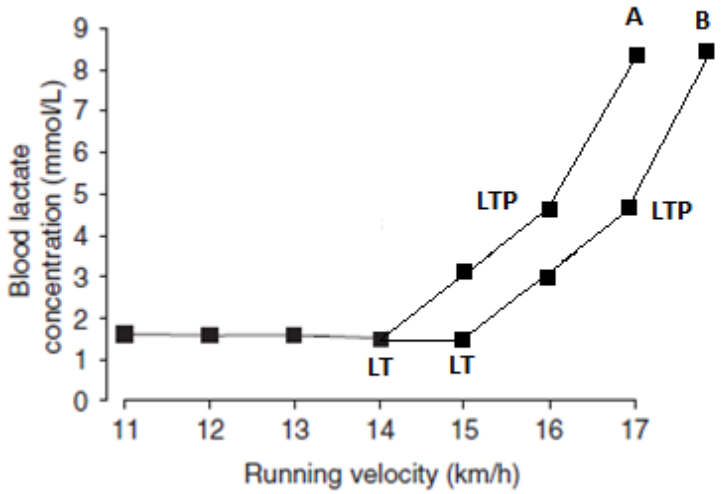

Fig. 2.3 The blood lactate concentration response to increasing exercise intensities (line A), and following a successful period of endurance training, with the line shifted to the right (line B). **LT**: Lactate threshold, **LTP**: Lactate turnpoint. (Adapted from Midgley et al., 2007).

Due to an enhanced O_2 delivery following endurance training, there is less reliance on anaerobic glycolysis to supply ATP at the same absolute exercise intensity. The result is a reduced lactate accumulation at absolute exercise intensities, and therefore, an enhanced LT due to a reduced rate of lactate production (Favier et al., 1986). Alongside the rate of lactate production, the blood lactate concentration is dependent upon the rate of efflux of lactate from the muscles to the blood and the clearance of lactate from the blood (Phillips et al., 1995; MacRae et al., 1992). Indeed, endurance training has been reported to increase the muscle specific clearing of lactate (Donovan & Brooks 1983). This is likely due to the increased abundance of the monocarboxylate transport (MCT) proteins, as endurance training has increased both the clearance capacity of lactate and the expression of MCTs (Juel 2001). MCT1 is the major MCT located in slow twitch muscle fibres, and is responsible for the import of lactate to the muscle for oxidation (McCullagh et al., 1996). MCT1 may also export lactate from skeletal muscle due to lactate movement being driven by the concentration gradient across the sarcolemma (Bonen et al., 2000). MCT4 is located in fast twitch muscle fibres, and is responsible for the efflux of lactate produced by anaerobic glycolysis (Wilson et al., 1998). The concentration of MCT4 does not increase as much as the concentration of MCT1 following

endurance training (Pilegaard et al., 1999a; Pilegaard et al., 1999b). Therefore, an increase in the LT is due to the capacity to transport lactate out of the muscle fibre, and the capacity of skeletal muscle to take up lactate. A muscle with primarily type II fibres has just 50% of the lactate transport capacity of a muscle composed of type I fibres (Hawley & Stepto 2001). Endurance training has been reported to cause a selected hypertrophy of type I muscle fibres, and it is also possible that long-term endurance training can cause a transformation of type IIb to type IIa (Spina et al., 1996; Andersen & Henriksson 1977) and from type IIa to type I (Simoneau et al., 1985; Sale et al., 1990).

Endurance exercise has been reported to lead to mitochondrial biogenesis (Morgan et al., 1971; Gollnick & Ianuzzo 1972; Hoppeler et al., 1973). Mitochondrial biogenesis is the increase in mitochondrial content, and is closely related to improved endurance (Irrcher et al., 2003; Adhihetty et al., 2003). This is due to an improved oxidative capacity and enhanced resistance to fatigue (Hood et al., 2000b). Indeed, VO_{2max}, LT and exercise economy have been found to be improved following mitochondrial biogenesis (Sjodin et al., 1982; Hoppeler et al., 1985; Holloszy et al., 1977). Mitochondrial biogenesis has been reported to change the phenotype of skeletal muscle from low-oxidative white muscle, to high oxidative red muscle (Hood 2001) and the sequence of events leading to mitochondrial biogenesis are shown in Fig.2.4.

Exercise training-induced mitochondrial biogenesis has been reported to cause a shift in substrate use (Holloszy & Coyle 1984). A decreased carbohydrate utilisation and a proportional increase in fat utilisation at the same absolute and relative exercise intensity is the result of a greater mitochondrial density (Coggan et al., 1995). The result of this shift in substrate use is a sparing of muscle glycogen (Hermansen et al., 1967), which can lead to improved endurance performance. In addition, the lowered reliance on carbohydrate

oxidation and anaerobic metabolism leads to a reduction in lactate production, and therefore an enhanced LT (Holloszy & Coyle 1984). Therefore, mitochondrial biogenesis is central to the improvement in LT following endurance training. Alongside the shift in substrate use causing a lowered lactate production, the increase in mitochondrial density also leads to an increase in lactate clearance through an enhanced lactate oxidation due to an increase in MCT1, which leads to lower rates of release of lactate from the muscle (Dubouchaud et al., 2000).

Endurance exercise causes an increase in cytosolic calcium concentration, as required for excitation-contraction coupling (Hood 2001; Fig.2.4). A high calcium concentration activates a number of kinases (calcium-calmodulin kinases) and phosphatases (calcineurin), which translocate their signals to the nucleus to alter the rate of gene transcription (Hood 2001); Fig.2.4). Endurance training also accelerates adenosine triphosphate (ATP) consumption and inhibits ATP synthesis, leading to an increased adenosine monophosphate (AMP):ATP ratio, which has been reported to activate adenosine monophosphate activated protein kinase (AMPK) (Hardie & Sakamoto 2006). Both calcium and AMPK-signalling mechanisms are involved in the initiation of mitochondrial biogenesis, through the regulation of peroxisome proliferator receptor-γ co-activator-1α (PGC-1α) expression and activity (Ojuka 2004; Puigserver & Spiegelman 2003). It is well established that endurance training increases PGC-1α mRNA and protein (Taylor et al., 2005). PCG-1α has been described as the master regulator of mitochondrial biogenesis (Baar 2004; Puigserver & Spiegelman 2003), as it coordinates the expression of the nuclear and mitochondrial genes needed for mitochondrial biogenesis (Baar 2006). An upregulation of oxidative enzymes and the conversion from 'white' glycolytic muscle to 'red' oxidative muscle was reported following muscle-specific overexpression of PCG-1α. In addition, a reduced expression of genes/proteins involved in oxidative metabolism occurred in both whole body and muscle specific PCG-1α knockout mice (Arany et al., 2005; Handschin et al., 2007; Leick et al., 2008).

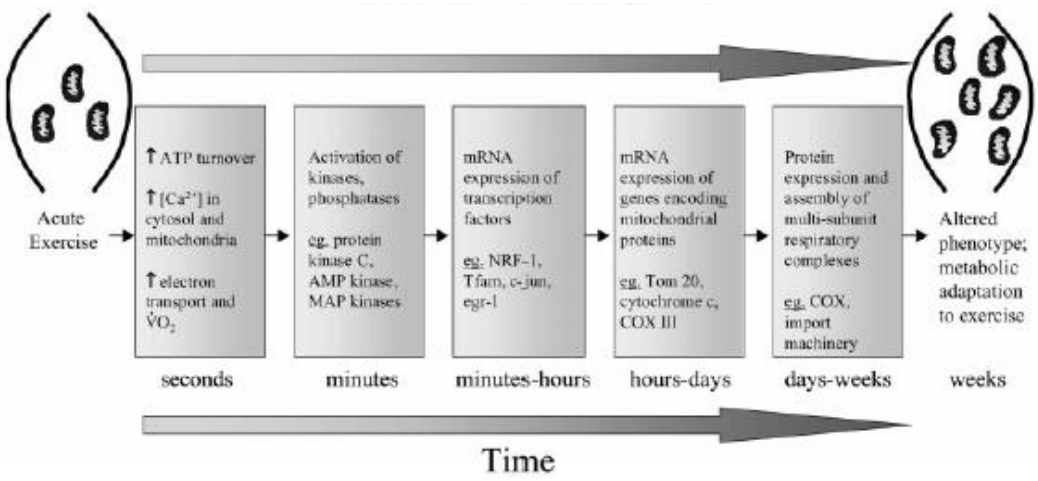

Fig.2.4 The sequence of events leading to mitochondrial biogenesis. Taken from Hood (2001)

2.1.2.3. Adaptations that Enhance Exercise Economy

Exercise economy is the oxygen uptake required at a given absolute exercise intensity (Jones & Carter 2000). An increase in exercise economy is associated with a change in the expression of fast twitch skeletal muscle fibres towards a more slow twitch phenotype (Dubouchaud et al., 2000). The result is a reduced energy cost for developing a particular level of force (Crow & Kushmerick 1982). As previously mentioned, endurance training can cause a transformation in muscle fibre type, and therefore, in exercise economy.

2.1.3 Summary of the Adaptations to Endurance Training Section

Long-term endurance training can cause numerous physiological adaptations, which in the most part can be detected by assessing the VO_{2max}, the LT and exercise economy. For long-term endurance training to lead to positive adaptations, adequate recovery is required. A failure to adequately recover from a period of endurance training before adding more training stress has the potential to lead to decreased exercise performance and overtraining. However, endurance athletes purposely stress themselves to decrease endurance performance, before resting and achieving supercompensation. Therefore, successful

endurance training is a careful balance between stressing the body adequately to improve, but not excessively, which could lead to overtraining.

2.2. TRAINING INTENSITY DISTRIBUTION

2.2.1 Introduction to Training Intensity Distribution

The main aim of endurance training is to improve performance and develop the components of endurance performance whilst avoiding overtraining (Fiskerstrand & Seiler 2004). In order to achieve this aim, there are many essential endurance exercise training variables that need to be considered. Probably the most critical and heavily debated is that of training intensity distribution (TID) (Seiler & Kjerland 2006). There is still a lack of consensus on the optimal TID required to achieve maximal performance gains. An optimal TID involves a training load which evokes maximal positive adaptations while avoiding excessive sympathetic stress leading to overtraining (Guellich et al., 2009). This is vital in the long-term development of endurance athletes as both the positive adaptations and the stress-related side-effects are cumulative (Seiler et al., 2007; Fig.2.1).

The TID of successful endurance athletes gives a good indication of an effective, but not necessarily optimal TID. Over time, each individual endurance athlete is involved in their own experimental setting. In response to specific blocks of training, an athlete and coach will make several iterative adjustments to the training programme in an attempt to optimise performance (Seiler & Kjerland 2006). How these changes to training affect athlete health, daily training tolerance and performance need to be closely assessed (Seiler & Tonnessen 2009). In agreement, it has been suggested that the International competition is an effective experimental arena, in a Darwinistic sense (Guellich et al, 2009). Endurance training is an individualised process, with each individual athlete responding slightly differently to different training stimuli (Gaskill et al., 1999; Vollaard et al., 2009). However, Seiler & Tonneson (2009) suggest that any consistent TID that emerges from different endurance sports is likely to be a result of successful self-organisation towards a population optimum. That is, an approach that results in most athletes staying healthy, making good progress and performing well in their

most important events. It has been proposed that TID may self-organise around two of the most important factors in elite endurance training: a high training volume and adequate exposure to or near race-pace intensity in training (Seiler et al., 2007). However, scientific evidence linking a specific TID to optimal improvements in endurance performance is lacking.

2.2.2 Measurement of Training Intensity Distribution

2.2.2.1 Measurement of Exercise Intensity

A linear relationship exists between heart rate and metabolic exercise intensity during dynamic exercise involving large muscle groups (e.g. running, cycling and swimming) (Gilman 1996). Therefore, exercise intensity during training and competitions can be estimated with the use of a heart rate monitor (HRM). Heart rate is an indicator of exercise intensity up to levels close to VO_{2max} (Gilman 1996). There are however, a few limitations with the use of heart rate monitoring, the main one being cardiac drift; this is the slow rise in heart rate that occurs during moderate to high workloads, when exercise duration is prolonged for >20min, indicating additional stress to the body due to an increase in heat storage (Gilman 1996). Despite these limitations, there are currently no other means of continuously and non-intrusively examining the exercise intensity in endurance athletes during both training and competition.

2.2.2.2 Establishing Training Intensity Zones

Training intensity is commonly organised into specific zones, which is vital in order to assess the TID. The most common methods of establishing exercise-intensity zones are blood lactate concentration thresholds (Fig. 2.6) and ventilatory thresholds (Fig. 2.7) at exercise of increasing intensity. Three specific zones can be established from blood lactate concentration using the aerobic-anaerobic transition, as first described by Kindermann et al (1979). Using blood lactate concentration thresholds, exercise intensities below the LT are in zone one

(Seiler & Kjerland 2006); Fig.2.6). The LT has been defined as the final exercise intensity before the blood lactate concentration increases distinctly from its resting concentration (Aunola & Rusko 1984). Exercise intensities above the LT but below the maximal lactate steady state (MLSS) or LTP are in zone two (Seiler & Kjerland 2006; Fig.2.6). The LTP has been defined as the final exercise intensity before the observation of a sudden and sustained increase in blood lactate concentration between the LT and the VO_{2max} (Smith & Jones 2001). The MLSS has been defined as the highest exercise intensity at which blood lactate concentration remains stable between 10 and 20 minutes of constant load exercise (Beneke, 1995; Fig. 2.5).

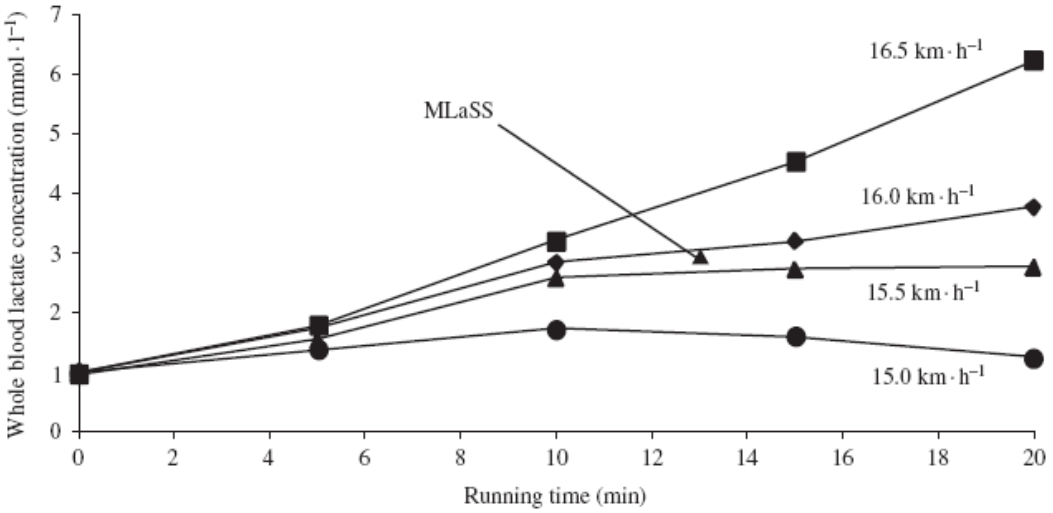

Fig.2.5 An example MLSS profile, showing the MLSS (MLaSS) at 15.5km.hr^{-1}, to the nearest 0.5km.hr^{-1}. (Taken from Almarwaey et al, 2004).

Exercise intensities above the MLSS/LTP are in zone three (Seiler & Kjerland 2006); Fig.2.6). Seiler & Kjerland (2006) have named these three intensity zones in terms of blood lactate concentration characteristics: first, a low lactate zone, second a lactate accommodation zone (where blood lactate concentration is elevated but production and removal rates re-establish equilibrium), and third a lactate accumulation zone, where blood lactate production exceeds maximum clearance rates, and muscle fatigue is imminent.

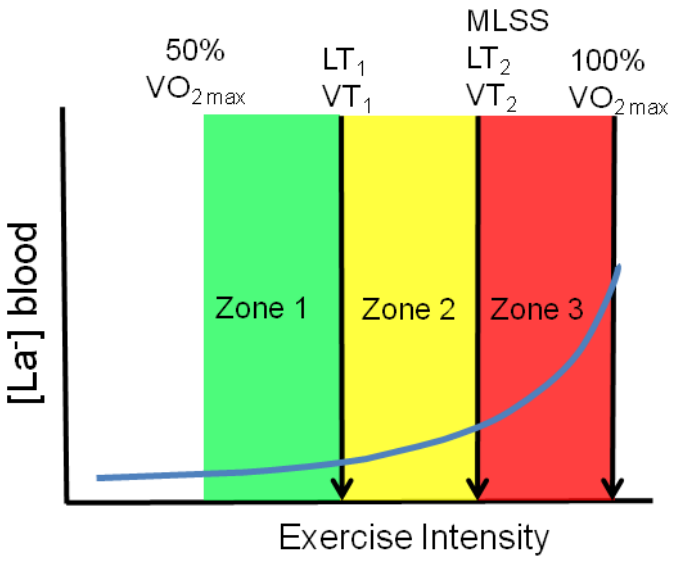

Fig.2.6 Blood lactate concentration response to increasing exercise intensities. (Taken from Seiler & Tonnessen (2009)

Lucia et al., (1999a); Lucia et al., (1998); Fig. 2.7) identified two specific ventilatory changes that correspond to the aerobic and anaerobic thresholds introduced by Kindermann et al (1979), from using breath-by-breath gas exchange measurements. These two changes have been called ventilatory threshold 1 (VT_1) and ventilatory threshold 2 (VT_2)/Respiratory Compensation Point (RCP): VT_1 occurs when there is an increase in both the ventilatory equivalent for oxygen ($VE{\cdot}VO_2^{-1}$) and end-tidal partial pressure of oxygen with no concomitant increase in the ventilatory equivalent for carbon dioxide ($VE{\cdot}VCO_2^{-1}$) (Davis 1985); Fig. 2.7). VT_2 occurs when there is an increase in both ($VE{\cdot}VO_2^{-1}$) and ($VE{\cdot}VCO_2^{-1}$) and a decrease in the end-tidal partial pressure of carbon dioxide (Davis 1985); Fig. 2.7). Therefore, at VT_2, blood lactate accumulation increases significantly, along with an additional hyperventilation in an attempt to buffer the additional hydrogen ions (Kindermann et al., 1979).

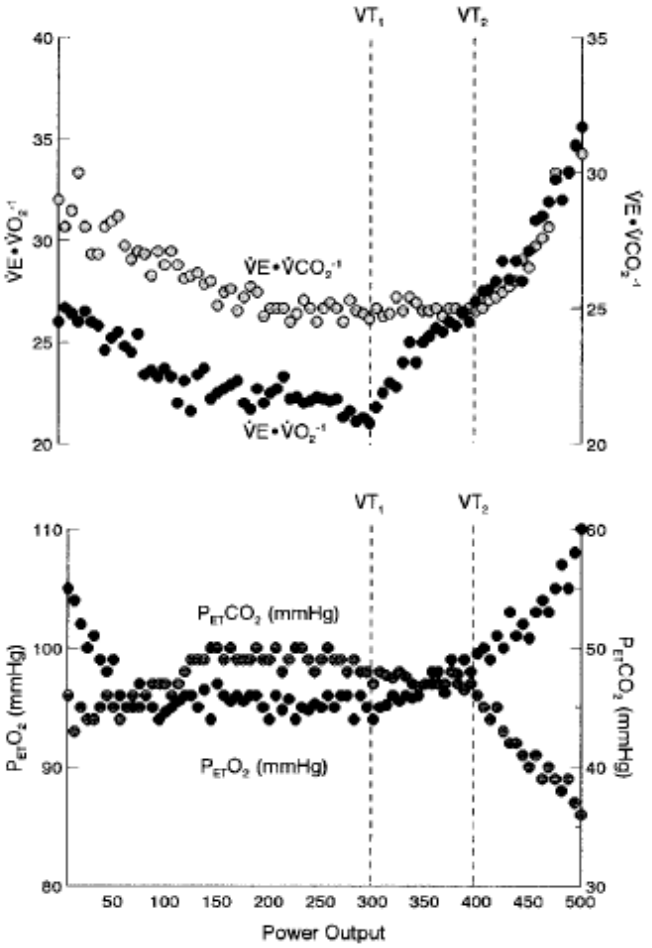

Fig.2.7 Determination of VT_1 and VT_2. Each gas-exchange data point corresponds to a 15-s interval. **$VE \cdot VO_2$**: Ventilatory equivalent for oxygen, **$VE \cdot VCO_2$**: Ventilatory equivalent for carbon dioxide, **$P_{ET}O_2$**: End-tidal pressure of oxygen, **$P_{ET}CO_2$,** End-tidal pressure of carbon dioxide. (Taken from Lucia et al., (2000a)).

The relationships between VT_1, LT_1 and VT_2, LT_2 have been demonstrated in a study by Lucia et al (1998). This study found no significant differences in the power output corresponding to VT_1 and LT_1 or VT_2 and LT_2 in a group of 28 professional or elite amateur cyclists undergoing a continuous incremental test to exhaustion. In this study, VT_1, VT_2 and LT_1 were defined as described previously and LT_2 was defined as the power output at a blood lactate concentration of $4 mmol \cdot L^{-1}$. This study also found no significant difference between both VT_1 and LT_1 and the first surface electromyography (sEMG) amplitude threshold in the vastus lateralis and rectus femoris muscles. In addition, the second sEMG amplitude threshold also occurred at similar time/power points as both VT_2 and LT_2 in the same muscle groups. In support, Chwalbinska-

Moneta et al (1998) have also demonstrated a close correlation between blood lactate concentration, EMG, and catecholamine thresholds. The EMG threshold could be the result of a progressive recruitment of motor units, with a possible participation of type IIa and type IIb fibres at the two thresholds, respectively. Seiler & Kjerland (2006) state that the aforementioned studies support the use of LT_1/VT_1 and LT_2/VT_2 as defensible physiological anchor points for the establishment of three training intensity zones. Although the determination of VT_1, VT_2 and LT seem to be consistent in the literature, the zone 2/3 boundary using blood lactate concentration is less clear. Therefore, care needs to be taken in interpreting results, as the onset of blood lactate accumulation (OBLA; 4mmol·L^{-1}); LTP or MLSS could all be used to demarcate zones 2 and 3 when using blood lactate concentrations. In addition, there is a potential limitation of using the HR corresponding to the two thresholds in the three intensity zone model to assess TID, as the HR is irrelevant at very high training intensities (Seiler & Kjerland 2006).

2.2.3 Polarised and Threshold Training Models

Seiler & Kjerland (2006) propose that two basic patterns of TID emerge from the research literature (Fig. 2.8). These authors state that the polarised and threshold training models provide a basic framework for future investigations exploring the endurance training process.

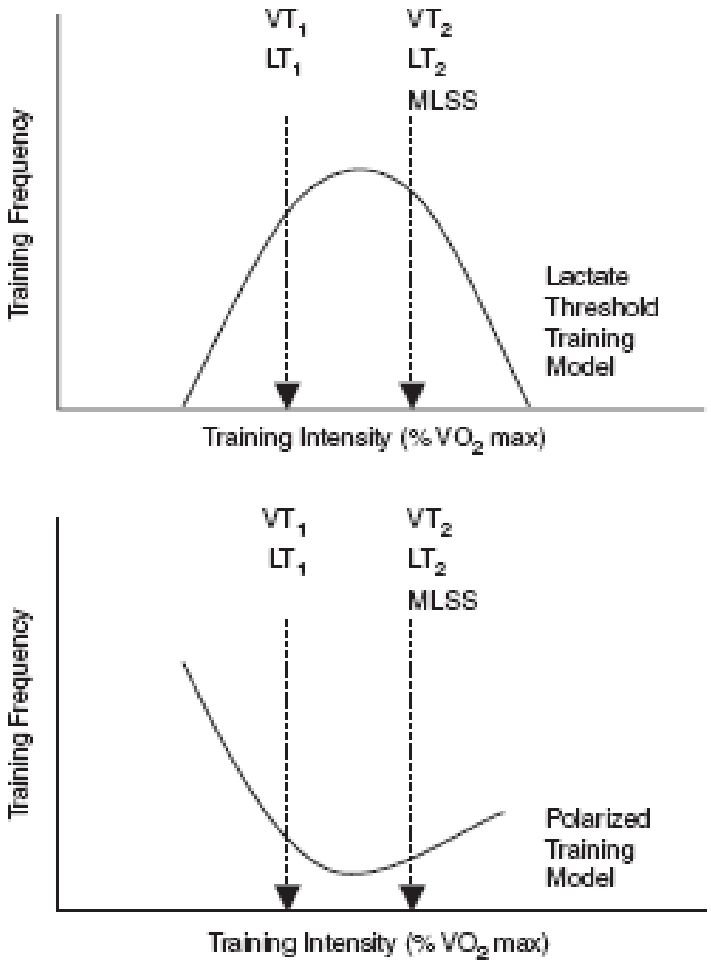

Fig.2.8. Conceptual TIDs associated with; the threshold training model (top) – emphasising training between the first and second lactate/ventilatory thresholds and the polarised training model (bottom) – emphasising a large volume of training below the first lactate or ventilatory threshold combined with significant doses of training with loads eliciting 90–100% of VO_{2max}. Taken from Seiler & Kjerland (2006).

2.2.4 Studies to have Assessed the Training-Intensity Distribution

2.2.4.1 Descriptive Studies

Table. 2.2 Descriptive studies assessing the training-intensity distribution in trained endurance athletes

Study	Subjects	Training Volume	Duration	Zones	Method	TID
Seiler & Kjerland 2006	12 elite XC skiers	10-12hrs·wk^{-1}	32d	VT/HR RPE/RPE Bla/BLa VT/HR	SGA SGA SGA TIZA	75-8-17 76-6-18 71-7-22 91-6-3
Esteve-Lanao et al., 2005	8 well-trained runners	~70km·wk^{-1}	24wk	VT/HR	TIZA	71-21-8
Guellich et al., 2009	36 elite rowers	13hrs·wk^{-1} (all) 6.5hrs·wk (rowing)	37wks	Bla/HR	TIZA	95-2-3
Lucia et al., 2000a; Lucia et al., 2000	13 elite cyclists	~750km·wk^{-1}	7mo (3 periods)	VT/HR VT/HR VT/HR	TIZA TIZA TIZA	88-11-1 78-17-5 77-15-8
Schumacher & Mueller 2002	7 elite cyclists	~400mi·wk^{-1}	7mo	Bla/HR	TIZA	94-4-2
Zapico et al., 2007	14 elite cyclists	~17hrs·wk^{-1}	7mo (2 periods)	VT/HR	TIZA	78-20-2 70-22-8
Mujika et al., 1995	18 elite swimmers	17-33km·wk^{-1}	44wk	Bla/Pace	SGA	78-12-10
Billat et al., 2001	20 elite runners	140-200km·wk^{-1}	12wk	RP/Pace	SGA	78-4-18
Billat et al., 2003	20 elite runners (2 groups)	120-200km·wk^{-1}	8wk	Bla/Pace	SGA SGA	84-7-9 85-14-1
Sandbakk et al., 2010	16 elite XC skiers (2 groups)	13-19hrs·wk^{-1}	6mo	HR/Bla/ HR	SGA SGA	88-7-5 88-5-7
Tjelta & Enoksen 2010	4 elite runners	115-145km·wk^{-1}	12mo (3 periods)	HR/HR/ Pace	SGA SGA SGA	78-20-2 81-12-7 78-18-4

SGA: Session Goal Approach, **TIZA:** Time in Zone Approach, **XC:** Cross-country, **HR:** Heart Rate, **RPE:** Rating of Perceived Exertion, **Bla:** Blood lactate concentration. **TID**: Training-Intensity Distribution (displayed as the percentage of time spent in zone 1, 2 and 3, respectively), **Zones:** How the training zones were determined, **VT:** Ventilatory Threshold, **RP**: Race Pace, **Pace:** Using the time for a particular distance to quantify the training zone the exercise took place.

To date, there have been a number of descriptive studies to have assessed the TID in elite and well-trained athletes in a number of different disciplines (Table 2.2). It is of significant use to coaches and exercise physiologists to observe how these elite athletes from different disciplines organise their training and can give some insight into effective TIDs. A high training volume, relative to the sport is common to all of these studies on elite endurance athletes.

2.2.4.1.1 Training Volume and Load

Elite and well-trained endurance athletes train with a high training volume and load (Table 2.2). This is presumably at the threshold of what the athlete can tolerate (at least temporarily) in order to maximise performance. It has been reported that higher performing athletes train with a higher volume (Billat et al., 2001; Fiskerstrand & Seiler 2004; Sandbakk et al., 2010), and a positive relationship has been reported between training volume and success in championships in rowers (Steinacker et al., 1998). It has been suggested that the benefits of high intensity training are only possible once the minimum training volume for the particular event is achieved (Jones & Carter 2000). In the long term, it has been suggested that a high training volume may facilitate endocrine adaptations (Kjaer 1998). However, there seems to be a ceiling for the amount of training volume that will continue to lead to positive adaptations. Studies have shown that in highly trained athletes with already high training volume, an additional increase through increased submaximal training does not appear to further enhance endurance performance or associated variables (Costill et al., 1988; Londeree 1997). For example, in rowing, this has been shown to occur at approximately 5000 to 6000 $km \cdot yr^{-1}$ (Steinacker 1993). In addition, with an increase in training load, there is an increased risk of overtraining, especially with monotonic training (Steinacker et al., 1998).

2.2.4.1.2 Methods of Assessing Training Intensity Distribution

2.2.4.1.2.1 Time in Zone Approach and Session Goal Approach

There are two methods of quantifying TID from HR recordings. Firstly the Time in Zone Approach (TIZA) whereupon the HRM is set with two threshold HRs corresponding to VT_1/LT_1 and VT_2/LT_2. The HRM then records all HR recordings for the entirety of each training session and determines how much time was spent in the three training zones (Seiler & Kjerland 2006). The TIZA is very user friendly, with most HRM manufacturers providing a function to record HR in different intensity zones. In addition, every training minute can be incorporated into the training analysis from the start to the finish of every training session. However, it has been suggested that a HR based TIZA will tend to underestimate the actual time and energetic and sympathetic stress at high exercise intensities such as interval training, due to delays in HR responses (Guellich et al., 2009). For example, in using this approach for a cycling interval training session, despite the power output corresponding to zone three for each bout, the HR may only enter zone three for a very short period of time during each interval.

Secondly, the Session Goal Approach (SGA) whereupon each training session is analysed according to the goals of steady state, threshold and interval training sessions. For steady state training sessions, the intensity zone of the average HR for the entire session is used. For threshold training sessions, the intensity zone corresponding to average HR of the specific threshold bout of training is used. For interval training, the intensity zone of the average peak HR attained during each interval bout is used. The intensity zone of the warm up and cool down for any training session is quantified in the same way as for the steady state training sessions.

Care needs to be taken when analysing the TID research, as differences in TID can occur for the same training period using either the TIZA or the SGA, based on HR recordings. Two

studies to date have analysed the TID using both the TIZA and the SGA. Seiler & Kjerland (2006) found that in a group of well-trained junior cross country skiers, training over 32 consecutive days, the TID using the TIZA was 91, 6.4 and 2.6% of training time spent in zones one, two and three, respectively, and using the SGA was 75, 8 and 17%. Similarly, Esteve-Lanao et al (2007) found that in a group of well-trained runners training over 5 months, the TID using the TIZA was 80, 12 and 8% and using the SGA was 74, 11 and 15%. These studies both show that the percentage of training time in zone one is considerably lower and the percentage of training time in zone three is considerably higher when using the SGA compared to the TIZA. The percentage of training time in zone two seems to be similar using both approaches.

2.2.4.1.2.2 Additional Methods to Quantify TID

In addition to HR recordings, the rating of perceived exertion (RPE) and blood lactate concentration measurements have been used to quantify the TID, using the SGA (Seiler & Kjerland 2006). Pilot work revealed that a 10-point RPE developed by Foster and colleagues (Foster et al., 1996; Foster 1998) can be demarcated into three training intensity zones (Seiler & Kjerland 2006). Using this approach, points 1 to 4 (easy to somewhat hard) are classified as zone one, points 5 and 6 are classified as zone two (hard) and points 7 to 10 are classified as zone three (very hard to maximal effort). The agreement between ventilatory threshold derived HR assessment of the TID using the SGA and this RPE approach was 92%, with no significant difference (Seiler & Kjerland 2006). There were also no significant differences between the ventilatory threshold derived HR assessment of the TID using the SGA, and the TID using the fixed blood lactate values of 2 and 4mmol.L^{-1} to demarcate zones one to two and two to three, respectively. This was the first study to use more than one method to analyse TID and to compare them, which adds substantially to the understanding of how to assess the TID in endurance athletes.

The studies reviewed in table 2.2 used different methods to determine the training intensity zones, with the HR associated with ventilatory thresholds (VT_1 and VT_2) and blood lactate concentration thresholds the most common. Fixed blood lactate concentrations of 2 and 4mmol·L^{-1} have been used to demarcate training intensity zones (Mujika et al., 1995; Seiler & Kjerland 2006; Guellich et al., 2009). However, there is considerable interindividual differences in the fixed blood lactate concentrations, which may over- or underestimate the true endurance capacity (Faude et al., 2009). The physiological stress might therefore be over- or underestimated for athletes with especially high or low lactate production, and this could be influenced by training and diet, as reduced muscle glycogen availability leads to lower lactate production (Faude et al., 2009). The pace of training sessions in relation to the previously established training zones by blood lactate concentration thresholds has been used to quantify TID (Mujika et al., 1995; Billat et al., 2003). The study by Mujika et al (1995) assessed the TID in swimmers, in which training paces are very easily quantified, and the training environment is very stable, and therefore it is likely that this was an accurate method to assess the TID. However, the study by Billat et al (2003) was in runners, and therefore is likely that this was less accurate, due to a less stable environment (hills, temperature, and humidity). Relating the pace adopted in training to that of racing has also been used to quantify the TID (Billat et al., 2001). In this study, the training of runners was related to marathon pace in order to quantify three training zones (below marathon pace, at marathon pace and above marathon pace). It has been reported that marathon pace is at or very close to the LT (Sjodin & Svedenhag 1985). The studies that assess TID using pace instead of HR should be treated with caution. This could potentially underestimate exercise intensity due to the so-called slow component, the gradual increase in VO_2 occurring after the third minute of exercise bouts performed over the LT/VT (Xu & Rhodes 1999), which obviously only effects the data for zones two and three. The percentage of maximum HR has been used to demarcate

three training zones (Tjelta & Enoksen 2010). This study used 62-82% HR_{max} as zone one, 82-92% for zone two and then used 10, 000m pace or quicker as zone three. The metabolic challenge of exercise in each of these zones may be different between individuals, and thus the results from this study also need to be treated with caution.

2.2.4.1.3 Training-Intensity Distribution of Different Performance Levels

It has been reported that in the 12 week lead up to the Olympic trials, the general TID of both male and female elite marathon runners of different standards was generally the same (78, 4 and 18% of training time below, at and above marathon pace, respectively) (Billat et al., 2001). However, the top-class male runners (marathon time: <2hr 11) had a 23% higher training volume (206 vs. 168km) and therefore spent more time in each training zone than the high-level male runners (marathon time: >2hr 11, <2hr 16). In contrast, there was no difference in the training volume between the top class female runners (marathon time: <2hr 31) and the high level female runners (marathon time: >2hr 31, <2hr 38). However, of the training performed at and faster than 10km race pace, the top class female runners spent more time at higher intensities (3km pace) than the high level female runners. Similarly, Sandbakk et al (2010) compared the training of World class and National standard male cross country skiers over 6 months and found essentially the same TID. However, the World class skiers trained with 30% more volume than the National standard skiers, a similar result to that of the males in the study by Billat et al (2001). In another study by Billat and colleagues (Billat et al., 2003), a group of elite male 10km runners were separated into those that train at the velocity associated with the VO_{2max} (vVO_{2max}) (HI) and those that complete no training at this intensity (LO) during an 8 week build up to an important race. HI completed 84, 7, 4 and 5% of training at a sub-LT intensity, at the LT intensity, between the LT and the VO_{2max} and at vVO_{2max}, respectively. In contrast, LO completed 85, 14, 1 and 0% of running at the same training intensities. Therefore, the major difference in the TID between the two groups was that HI

completed more high intensity training: 5 and 4% of training at vVO_{2max} and halfway between the LT and the vVO_{2max}, respectively in comparison to 0 and 1%, respectively in LO. In addition, LO completed more training time at the LT intensity (14%) in comparison to HI (7%). There was no difference in the training time spent at a sub-LT intensity between HI and LO. LO achieved a significantly higher training volume (174km.wk^{-1}) than HI (158km.wk^{-1}). It is likely, however, that HI achieved a greater training load, due to more training at high intensities than LO, although this is not reported. HI ran faster over 10km (28min 15s) than LO (28min 54s), and also had a higher VO_{2max} and vVO_{2max}. However, the runners in LO were more economical. This study demonstrates that despite a significantly lower training volume, a training programme with more training at high intensities (zone three) in place of training at the LT intensity (zone two) can be beneficial. In addition, in relation to the TID models proposed by Seiler & Kjerland (2006), the training completed by HI was more polarised than that in LO.

With the exception of period two in the study by Zapico and colleagues (Zapico et al., 2007), the descriptive studies assessing the TID using the TIZA in elite athletes seem to follow a general pattern of ~80% and ~20% of training time below and above zone one (VT_1, LT). The elite athletes also all train with very high volume. It may be relevant that the only study assessing sub-elite athletes (Esteve-Lanao et al., 2005) revealed that these athletes spend more time in zones two and three, combined (~30%) and consequently, less time in zone one (~70%) than the elite athletes. They also have a lower training volume in relation to their discipline, and so this has the effect of increasing the time spent at higher intensities (zones two and three). A significant relationship has been found between the time spent in zone one and performance (Esteve-Lanao et al., 2005). In addition, no significant correlation was found between the training time spent in zones two and three, and performance. It would therefore seem that the sub-elite athletes in the study by Esteve-Lanao et al (2005) would benefit from increasing training volume through an increase in zone one training time and this would also

decrease the percentage of training time spent in zones two and three to <20%, in line with the studies on elite endurance athletes.

2.2.4.1.4 Comparing Different Training-Intensity Distributions in Similar Studies

Elite male road cyclists have been reported to gain large physiological adaptations following a winter mesocycle of training, with high training volume (Zapico et al., 2007). The TID of these cyclists was 78, 20 and 2% of training time in zones one, two and three, respectively, analysed using the TIZA. Physiological adaptations included increases in the power corresponding to VT_1 and VT_2, the peak power output, the MLSS and the VO_{2max}. The training volume of these cyclists significantly increased during the spring mesocycle, and the TID shifted to 70, 22 and 8% of training time in zones one, two and three, respectively. None of the previously improved physiological adaptations were further improved following the spring mesocycle and in fact, the mean peak power output decreased back to a pre-training value. The authors suggest that the lack of further improvement following the spring mesocycle reflects either a physiological ceiling was reached, or an accumulation of training volume. A very similar study on a group of elite male road cyclists assessed the effect of TID on physiological adaptation, using the TIZA (Lucia et al., 2000a; Lucia et al., 2000b). This study also assessed the cyclists over an entire season, but was split into three training periods. The training volume increased in each training period, and was very high in the final two training periods. The TID was 88, 11 and 1% of training time in zones one, two and three, respectively in period one, 78, 17 and 5%, respectively in period two and 77, 15 and 8%, respectively in period three. The most striking difference between this study and the study by Zapico et al (2007) is the continual improvement in LT. The power associated with the LT was 319, 350 and 379W, respectively in periods one, two and three in the study by Lucia and colleagues (Lucia et al., 2000a; Lucia et al., 2000b). In both of these studies (Zapico et al., 2007; Lucia et al., 2000a; Lucia et al., 2000b), the cyclists trained with a high volume and this increased throughout the season. In

the study by Lucia and colleagues (Lucia et al., 2000a; Lucia et al., 2000b) and in the first period of training in the study by Zapico et al (2007), the training time in zone one is high (>77%), which has been suggested to be beneficial (Ingham et al., 2008; Esteve-Lanao et al., 2007; Esteve-Lanao et al., 2005; Fiskerstrand & Seiler 2004) and was beneficial in both of these studies. However, in the second period of training in the study by Zapico et al (2007), the time spent in zone one decreased to 70%, and this is a potential explanation of the lack of physiological adaptation following this second period of training. In addition, the time spent in zone two in the second period of training in the study by Zapico et al (2007) rose to above 20%, which has been suggested to result in a physiological adaptation plateau as opposed to increasing training time in zone one (Esteve-Lanao et al., 2007). This therefore provides another potential explanation for the lack of physiological adaptation following the second period.

2.2.4.2 Experimental Studies

Experimental studies have attempted to manipulate the TID in elite and well-trained endurance athletes, to assess if different responses are present, due to the TID (Table 2.3). A group of elite rowers were found to have a greater improvement in the power corresponding to both the LT and to 4mmol·L^{-1} blood lactate concentration following 12 weeks of a predominantly low intensity training programme compared to a group following a mixed intensity training programme of equal training volume (Ingham et al., 2008; Table 2.3.). The low and the mixed intensity groups spent 98 and 72% of training time sub-LT, respectively. However, there were no changes in the performance (2km time trial) between the groups. As the groups were matched for training volume, the low intensity group would have been training with a lower training load, and so this result is intriguing. In addition, the mixed intensity group showed no signs of overtraining, and therefore this result was unlikely to be caused by excessive sympathetic stress in the mixed group. This study provides further

evidence of the importance of training in zone one. The manipulation of training time spent in zones one and two was assessed in two groups of well-trained runners over a five month training period (Esteve-Lanao et al., 2007; Table 2.3). The two groups were matched for performance and physiological attributes before the training period and were prescribed training with no difference in the total training load or the time spent in zone three, but differences in the time spent in zones one and two. The TID for group 1 was 80, 12 and 8% of training time in zones 1, 2 and 3, respectively and the TID for group 2 was 67, 25 and 8%, respectively. The major difference in the training between the groups was that group 2 did zone two 'tempo runs' whilst group 1 did longer runs in zone one, and there was no sign of overtraining in either group. The magnitude of improvement in a simulated 10.4km cross-country race was significantly greater in group one (7%) than group two (5%). In agreement with Esteve-Lanao et al (2005) and Ingham et al (2008), this study shows a greater improvement with a training programme including more training in zone one.

Table. 2.3 Experimental studies to have manipulated TID in well-trained endurance athletes.

Study	**Subjects/ Study Duration**	**Groups**	**Results**
Ingham et al., 2008	Elite rowers/ 12wks	1. 98% <LT, 2% >LT 2. 78% <LT, 22% >LT	Power at LT: 1>2 Power at 4mmol.L^{-1} Bla: 1>2 2km rowing performance: nsd
Esteve-Lanao et al., 2007	Well-trained runners/ 5mo	1. 80-12-8 2. 67-25-8	Improvement in XC race: 1>2

> One group performed better than the other, **nsd**: No significant difference, **XC**: Cross-Country

Two case studies illustrate how increasing training volume and decreasing the intensity of high intensity training sessions can provide large improvements in performance in already well-trained endurance athletes (Seiler & Tonnessen 2009). In both case studies, the athletes replaced a high intensity, low volume programme with a high volume, low intensity programme. In the first case study on a cyclist, training volume was roughly doubled, training

time in HR zone five was replaced with training time in HR zones three and four and training time in HR zone two was reduced (Table 2.4). Following the change in training, there were large improvements in an already high VO_{2max} (11%; 81-88ml.kg^{-1}.min^{-1}) and LT power (14%; 375 – 440W). In the second case study on a runner, a similar redistribution took place (Table 2.4). This change in training also resulted in an increase in an already high VO_{2max} (9%; 76-83mL·kg^{-1}·min^{-1}) and LT velocity (5%; 16.9-17.7km·hr^{-1}).

Table.2.4 Case studies 1 and 2. TID and training volume for the two seasons assessed. (Data from Seiler & Tonnessen (2009)

Intensity Zone (%HR_{max})	**Case Study 1 Season One (h:min·wk^{-1})**	**Case Study 1 Season Two (h:min·wk^{-1})**	**Case Study 2 Season One (h:min·yr^{-1})**	**Case Study 2 Season Two (h:min·yr^{-1})**
5 (95-100%)	0:45 (8.5%)	0:05 (0.5%)	8:00 (3.0%)	2:00 (0.5%)
4 (90-95%)	-	0:40 (4.0%)	33:00 (12.0%)	13:00 (2.5%)
3 (85-90%)	0:30 (5.5%)	1:00 (5.5%)	36:00 (13.0%)	50:00 (10.0%)
2 (75-85%)	3:05 (36%)	1:00 (5.5%)	49:00 (18.0%)	20:00 (4.0%)
1 (55-75%)	4:20 (50%)	15:20 (85%)	149:00 (54%)	412:00 (83.0%)
Weekly total	8:40	18:05		
Annual total	420:00	850:00	275:00	497:00

The effectiveness of a large volume of low intensity training is challenged by a study manipulating the training of well-trained cross-country skiers over two years (Gaskill et al., 1999; Table 2.5). All of the athletes completed the same high volume training in the first year, consisting of 84 and 16% of training below and above the LT, respectively. Half of the group responded positively to this training, with increases in VO_{2max} and LT of >7 and >10%, respectively, and these athletes maintained the same training for year two. The athletes that did not respond, more than doubled their high intensity training time above the LT to 45%, subsequently decreasing sub-LT training to 55%. There was no difference in the overall training volume between the groups in the second year of training, although the increased high intensity training meant that the treatment group had a significantly higher training load.

In the second year of training, both groups improved VO_{2max} and LT (Table. 2.5). The TID in the first year of training is very similar to that of other elite endurance athletes (Table. 2.2). The response of the treatment group is in contrast to the studies that have reported a greater improvement in performance and physiological adaptation following more training in zone one (Ingham et al., 2008; Esteve-Lanao et al., 2007; Esteve-Lanao et al., 2005).

Table.2.5 Results of the study by Gaskill et al., (1999)

Year	Group	TID	Result
One	Both	84% <LT, 16% >LT	Half of the group responded positively (Responders) Half of the group did not respond (Non-responders)
Two	Responders	84% <LT, 16% >LT	Responded positively
	Non-responders	55% <LT, 45% >LT	Responded positively

LT: Lactate Threshold (sudden increase in blood lactate concentration at 3-5mmol·L^{-1})

The concept that a more effective training programme includes both a high overall training volume and a high training volume in zone one is also challenged by Evertsen et al (1999); Table. 2.6). For two months, the training of a group of elite cross-country skiers trained was the same. For the following five months, two training groups were formed, that differed in both training volume and training intensity. A moderate intensity group trained with 86% of training time in zone one (<1.5mmol.L^{-1} blood lactate concentration) and 14% in zone two (3-4mmol.L^{-1} blood lactate concentration). In contrast, a high intensity group trained with 17 and 86% of training in zones one and two, respectively. The performance in a simulated 20min race was improved significantly more in the high intensity group. In addition, the LT improved in the high intensity group, but not the moderate intensity group. Therefore, despite the training volume being lower in the high intensity group, and this group only using zone one training for restitution, the performance and physiological adaptation was higher in this group than a group training with a greater overall volume and a greater volume in zone one.

Table.2.6 Results of the study by Evertsen et al., (1999)

Group	Duration	Volume ($hrs \cdot wk^{-1}$)	Zone 1 (%)	Zone 2 (%)	Performance improvement (%)
Both	2mo	10	84	16	-
Mod	5mo	16	86	14	1.9
High	5mo	12	17	83	3.8*

Mod: Moderate intensity group, **High**: High intensity group, **Performance**: 20min simulated race,
* Significantly higher than Mod group.

2.2.5 The Polarised Training Model

Studies using the SGA have shown that a polarised training model (Fig. 2.8) is effective in elite and well-trained endurance athletes (Esteve-Lanao et al., 2007; Seiler & Kjerland 2006; Fiskerstrand & Seiler 2004; Billat et al., 2001). Indeed, Fiskerstrand & Seiler (2004) reported how the training in elite rowers had become more polarised in the past 30 years. In addition, a polarised training model was reported to be more effective than a training model emphasising more training time in zone two (Esteve-Lanao et al., 2007). However, it is not possible to tell if a training programme was polarised when analysing the training using the TIZA (Sandbakk et al., 2010; Guellich et al., 2009; Zapico et al., 2007; Lucia et al., 2000a; Lucia et al., 2000b). The TIZA underestimates time in zone three, and can therefore make a training programme that is polarised in nature, appear otherwise (Esteve-Lanao et al., 2007; Seiler & Kjerland 2006).

It has been reported that the periodisation of TID over the course of a season involves shifting the zone one training to a lower intensity and the higher intensity training (zones two and three) to higher intensities (Guellich et al., 2009). This can be achieved through race selection in elite cyclists, with races containing mainly flat profiles early in the season and mountainous races as the season progresses (Schumacher & Mueller 2002). It therefore seems that training becomes more polarised throughout a training season.

Less experienced athletes have been found to train harder than prescribed (zone two) during prescribed low intensity sessions (zone one) and not hard enough (zone two) during prescribed high intensity sessions (zone three) (Foster et al., 2001b). The zone three training sessions are believed to be essential in eliciting maximal performances, yet they cannot be performed optimally if prescribed zone one training sessions are actually performed in zone two (Bruin et al., 1994), at least in part due to the delayed recovery (Seiler et al., 2007).

2.2.6 The Threshold Training Model

2.2.6.1 Well-Trained Endurance Athletes

Groups of cross country skiers and marathon runners have been reported to spend less time in zone two than in zone three, using the SGA (Seiler & Kjerland 2006; Billat et al., 2001); Table. 2.2). Even though it was the intensity of their specialist distance, elite marathon runners have been shown to spend just 4% of training time at the intensity corresponding to the LT (Billat et al., 2001). A group of elite runners spending more time in zone two (14%) and a higher training volume (174km) were reported to be slower over 10km and had a lower VO_{2max} than a group with a lower training volume (158km) and less time spent in zone two (7%) (Billat et al., 2003). This could be suggestive that training in zone two could have a negative effect upon performance and physiological adaptation.

It has been suggested that in elite endurance athletes training with high training loads, too much training in zone two might generate excessive sympathetic stress (Chwalbinska-Moneta et al., 1998) while still providing a sub-optimal stimulus for eliciting further gains in capacity (Londeree 1997). This may lead to excessive fatigue, potentially via a down-regulation of the sympathetic nervous system (Esteve-Lanao et al., 2007). The overstrain due to training excessively in zone two has also been suggested to be due to substrate depletion and incomplete recovery (Steinacker et al., 1993). With very high training loads, the intensity has

to be reduced, and thus there has been more attention on training in zone one (Steinacker et al., 1993). In well-trained runners with a mean weekly time of ≥6 hours, when training time in zone two exceeded 20%, no further beneficial adaptations occurred as opposed to increasing time in zone one (Esteve-Lanao et al., 2007). In addition, it has been suggested that a high percentage of tempo training in zone two (>30%) does not improve performance and VO_{2max} compared to training with only 5-10% tempo training (Jensen et al., 1993).

2.2.6.2 Untrained Individuals

Moderate intensity training (~55-85% HR_{max}) for 20 to 60 minutes (at least $3d \cdot wk^{-1}$) is recommended for improving and maintaining cardiorespiratory fitness in non-athletes (ACSM, 1998). This type of training has been reported to be effective at inducing large gains in physiological adaptation in previously untrained individuals (Table. 2.7). The highest exercise intensity that can be maintained for a prolonged period of time is the intensity at the zone 2/3 boundary (Beneke, 1995), and is therefore very attractive to untrained individuals. This TID corresponds to the threshold training model (Seiler & Kjerland 2006).

Table.2.7 Studies showing a physiological adaptation following training in zone two in untrained subjects. All studies used cycling and 100% of training was completed in zone two.

Study	Frequency ($d \cdot wk^{-1}$)	Duration (wks)	Training duration and intensity	Improvements
Yoshida et al., 1982	3	8	15min at an intensity corresponding to 4mmol.L-1 Bla	VO_{2max} (14%) VO_2 at LT (37%)
Denis et al., 1984	3-4	20	60min at 80-85% VO_{2max}	VO_{2max} (19%) Power at 4mmol.L^{-1} Bla (42%)
Takeshima et al., 1993	3	8	30min at LT	VO_{2max} (10%) VO_2 at LT (18%)
Gaskill et al., 2001	3	6	50min at VT	VO_{2max} (16%) VT (46%)

Bla: Blood lactate concentration.

The large increase in physiological adaptation in previously untrained individuals (Table 2.7) does not appear to be limited to training in zone two (Londeree 1997). Indeed, a meta-

analysis coded training status and training intensities from 85 study groups and reported that in sedentary subjects, every training intensity from below LT to above 4mmol·L^{-1} blood lactate concentration improved performance, with no significant difference between the intensities (Londeree 1997).

2.2.7 Training in Zone 1

Training in zone one has been reported to be effective in elite and well-trained endurance athletes (Ingham et al., 2008; Esteve-Lanao et al., 2005; Fiskerstrand & Seiler 2004), but the physiological mechanisms underlying this effectiveness are not well understood. Zone one represents a wide intensity range in elite endurance athletes, due to the LT/VT_1 being so high. It has been suggested that a large fraction of zone one training takes place at ~60-65% VO_{2max} (Seiler & Tonnessen 2009). An elite endurance athlete (VO_{2max}: 70-80mL·kg^{-1}·min^{-1}) training at 65% of VO_{2max} (zone one) would have about the same muscular oxidative flux as an untrained person training at or near VO_{2max}, assuming similar active muscle mass (Seiler & Kjerland 2006). An elite athlete can train at 65% VO_{2max} (zone one) for a long duration of time, and so along with a large cellular energy turnover, this seems to be sufficient to provide an effective stimulus for the induction of the various genes involved in mitochondrial biogenesis (Hood et al., 2000a). Indeed, it has been reported in animals that low intensity training of approximately 50% VO_{2max} is sufficient to maximise the increase in mitochondria in type I muscle fibres, but to induce significant increases in type II muscle fibres, much higher intensities are needed (Dudley et al., 1987; Harms & Hickson 1983). As elite endurance athletes have a large proportion of type I fibres, and maximising mitochondrial volume in these fibres can occur with sub-LT training, it is no surprise that this constitutes the majority of the training. Low intensity exercise of approximately 65% of VO_{2max} has also been shown to approximate the same intensity associated with maximal fat utilisation in trained subjects (Achten & Jeukendrup 2003). It is possible that this stimulates an increase in lipolysis, which

will consequently decrease the blood lactate concentration, due to decreased carbohydrate oxidation. Indeed, it has been reported that long distance runners have higher LTs than middle distance runners (MacDougall et al., 1977), which is indirect evidence that a large volume of sub-LT training is effective at increasing the LT. It is also possible that low intensity training enhances recovery from high intensity training through in increase in peripheral blood flow (Steinacker et al., 1998).

Zone one training causes less stress than training in zone two. It has been reported that in a group of elite female rowers, a 2hr training session at approximately 60% VO_{2max} (zone one) caused minimal changes in blood hormones (cortisol, growth hormone, epinephrine, norepinephrine) and immune-function measures (Nieman et al., 1999). A 'J shaped curve' has been used to model the relationship between exercise and susceptibility to infection (Nieman 1994). An enhanced immune function above sedentary levels might be achieved with moderate exercise (Matthews et al., 2002). In contrast, impaired immune function might result from excessive amounts of prolonged, high-intensity exercise. Mechanisms behind these changes are not well understood, but may relate to an increased level of catecholamines (Moynihan et al., 1998). A catecholamine threshold occurs at the zone one-zone two boundary (Chwalbinska-Moneta et al., 1998), and it is therefore possible that sub-LT training causes less of an increase in catecholamines and therefore less chance of picking up an infection.

A group of well-trained runners were found to have a faster recovery of the autonomic nervous system (ANS; heart rate variability (HRV)) following up to 2hrs of training in zone one, compared with training in zones two and three (Seiler et al., 2007). This study also showed no difference in the recovery time of the ANS following training sessions in zones two and three. The authors suggest that VT_1 may demarcate a 'binary' threshold for ANS/HRV recovery in

highly trained athletes; because further delays in HRV recovery with even higher training intensities were not observed. The results of this study could explain the results of some of the descriptive studies (Table 2.2). The elite athletes spent a high percentage of training time in zone one and this could have been because they are able to recover so quickly from zone one training, whereas a large amount of training in zone two would have led to a longer recovery time for the ANS.

2.2.8 Training in Zone 2

Training in zone 2 (at the MLSS) twice per week for six weeks has been reported to lead to increases in physiological adaptation in trained endurance runners (Billat et al., 2004). Training time at the MLSS increased from 30 to 60 min and the result was increases in VO_{2max}, LT, MLSS and time to exhaustion at the MLSS. The authors suggest that the most probable explanation for this improvement was an increase in lactate clearance. Improvements in the running speed associated with a blood lactate concentration of 4mmol·L^{-1} have been reported in a group of well-trained runners following 14wks of training, including one 20min training session with a blood lactate concentration of 4mmol.L^{-1} (Sjodin et al., 1982). This study also reported a negative relationship between the change in the blood lactate concentration during the 20min training session and the improvement in the speed associated when it was 4mmol.L^{-1}, suggesting the training stimulus was greatest at this sustained intensity. Improvements in both performance and physiological adaptation have been reported to be higher when 83% of training time was spent in zone two as opposed to 14% for five months in well-trained cross-country skiers (Evertsen et al., 1999). In fact, other studies have shown elite athletes to spend more time in zone two than zone three during a period of training (Tjelta & Enoksen 2010; Billat et al., 2003; Mujika et al., 1995). Performance was improved by 4-11% in the elite swimmers in the study by Mujika et al (1995). These studies provide evidence that

training in zone two can be beneficial in trained endurance athletes, but the reported time spent in zone 2 is still relatively low.

Two similar studies reported no difference in physiological adaptation after training continuously at the MLSS/anaerobic threshold (AnT) (zone two) compared with intervals at alternating intensities above and below the MLSS/AnT (Philp et al., 2008; Keith et al., 1992). Both studies used moderately trained males, training for 8wk. In the study by Keith et al (1992), the subjects progressed from cycling 2 to 4d·wk^{-1}, with the continuous group cycling for 30min at the AnT, while the interval group alternated between intensities 30% above and below the AnT for 7.5min each, for the same total duration. Improvements were found in both groups for VO_{2max}, AnT and TTE at AnT, with no differences between the groups. In the study by Philp et al (2008), the subjects trained on a treadmill for 2d·wk^{-1}, progressing from 21 to 33min·session^{-1}. The continuous group ran at the MLSS, while the interval group alternated between 0.5km.hr^{-1} above and below the MLSS for the same total duration. Improvements were found in both groups for VO_{2max}, LT and MLSS, with no differences between the groups.

It has been suggested that the optimum training intensity for eliciting maximum gains in endurance performance is from the highest intensity maintained in a steady state (Kindermann et al., 1979). At this exercise intensity, the skeletal muscle cells experience a high stimulation of oxidative metabolism and can be held for a prolonged period of time, as the mechanisms which lead to blood lactate production are only being used minimally (Kindermann et al., 1979). It has been proposed that the exercise intensity which elicits the maximal blood lactate removal from the circulation (MLSS) should induce specific adaptations of the lactate removal mechanism. However, as seen previously, Keith et al (1992) found no support for the blood lactate at the AnT representing the optimal training intensity.

2.2.9 Training in Zone 3

High intensity training (HIT) needs to be carefully incorporated into a training programme, as large volumes of HIT can lead to a down regulation of the central nervous system (Esteve-Lanao et al., 2007). This is consistent with a hormonal exhaustion syndrome (Lucia et al., 2001). In a pilot study, Esteve-Lanao et al (2007) assessed the effect of an increased amount of zone three training. When zone three training accounted for up to 15% using the TIZA (this would be a considerably larger percentage for the SGA), the training was too demanding for well-trained runners to be followed for more than two to three weeks, and resulted in most of the runners showing signs of overreaching/overtraining. In fact, during highly demanding endurance events such as the Tour de France, the amount of time spent in zone three during the race does not surpass 10% of the total race time, using the TIZA (Foster et al., 2005; Lucia et al., 2003; Lucia et al., 1999b). However, with the correct amount of training in zone three, large performance gains are possible in already well trained endurance athletes (Westgarth-Taylor et al., 1997; Weston et al., 1997; Lindsay et al., 1996). Indeed, it has been reported that for well-trained endurance athletes, some training needs to take place in zone three for an enhancement in physiological adaptation and performance (Londeree 1997). However, it seems that for HIT to be as effective as possible, the minimal training volume for the particular event first needs to be achieved (Iaia et al., 2009). High intensity sessions appear to be well tolerated when variation in intensity of training is ensured (Lehmann et al., 1992). Therefore, the effectiveness of a polarised training model could be in some way due to the very different training intensities (zone one and zone three), as opposed to a monotonic zone two training in the TTM, as this has been shown to protect against overtraining (Foster 1998). Research on TID has shown that the training programmes of all elite and well trained endurance athletes include high intensity training (zone three) (Table. 2.2). Training in zone three has been reported to be the most effective training for inducing increases in VO_{2max} (Rusko & Bosco 1987), which has been shown to be an important determinant of endurance performance

(Billat et al., 2003). HIT normally involves repeated bouts of exercise at an intensity corresponding to zone three, in a three-zone training intensity model. These bouts are usually ten seconds to five minutes in duration, with a short period of low intensity exercise or rest in between to allow a partial, but often not a full recovery (Laursen & Jenkins 2002b).

2.2.9.1 Well-Trained Endurance Athletes

Three studies from South Africa were the first to experimentally assess the effectiveness of HIT in well-trained athletes (Westgarth-Taylor et al., 1997; Weston et al., 1997; Lindsay et al., 1996); Table 2.8). No HIT was completed in the 3-4 months before the commencement of the studies, and HIT replaced ~15% of the ~300km training volume in this group of cyclists, and this training volume was not different during the intervention as before. Moderate intensity, prolonged training at 70-75% VO_{2peak} made up the rest of the training. These studies show the effectiveness of this interval training programme at improving performance in already well-trained cyclists. The significant improvements in the 40km TT were associated with the subjects being able to sustain a significantly higher absolute and relative power output (Westgarth-Taylor et al., 1997; Lindsay et al., 1996). The improvements in TTE at 150% PPO demonstrate an improvement in muscular resistance to fatigue and therefore, anaerobic performance (Lindsay et al., 1996). The acute responses and effects to an interval training session might give insight into the effectiveness of these interval training sessions. An interval training session involving 8*5min at 82.5% PPO (1min active recovery) was assessed in highly trained endurance athletes (Stepto et al., 2001); Table 2.9). These findings demonstrate that this particular interval training session involves high rates of glycogenolysis and total energy expenditure.

Table. 2.8 Performance and physiological changes following a period of high intensity interval training in trained endurance athletes

Study	Duration (wks)/ No. of HIT sessions	Interval session Discipline Reps: (no*duration@intensity) (Rest duration)	Performance improvements	Physiological changes
Lindsay et al., 1996	4 6	Cycling 6-8*5min@80%PPO (1min)	• 40km TT (8%) • PPO (4%) • TTE at 150% PPO (20%)	
Weston et al., 1997	4 6	Cycling 6-8*5min@80%PPO (1min)	• 40km TT (2%) • PPO (3%) • TTE at 150% PPO (22%)	
Westgarth-Taylor et al., 1997	6-7 12	Cycling 6-9*5min@80%PPO (1min)	• 40km TT (12%) • PPO (5%) • TTE at 150% PPO (22%)	
Creer et al., 2004	4 8	Cycling 4-10*30s@all-out (4min)	• PPO (6%)	• VO_{2max} (5%) • Motor unit activation
Smith et al., 2003	4 8	Running 6*60%Tmax@Pmax (1:2)	• 3km run time (3%) • Tmax (23%)	• VT (7%)
Driller et al., 2009	4 8	Rowing 8*2.5min@90%PPO (≤70%HR_{max})	• 2km TT power (6%)	• VO_{2peak} (7%)

HIT: High Intensity Training, **PPO:** Peak Power Output, **TT:** Time Trial, **TTE:** Time To Exhaustion, **Vmax:** The minimum intensity during an incremental test in which VO_{2max} is attained, **Pmax:** The minimum power to elicit VO_2, **Tmax:** The TTE at Vmax/P_{max}, **VT:** Ventilatory Threshold, **LT:** Lactate Threshold.

Table.2.9 Responses to an acute interval training session (Stepto et al., 2001)

Physiological measure	Value (rep no.)
Bla ($mmol \cdot L^{-1}$)	5-6 (1-8)
HR (bpm)	156(1), 162-167(2-8)
Arterial oxygen saturation (%)	95.6 (1), 94 (2-7)
CHO oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	340 (1-8)
Fat oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	16-25 (1-8)
RER	0.97-0.92 (1-8)
Muscle glycogen ($mmol \cdot kg^{-1}$) dry mass	501-243 (pre to post)

BLa: Blood Lactate Concentration, **HR:** Heart Rate, **CHO:** Carbohydrate, **RER:** Respiratory Exchange Ratio.

2.2.9.2 Optimising High Intensity Training

2.2.9.2.1 Programme

It is not possible to assess whether the interval training sessions presented in Table. 2.8 are optimal at inducing performance and physiological gains. Intervals of 4min duration at 85% peak power output (PPO) were found to be the most effective at inducing improvements in both 40km TT performance and PPO in trained cyclists (Stepto et al., 1999); Table. 2.10). Intervals of 30s duration at 175% PPO with 4.5 min recovery were also found to be effective at inducing an improvement in 40km TT performance, but not for inducing an improvement in PPO. This is intriguing, as it does not conform to the concept of specificity, which states that training has to be at or near the intensity of the event to have the greatest benefit. Little or no enhancement in 40km TT performance was found following the 1min and the 8min intervals. These different responses between the 4min and the 30s intervals of different intensities suggests that different mechanisms are responsible for the improved performance following HIT. P_{max} has been defined as the minimum power required to elicit VO_{2max} and T_{max} is the time to exhaustion at P_{max}. Intervals of 60%T_{max} duration at an intensity of P_{max} with a recovery consisting of the HR dropping to 65% HR_{max} (mean 2.6min) were shown to provide the most consistent improvements in endurance performance (Laursen et al., 2002b); Table. 2.10). All three interval training groups in the study by Laursen et al (2002b) improved 40km TT performance, PPO and VO_{2peak}, but group 2 improved more than group 3 in PPO and VO_{2peak}, whereas group 1 did not improve these measures to a greater extent than group 3 (Table. 2.10). Although these interval training sessions have been found to be effective, it should be stressed that the minimum volume of training should also be completed in a particular event for the high intensity training to be most effective (Iaia et al., 2009; Jones & Carter 2000).

Table.2.10 Studies to have assessed the optimisation of interval training in well-trained endurance cyclists

Study	Duration (wks)/ No. of HIT sessions	Interval session Discipline Reps: (no*duration@intensity) (Rest duration)
(Stepto et al., 1999)	3 6	1. 12*30s@175%PPO (4.5min) 2. 12*1min@100%PPO (4min) 3. 12*2min@90%PPO (3min) 4. 8*4min@85%PPO (1.5min) 5. 4*8min@80%PPO (1min)
(Laursen et al., 2002b)	4 8	1. 8*60%Tmax@P_{max} (1:2) 2. 8*60%Tmax@P_{max} (65%HR_{max}) 3. 12*30s@175%PPO (4.5min) 4. No intervals

PPO: Peak Power Output, Pmax: Minimum power to elicit VO_{2max}, **Tmax:** Time to exhaustion at Pmax.

2.2.9.2.2 A Single High Intensity Training Session

Changes can be made to the oxygen delivery to the muscle and the relative demands on particular metabolic pathways within muscle cells by altering the intensity and the duration of the work and the rest periods of a high intensity interval training session (Laursen & Jenkins 2002a). The particular training session that is chosen leads to specific adaptations at the cellular and systemic level (Laursen & Jenkins 2002a). Performance improvements have been demonstrated following a variety of different HIIT programmes in already well trained endurance athletes (Tables 2.8 and 2.10). Therefore, the separate areas of work and rest intensities and durations will briefly be discussed.

It has been shown previously that optimum improvements in cardiorespiratory fitness occur when training is at an intensity corresponding to 90-100% of VO_{2max} (Thomas et al., 1985; Thomas et al., 1984; Wenger & Macnab 1975). It was suggested that training intensities exceeding VO_{2max} would be less effective, due to the fatigue from the intensity reducing the volume of the training session (Magel et al., 1975), and not inducing any greater hypoxia in muscle than at 90 to 100% VO_{2max} (MacDougall & Sale 1981). Indeed, training at or near the

VO_{2max} should provide the optimal stimulus for adaptation in this measure, as this is the intensity whereby the physiological processes and structures are under maximal stress (Midgley & Mc Naughton 2006). Interval training has been reported to be more effective than continuous training at eliciting VO_{2max}, and VO_{2max} has been shown to be maintained for longer during interval training (Billat et al., 2000). To achieve peak cardiovascular responses, the optimal duration of each interval appears to be 4min (Seiler & Sjursen 2004). In the study by Seiler and Sjursen (Seiler & Sjursen 2004), a group of well-trained runners replaced one HIT session per week for four weeks with a training session in the laboratory. These sessions were self-paced, and performed with a 1:1 work: recovery ratio; 24*1min, 12*2min, 6*4min and 4*6min. The highest average VO_2 and HR was achieved by 6 of the 12 runners during the 4min intervals. In an individual interval training session involving 4 min intervals, it has been suggested that 30min of work in the near VO_{2max} range is the upper limit (Seiler & Hetlelid 2005).

The research therefore suggests that 4min intervals at an intensity of 95-100% VO_{2max} are most likely to lead to optimal performance benefits. During a 6*4min interval training session in well trained runners, a 2min recovery period has been reported to be optimal (Seiler & Hetlelid 2005). In this study, the intensity of the 4min work periods were self-selected and the subjects used either 1, 2 or 4min recovery periods during separate training sessions, one week apart. The instruction given for the work periods was to maintain the highest average running speed. From 1 to 2min recovery, there was an increase in running speed and VO_2, but there was no additional effect from increasing the recovery period to 4min. In addition, there was no difference between the different recovery periods for blood lactate concentration or HR. The average work intensity was 95-100% VO_{2max} and 6-7mmol·L^{-1} blood lactate concentration. To further emphasise the effectiveness of a 2min recovery period, the same authors (Seiler & Hetlelid 2005), asked the same runners to complete 6*4min intervals at the highest average

speed from the original study. The runners were instructed to self-select a recovery period that would allow them to complete the interval session and were provided no feedback (time, HR). Despite RPE and blood lactate concentration increasing throughout the intervals, the recovery period remained essentially unchanged between each interval, with the mean recovery time being 118s. The authors suggest that this recovery may be effective, as it allows for the re-establishment of intracellular concentrations of [Pi] and [H_2PO_4], which are important in contractile fatigue. In support of the 2min rest periods, two groups of well-trained athletes completing the same interval training session with the exception of the rest duration found that a recovery period of 2.6min was more effective at inducing increases in performance and physiological adaptation than a recovery period of 4.2min (Laursen et al., 2002a).

2.2.10 Training Intensity Distribution During Competition

Assessing the training load and the TID of world class endurance cyclists during a three week cycle stage race may give us an enhanced understanding of what an elite endurance athlete is capable of (Table. 2.11). The training load (TRIMP) is very similar between different 3wk cycle tour events, despite the different lengths of the tours (Lucia et al., 2003) and also from the same tour but in different years (Foster et al., 2005). Therefore, there might be an upper limit to the sustainable rate of relative energy expenditure in these events (Foster et al., 2005). It seems that athletes pace themselves over these multi-day events (Foster et al., 2005; Foster et al., 2004)), just as they would over a single event (Foster et al., 2004). Indeed, it has been suggested that anticipation of the total activity and metabolic changes required to complete a given exercise task uses a central 'programmer' (Ulmer 1996). The athletes' body avoids damage and premature fatigue by allowing the programmer to regulate the systems. This type of regulation from the central nervous system is known as "teleoanticipation" (St Clair et al., 2001).

Table. 2.11 The TID in 3wk cycle tour races in professional road cyclists. All studies used VT to demarcate three intensity zones assessed the TID using HR and the TIZA.

Study	Race	TID
Lucia et al., 1999b	TdeF	70-23-7
Lucia et al., 2003	TdeF	75-23-2
	VaE	72-25-3
Foster et al., 2005	TdF and VaE	Yr 1: 70-24-6
		Yr 2: 71-24-5

TdeF: Tour De France, **VaE**: Vuelta a Espana.

The TID in different 3wk cycle tours and between different years is remarkably similar (Table. 2.11). This is presumably what an elite endurance cyclist is capable of over a prolonged period of time. It is interesting that the TID in these 3wk races is similar to that during a ~3month period of training in elite road cyclists (Zapico et al., 2007). There was no improvement in physiological adaptation after this training period in the study by Zapico et al (2007), but there was in another group of elite road cyclists spending more time in zone one and less time in zone two (Lucia et al., 2000a; Lucia et al., 2000b). During the 3wk cycle tour races, there is a progressive decrease in HR_{max} (Lucia et al., 2003). This is a sign of decreased sympathetic nervous activity and overreaching (Hedelin et al., 2000). As elite road cyclists are accustomed to the training volume required during a 3wk cycle tour race (Lucia et al., 2000a;Lucia et al., 2000b), the overreaching must be caused by the increased training load. The cyclists in the study by Zapico et al (2007) had a lower HR_{max} following the training, and therefore were likely to be overreaching. It is therefore likely that the TID in a 3wk cycle tour race may not be optimal to maximise the physiological adaptation and performance gains during training.

2.2.11 Summary of the Training-Intensity Distribution Section

Descriptive studies assessing the training-intensity distribution in elite endurance athletes in a number of different sports have reported that these athletes train with a high training volume. These athletes spend more than 80% of their training time at exercise intensities lower than the LT, and therefore, less than 20% of training time below the LT. This training-intensity

distribution is therefore effective, as it is adopted by elite endurance athletes, but not necessarily optimal. In addition, this training-intensity distribution may be effective in only the most successful endurance athletes. Experimental studies support the effectiveness of training in zone one, and this is likely due to physiological adaptations that can be achieved with high volume endurance training, and an enhanced recovery compared with training in zones two or three. There are however, some studies that have reported greater improvements in performance following an increase in the proportion of higher intensity training. The research assessing an optimal training-intensity distribution is therefore inconclusive.

2.3 TRAINING-INTENSITY SPECIFIC ADAPTATIONS

2.3.1 Studies Assessing the Impact of Exercise Intensity

When the total workload is matched, several responses to exercise of different intensities do not show a consistent trend (Tables 2.12-2.15). However, the literature reveals that there is a more consistent increase in performance and physiological adaptation following higher-intensity exercise (Tables 2.12 and 2.15). The lack of difference in response from exercising at different intensities could be due to the differences in the exercise intensity not being sufficiently high enough in these particular studies. If the metabolic stresses on the different exercise intensities are too similar, this may not be sensitive enough to detect a different response. For example, if two different exercise intensities are between the LT and the MLSS (zone 2), the metabolic stress will be similar, and therefore responses are likely to be similar. However, by training in the different training zones in a three zone training-intensity model, demarcated with either blood lactate concentration or ventilatory equivalents, differences are more likely.

Table. 2.12 Studies to have assessed responses to exercise of different intensity with matched total work and frequency and found intensity-dependent responses in favour of higher exercise intensities.

Study/ Training Status/ (Study no.)	Frequency/ Duration	Discipline/ Groups	Results
(Helgerud et al., 2007) Moderately trained (1)	3d.wk^{-1} 8wk	Running/treadmill 1. 47*15s at 90-95% HR_{max} 2. 4*4min at 90-95% HR_{max} 3. 45min at 70% HR_{max} 4. 24.25min at 85% HR_{max}	VO_{2max}: 1,2 > 3,4 SV: 1,2 > 3,4 LT: nsd Economy: nsd
Daussin et al., 2007) Untrained (2)	3d.wk^{-1} 8wk	Cycling Randomised cross-over study design 1. 1min at 90% P_{max}, 4min at VT 2. 61% P_{max} continuously	VO_{2max}: 1>2 CO_{max}: 1>2 a-$VO2_{diff}$: nsd PPO: 1>2 Peak BLa: 1>2
(Driller et al., 2009) Well-trained (3)	2d.wk^{-1} 4wk	Rowing Randomised cross-over study design 1. 8*2.5min at 90% all-out 2. 35-40min at 2-3mmol.L^{-1} Bla	VO_{2peak}: nsd 4min rowing power: nsd Power at 4mmol.L^{-1} Bla: nsd 2km TT: 1>2
(Tabata et al., 1996) Untrained (4)	5d.wk^{-1} 6wk	Cycling 1. 7-8*20s at 170% VO_{2max} 2. 60min at 70%VO_{2max}	VO_{2max}: nsd Anaerobic capacity (MAOD): 1>2
(Edge et al., 2005) Untrained (5)	3d.wk^{-1} 5wk	Cycling 1. 6-10*2min at 120-140% LT (90-100% VO_{2max}) 2. 20-30min at 80-95% LT (60-70% VO_{2max})	VO_{2peak}: nsd LT: nsd PPO: nsd Peak Bla: nsd RSA: 1>2
(Cunningham et al., 1979) Untrained (6)	4d.wk^{-1} 12wk	Cycling 1. 2min at 90-100% VO_{2max} 2. 20min at 70-80% VO_{2max}	VO_{2max}: nsd a-VO_{2diff}: 1>2
(Poole & Gaesser 1985) Untrained (7)	3d.wk^{-1} 8wk	Cycling 1. 10*2min at 105% VO_{2max} 2. 35min at 70% VO_{2max} (4-8mmol.L^{-1} Bla) 3. 55min at 50% VO_{2max} (<2mmol.L^{-1} Bla)	VO_{2max}: nsd LT: nsd VT: 1>2,3 VE_{max}: 1>2,3
(Edge et al., 2006) Moderately trained (8)	3d.wk^{-1} 5wk	Cycling 3 6-10*2min at 90-100% VO_{2peak} (120-140% LT) 4 20-30min at 60-75% VO_{2peak} (80-95% LT)	VO_{2peak}: nsd LT: nsd Buffering capacity: 1>2

>: A greater improvement, **VO_{2max}**: Maximal Oxygen Consumption, **SV:** Stroke Volume, **CO:** Cardiac Output, **LT:** Lactate Threshold, **VT**: Ventilatory Threshold, **HR**: Heart Rate. **nsd**: No significant difference, **Bla:** Blood lactate concentration, **TT:** Time trial, **a-VO_{2diff}**: Arterio-venous oxygen difference, **MAOD**: Maximal Accumulated Oxygen Deficit, **PPO:** Peak Power Output, **RSA:** Repeated Sprint Ability, **VE:** Ventilation.

Table 2.13 Studies to have assessed responses to exercise of different intensity with matched total work and frequency and found mixed results for intensity-dependent responses

Study/ Training Status	Frequency/ Duration	Discipline/ Groups	Results
(Franch et al., 1998) Recreational runners (9)	3d.wk^{-1} 6wk	Running 1. 30-40*15s (20.4km.hr^{-1}) 2. 4-6*4min (16.6km.hr^{-1}) 3. 26min at 15km.hr^{-1}	VO_{2max}: 3,2>1 TTE at 85% VO_{2max}:3>2,1
(Gorostiaga et al., 1991) Untrained (10)	3d.wk^{-1} 8wk	Cycling 5 30s at 100% VO_{2max}, 30s rest 6 30min at 50% VO_{2max}	VO_{2max}: 1>2 PPO: 1>2 ADK activity: 1>2 Bla and RER at a relative work rate: 2>1 CS activity: 2>1
(Daussin et al., 2008) Untrained (11)	3d.wk^{-1} 8wk	Cycling Randomised cross-over study 7 1min at 90%$_{Pmax}$, 4min at VT 8 61% P_{max} continuously	VO_{2max}: nsd HR_{max}: 1>2 SV: 1>2 TTE: 1>2 VO_2 kinetics: 1>2 MOC: 1>2 Capillary density: 2>1

MOC: Skeletal muscle mitochondrial oxidative capacity, **TTE**: Time to exhaustion, **VT:** Ventilatory Threshold, **PPO:** Peak Power Output, **ADK:** Adenylate Kinase, **Bla:** Blood lactate concentration, **RER:** Respiratory exchange ratio, **CS:** Citrate Synthase, **HR:** Heart Rate, **SV:** Stroke Volume.

Table 2.14 Studies to have assessed responses to exercise of different intensity with matched total work and frequency and found no intensity-dependent responses.

Study/ Training Status	Frequency/ Duration	Discipline/ Groups	Results
(Eddy et al., 1977) Untrained (12)	4d.wk^{-1} 7wk	Cycling 1. 1min at 100% VO_{2max} 2. 70% VO_{2max}	VO_{2max}: nsd TTE at 80% VO_{2max}: nsd Submax HR, VO_2, Bla: nsd Peak Bla: nsd
(Overend et al., 1992) Untrained (13)	4d.wk^{-1} 10wk	Cycling 1. 30s at 120%VO_{2max} 2. 3min at 100% VO_{2max} 3. 80% VO_{2max}	VO_{2max}: nsd VT: nsd
(Keith et al., 1992) Moderately trained (14)	2-4d.wk^{-1} 8wk	Cycling 1. AnT 2. 30% above and below AnT	VO_{2max}: nsd AnT: nsd TTE at AnT: nsd CS, HAD, PFK activity: nsd
(Berger et al., 2006) Untrained (15)	3-4d.wk^{-1} 6wk	Cycling 1. 60% VO2max 2. 20*1min at 90% VO_{2max}	VO_{2peak}: nsd VT: nsd PPO: nsd
(Philp et al., 2008) Moderately trained (16)	2d.wk^{-1} 8wk	Running/treadmill 1. MLSS 2. 0.5km.hr^{-1} above and below the MLSS	VO_{2max}: nsd LT: nsd MLSS: nsd

AnT: Anaerobic Threshold, **MLSS:** Maximal Lactate Steady State, **TTE:** Time To Exhaustion**, HR:** Heart Rate**, Bla:** Blood lactate concentration, **VT:** Ventilatory Threshold**, PPO:** Peak Power Output, **CS:** Citrate Synthase, **HAD:** β-hydroxyacyl-coenzyme A dehydrogenase, **PFK:** Phosphofructokinase.

Table 2.15 Responses to different exercise intensities on key measures of endurance, from the studies reviewed in tables 2.12 to 2.14

Measure	Higher intensity>lower intensity	No difference between exercise intensities	Lower intensity>higher intensity
VO_{2max}/VO_{2peak}	1, 2, 10	3-9, 11-16	
LT/VT_1	7	1, 2, 7, 8, 13, 15, 16	
VT_2/AnT/MLSS	2	3, 14, 16	
Economy		1, 2	
Maximal performance	1, 3, 5, 10, 11	3, 5, 12, 14, 15	

LT: Lactate Threshold, **VT:** Ventilatory Threshold, **AnT:** Anaerobic Threshold, **MLSS:** Maximal Lactate Steady State.

2.3.2 Exercise Intensity Effects on Physiological Adaptation

2.3.2.1 VO_{2max}

When the total work performed during a period of training is matched, it has been reported that the VO_{2max} is increased more following exercise of a higher intensity (Helgerud et al.,2007; Daussin et al., 2007; Gorostiaga et al., 1991). These three studies compared high intensity interval training with moderate intensity continuous training (Table. 2.12). It would appear that the greater increase in VO_{2max} in these studies following the higher exercise intensity was due to cardiovascular adaptations. Indeed, Helgerud et al (2007) and Daussin et al (2007) both reported greater increases in stroke volume following the higher intensity training. In addition, Gorostiaga et al (1991) reported lower blood lactate concentrations at relative intensities, lower respiratory exchange ratio and an enhanced citrate synthase activity following the moderate intensity training, despite the VO_{2max} increasing more following high intensity training. This would suggest that cardiovascular adaptations (SV, CO) were the cause of the greater increase in VO_{2max}. Indeed, despite reporting no difference in the increase in VO_{2max} between high and moderate intensity exercise training, Daussin et al (2008) also found a greater increase in SV following high intensity training. However, Cunningham et al (1979) reported the a-vO_{2diff} to be improved more following high than moderate intensity training, but there were no differences in the increase in VO_{2max}. The increased SV following high intensity exercise training in these studies is likely to be due to an increased left ventricular size and wall thickness, along with increases in erythrocyte mass and plasma volume (Table. 2.1). Indeed, high intensity training (85-90% VO_{2max}) $5d \cdot wk^{-1}$ for 10wk led to a 71% increase in VO_{2max} compared to training at a lower intensity (60-70% VO_{2max}) in rats and mice (Kemi et al., 2005). Isolated heart muscle cells (cardiomyocytes) from the left ventricle were analysed to assess the effectiveness of these different training intensities on adaptations to the heart. High intensity interval training lead to a greater increase in contractile capacity of the cardiomyocytes than moderate intensity training. This was reported to be due to increasing

the extent and the rate with which it shortens during systole and relaxes during diastole and by improving its ability to generate force, independent of neurohormonal influences. In addition, high intensity exercise has been reported to induce a hypertrophic response in the cardiomyocytes (Wisloff et al., 2001). The intensity specific adaptations to an isolated cardiomyocyte are shown in Fig. 2.9.

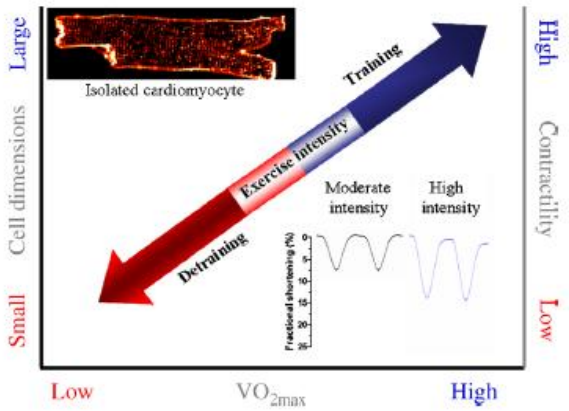

Fig. 2.9 Exercise intensity dependent adaptations of VO_{2max} and cardiomyocyte function/structure. Taken from Wisloff et al (2009).

2.3.2.2 Lactate Threshold

The majority of studies to have compared exercise training of different intensities, but with the total work matched, have found no difference in the improvement in LT following high intensity versus moderate intensity exercise (Tables 2.12 to 2.15). An increase in LT has been linked with an increased concentration of the lactate transporters, MCT1 and MCT4 (Wilson et al., 1998; McCullagh et al., 1996). It has been reported that 5 months of training in a group of elite cross country skiers, including 83% of training at a high intensity (3-4mmol·L^{-1} blood lactate concentration) and 17% of training at a lower intensity (<1.5mmol·L^{-1} blood lactate concentration) significantly improved the LT (Evertsen et al., 2001). However, following 86% low intensity training and 14% high intensity training, the LT did not improve and concentration of MCT1 actually fell over the 5 month training period. The authors suggest this

was due to the training not including as much high intensity as during the season, as the pre-training biopsies were taken soon after the racing season, and so the concentration of MCT1 was likely to be high due to the high intensity racing. Therefore, although neither group improved the concentration of either MCT1 or MCT4, the concentration of MCT1 was maintained more effectively with the higher intensity training. It has been reported that MCT4 is less active at lactate concentrations of less than 10mmol.L^{-1}(Juel & Halestrap 1999), suggesting that high intensity training needs to be at an intensity to elicit a lactate concentration of over 10mmol·L^{-1}. In addition, it has been reported that the expression and concentration of MCT1 in both rat and human skeletal muscle is increased following intense training, but not following less intense training (Bonen et al.,2000; Pilegaard et al., 1999a; Pilegaard et al., 1999b; Wilson et al., 1998; Baker et al., 1998; Bonen et al., 1998; McCullagh et al., 1996).

Buffering capacity has been reported to be increased more following high intensity than moderate intensity training in moderately trained individuals (Edge et al., 2006). Indeed, high intensity training has been reported to increase the buffering capacity in well-trained endurance athletes (Stepto et al., 2001; Weston et al., 1997). During high intensity interval training, as each subsequent exercise bout is performed at a progressively lower pH, the mechanism controlling pH is placed under large stress (Costill et al., 1984). The lower pH is due to an accumulation of H+, due to the intensity being above the LT (Chwalbinska-Moneta et al., 1989). This leads to a greater buffering capacity to help maintain the pH, and therefore an enhanced LT (Costill et al., 1984).

The lactate exchange ability could be improved from an increased capillary density, due to an increased exchange area and a decreased distance between the site of lactate production and the capillary wall (Messonnier et al., 2002). Exercise intensity seems to provide a larger

stimulus for capillary growth than exercise duration (Iaia et al., 2009). Changes in angiogenesis have been suggested to be related to the increase in blood flow and thereby shear stress (Hellsten et al., 2008; Milkiewicz et al., 2001), and to the mechanical stretch associated with muscle contractions (Hellsten et al., 2008; Rivilis et al., 2002). Indeed, high intensity training at 150% VO_{2max} has been reported to increase muscle capillarization (Jensen et al., 2004).

2.3.2.3 Exercise Economy

An increase in exercise economy is associated with a change in the expression of fast twitch skeletal muscle fibres towards a more slow twitch phenotype (Dubouchaud et al., 2000). There is evidence to suggest that a change in the ratio of type IIa to type IIb fibres occurs with endurance training (Saltin 1977). However, the effect of exercise intensity on the conversion of muscle fibre types is unknown. However, it is possible that training at too high an intensity can be detrimental to exercise economy in runners (Franch et al., 1998). Interval training took place at 94%, 106% and 132% VO_{2max}, and while the running economy was significantly improved in the 94% and 106% groups, it was not improved in the 132% group.

2.3.2.4 Mitochondrial Biogenesis

Endurance training of different intensities can lead to different molecular signals that induce mitochondrial biogenesis (Laursen 2010). Prolonged endurance exercise and high volume endurance training have been reported to increase intramuscular calcium, which can activate calcium calmodulin kinases and lead to mitochondrial biogenesis (Rose et al., 2007). In contrast, it has been reported that a single bout of HIT (>100% VO_{2max}) generates a 20-50% transient decrease in Ca^{2+} uptake and release (Matsunaga et al., 2002). As previously mentioned, the increased AMP:ATP ratio due to endurance exercise has been shown to activate AMPK (Hardie & Sakamoto 2006), which has been reported to be involved in the initiation of mitochondrial biogenesis (Ojuka 2004; Puigserver & Spiegelman 2003). The AMP:ATP ratio is increased most rapidly during HIT (Coffey & Hawley 2007). Indeed, 4*30s

'all-out' sprint efforts on a cycle ergometer have been reported to increase AMPK immediately (Gibala et al., 2009). Three hours later, there was a 2-fold increase in PGC-1α mRNA and no increase in calcium calmodulin kinases. An increase in AMPK has also been reported following HIT (~90%VO_{2max}) in a group of highly trained cyclists (Clark et al., 2004). A similar increase in PGC-1α has been reported in rats following either $6hr \cdot d^{-1}$ of swimming carrying 2% body weight and 14*20s with a weight equal to 14% body weight (Terada et al., 2005). However, the activation of AMPK was almost twice as high in the HIT group. These findings suggest that different molecular pathways are activated based on exercise-intensity. It is therefore possible that by maximising these signalling pathways with the most effective training programme could maximise mitochondrial biogenesis and therefore endurance performance.

An increase in enzyme activity may be exercise intensity dependent, as enzymatic processes re-synthesise ATP during exercise. The higher the exercise intensity, the greater the ATP turnover rate, and therefore, the activity of the enzymatic machinery may be increased in skeletal muscle to maintain homeostasis (Evertsen et al., 1999). The intensity of exercise needed for an increase in cytochrome c concentration in different fibre types is shown in Table. 2.16. The authors (Dudley et al., 1982) attribute these finding to muscle recruitment at increasing exercise intensities. Type I muscles are recruited up to ~95% VO_{2max}, beyond which less type I fibres are recruited. Type IIa muscle fibres are recruited up to ~85% VO_{2max}, beyond which there is no further recruitment in these muscle fibres. Finally, type IIb muscle fibres are only recruited at exercise intensities over 73% VO_{2max}. In addition, the study found that for the same response of cytochrome c, the duration of exercise necessary to bring about the change becomes less as the intensity is increased. It has also been suggested that high intensity training could improve the aerobic potential of type IIa muscle fibres by making them more fatigue resistant (Billat et al., 2003). Indeed, the recruitment of more fast-twitch fibres at increasing exercise intensities has also been reported in humans (Gollnick 1985). Importantly,

it has been reported that increases in mitochondrial density and oxidative enzyme activities are greatest in those muscles that are directly activated in training, with little or no adaptation in the untrained limbs (Gollnick & Ianuzzo 1972). This is consistent with the idea that the stimulus for mitochondrial biogenesis originates within the contractile muscle, independent of humoral influences (Hood 2001). This idea is supported by findings that have shown high intensity training causes increases in oxidative capacities in those specifically recruited skeletal muscle fibres in well trained rats (Laursen et al., 2007). This was demonstrated with an increase in the activity of citrate synthase in the white vastus muscle in the group that trained at a high intensity, but not those that trained at a moderate intensity. Indeed, high intensity training has been reported to increase the volume of muscle mass recruited in well-trained cyclists (Creer et al., 2004; Lucia et al., 2000a).

Table. 2.16 Data from Dudley et al (1982). The response of cytochrome c concentration in different muscle fibre types to exercise of different intensities. Rats trained $5d \cdot wk^{-1}$ for 8wk at different intensities.

Muscle type	Response of cytomchrome c oxidase activity to different exercise intensities
Type I	↑up to 95% VO_{2max}, ↓>95% VO_{2max}
Type IIa	↑up to 85% VO_{2max}, nsd >85% VO_{2max}
Type IIb	↑ >73% VO_{2max}

VO_{2max}: Maximal oxygen consumption, **nsd**: No significant difference.

Including high intensity training into the training programme of already well-trained endurance athletes leads to a positive improvement in performance, as previously shown (Table. 2.8). However, it seems unlikely that this increase in performance is due to the increase in the activity of oxidative enzymes (Table. 2.17). This could be because the aerobic demands of the high intensity training were already sufficiently met by the pre training oxidative capacity. Two studies (Hulston et al., 2010; Yeo et al., 2008) have shown an increase in mitochondrial oxidative enzyme activity or total protein content in well-trained cyclists following high intensity training, but only after training with reduced muscle glycogen, induced by prior exercise. The performance improvement however, was not different between the

high and low glycogen training groups, and therefore there seems to be no advantage to the adaptations to the enzymes reported in these studies.

In contrast, high intensity training has a large effect on oxidative enzyme activity in moderately trained individuals (Table. 2.18). These three studies all used the same HIT training session, which included 10 intervals of 4 minutes duration at ~90% VO_{2peak} with a 2 minute rest between each interval. It has also been reported that fewer intervals of shorter duration, but higher intensity also induce a large improvement in both endurance performance and oxidative enzyme activity (Table. 2.19). However, the increase in mitochondrial oxidative enzyme activities does not seem to be dependent upon exercise intensity. With the total work matched between groups, the maximal activities of citrate synthase and β-Hydroxyacyl-Coenzyme A Dehydrogenase were increased, but not different in two groups training at different intensities (Keith et al., 1992); Table. 2.14). In contrast, lower intensity exercise has been shown to cause a greater increase in citrate synthase activity than high intensity exercise, with the total work matched between the groups (Gorostiaga et al., 1991); (Table. 2.13).

The impact of training with very different exercise intensities and with very different total work performed during the training period has been assessed (Burgomaster et al., 2008; Gibala et al., 2006); Table. 2.20). These studies compared a group training with very high intensity and low total work (high-intensity, low-volume) with a group training with low intensity and high total work (low-intensity, high-volume). Following the training, both high-intensity, low-volume and low-intensity, high-volume groups gained large improvements in endurance performance and markers of enhanced exercise metabolism (Table. 2.20). The authors demonstrated that an increase in exercise intensity can compensate for a lower training volume in previously untrained individuals.

Table. 2.17 The influence of HIT on enzyme activities in well-trained endurance athletes

Study	Duration/ No. of HIT sessions	HIT	Result
Weston et al., 1997	4wks 6	6-8*5min@80%PPO	Endurance performance: ↑ CS: nsd HAD: nsd
Evertsen et al., 1999	5mo	Training at 80-90% VO_{2max}: 83% Training at 60-70% VO_{2max}: 17%	Endurance performance: ↑ CS: nsd SDH: ↑
Sjodin et al., 1982	14wks	0, 0.4 and 6.1hrs.wk^{-1} below, at and above 4mmol.L^{-1} Bla	VO_{2max}: ↑ CS: ↑
Kohn et al., 2010	6wks 12	6*60Tmax@94% peak treadmill speed	Endurance performance:↑ CS: nsd HAD: nsd

VO_{2max}: Maximal oxygen consumption, **PPO:** Peak Power Output**, CS:** Citrate Synthase**, HAD:** β-Hydroxyacyl-Coenzyme A Dehydrogenase, **SDH:** Succinate Dehydrogenase**, Bla:** Blood lactate concentration**, Tmax:** Time to exhaustion at VO_{2max}.

Table. 2.18 The influence of aerobic HIT on enzyme activities in moderately trained individuals

Study	Duration/ No. of HIT sessions	Result
Talanian et al., 2007	13d 7	VO_{2peak}: ↑ CS: ↑ HAD: ↑
Perry et al., 2008	6wks 18	VO_{2peak}: ↑ TTE at 90% VO_{2peak}: ↑ CS: ↑ HAD: ↑
Gurd et al., 2010	6wks 18	VO_{2peak}: ↑ CS: ↑ HAD: ↑ COX IV: ↑

VO_{2max}: Maximal Oxygen Consumption, **TTE**: Time to Exhaustion, **CS:** Citrate Synthase**, HAD:** β-Hydroxyacyl-Coenzyme A Dehydrogenase, **COX IV:** Cytochrome C Oxidase Subunit IV.

Table. 2.19 The influence of short, very high intensity training on enzyme activities in untrained or moderately trained individuals

Study	Duration (wks)/ No. of HIT sessions	HIT	Result
Hood et al., 2011	2 6	10*1min @ 60% PPO	CS (protein content): ↑ COX IV (protein content): ↑
Little et al., 2010	2 6	8-12*60s@~100% PPO	Endurance performance: ↑ CS: ↑ COX: ↑
Burgomaster et al., 2008	6 18	4-6*30s Wingates	HAD:↑ PDH: ↑
Burgomaster et al., 2005	2 6	4-7*30s Wingates	Endurance performance: ↑ CS:↑

PPO: Peak Power Output, **CS:** Citrate Synthase**, HAD:** β-Hydroxyacyl-Coenzyme A Dehydrogenase, **COX IV:** Cytochrome C Oxidase Subunit IV, **COX:** Cytochrome C Oxidase, **PDH:** Pyruvate Dehydrogenase.

Table. 2.20 Studies to have assessed the impact of training at different intensities in untrained individuals, but with very different total work.

Study	Duration/ Frequency	High/Low Intensity Training	Total Work ($kJ.wk^{-1}$)	Results
Gibala et al., 2006	2wks $3.wk^{-1}$	HIT: 4-6*30s 'all-out' with 4min rec. LIT: 90-120min@65%VO_{2peak}	HIT: 475 LIT: 3250	Endurance performance: ↑nsd COX Act: ↑nsd COX II PC: ↑nsd COX IV PC: ↑nsd BC: ↑nsd GC: ↑nsd
Burgomaster et al., 2008	6wks $3/5.wk^{-1}$	HIT: 4-6*30s 'all-out' with 4.5min rec. ($3d.wk^{-1}$) LIT: 40-60min@65%VO_{2peak} ($5d.wk^{-1}$)	HIT: 225 LIT: 2250	PDH PC: ↑nsd CS Act: ↑nsd HAD Act: ↑nsd PGC-1α PC: ↑nsd

HIT: High Intensity Training, **LIT:** Low Intensity Training, **rec:** Recovery, **nsd:** No Significant Difference between HIT and LIT, **↑**: Increased from pre-training, **VO_{2peak}:** Maximal Oxygen Consumption, **COX:** Cytochrome C Oxidase, **Act:** Activity, **PC:** Protein Content, **BC:** Buffering Capacity, **GC:** Glycogen Content, **PDH:** Pyruvate Dehydrogenase, **CS:** Citrate Synthase, **HAD:** β-Hydroxyacyl-Coenzyme A Dehydrogenase, **PGC-1α**: Peroxisome proliferator receptor-γ co-activator-1α.

2.3.3 Summary of the Training-Intensity Specific Adaptations Section

When the total workload of training is matched between two interventions with different exercise intensities, it is often, but not always the intervention with the higher exercise intensity that leads to greater increases in physiological adaptation and endurance performance. VO_{2max} has been reported to increase more following higher intensity training,

and this seems to be due to cardiovascular adaptations, including an increased SV and an increased contractile capacity of cardiomyocytes. Research suggests that improvements in LT are not exercise intensity-specific. However, it has been suggested that the activity of MCT1, buffering capacity and capillarisation are increased more following higher intensity training, which would lead to a greater increase in LT. It is possible that different molecular pathways are activated when training at different exercise intensities, but the effect of training-intensity on these pathways is unclear at present. Finally, as muscle fibre recruitment is different at different exercise intensities, it is possible that specific adaptations only take place in those muscle fibres that are recruited during training.

2.4 OVERTRAINING

2.4.1. The Overtraining Process

At certain times in the training season, endurance athletes will intensify training with the aim to increase performance. Following a single intense training session, there may be a decrease in performance and the athlete may experience acute feelings of fatigue. Following a series of intense acute training sessions, the athlete may experience similar or greater symptoms of fatigue and declined performance. This is due to an absence of appropriate recovery (Lehmann et al., 1993) and the athlete is identified as overreached (Fig 2.1). Recovery from overreaching is possible within two weeks, and therefore it has been previously suggested to be a relatively normal and harmless stage of the training process (Jeukendrup et al., 1992; Halson et al., 2002). In fact, following a period of overreaching, if the athlete carries out an adequate period of recovery, then a 'supercompensation' effect could be achieved, resulting in a higher level of performance following the recovery period than before the overreaching period (Budgett 1998; Fig.2.1). If intensified training continues following overreaching, this could result in overtraining (Urhausen & Kindermann 2002; Fig.2.1). The fatigue, performance decline and mood disturbances have been suggested to be more severe in overtrained than in overreached athletes, but scientific evidence is lacking to either prove or disprove this suggestion. Whereas overreached athletes may be fully recovered within two weeks, full recovery from overtraining may take months or possibly years (Budgett 1998).

2.4.2 Causes of Overtraining

Overtraining is caused by an excessive training load, together with inadequate recovery (Hedelin et al., 2000). In well-trained rowers, although the success in Championships is positively related to the training volume (Steinacker et al., 1993), the risk of overtraining also increases with an increase in training volume, and in particular with monotonic training (Fry et al., 1992; Bruin et al., 1994). Indeed, it is well established that the risk of overtraining can be

reduced by alternating hard and easy sessions (Steinacker et al., 1998). It would therefore seem beneficial to endurance athletes to design a training programme involving alternating high and low intensities.

A decrease in muscle glycogen stores has been suggested to be at least partly attributable to the fatigue and underperformance associated with overtraining (Halson & Jeukendrup 2004). Indeed, following 10d of overload training in 12 well-trained swimmers, 8 responded with positive performance gains, while in 4 swimmers, there was no change in performance (Costill et al., 1988). In the non-responders, there was a significant decrease in muscle glycogen, caused by significantly less carbohydrate consumed than the responders. However, another study has reported that decreased muscle glycogen content is not the cause of overtraining (Snyder et al., 1995). A group of well-trained cyclists were classified as overreached following overload training for 15d, despite maintained muscle glycogen levels. It therefore appears that a mechanism other than a decrease in muscle glycogen is responsible for overtraining.

2.4.3 Types of Overtraining

Two types of overtraining have been distinguished; a sympathetic type and a parasympathetic type (Israel 1976). Heart rate variability (HRV) can be used to detect these two types of overtraining, as it has been shown to detect changes in the autonomic nervous system (ANS) (Pichot et al., 2002). There are regular fluctuations in resting HR, which are primarily due to the changing level of both parasympathetic and sympathetic neural control of the heart (Yamamoto et al., 1991). HRV is measured by examining the intervals between successive R waves from the QRS complex (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). There is a high frequency and a low frequency HRV, which reflect autonomic balance status (Portier et al., 2001). The high frequency (0.15-0.45Hz) reflects parasympathetic (vagal) activity, and is mediated through

respiration, whilst the low frequency (0.04-0.15Hz) seems to reflect both parasympathetic and sympathetic activity (Akselrod et al., 1985).

2.4.3.1 Sympathetic Type Overtraining

Overreaching has been associated with a decreased HRV, and therefore parasympathetic inhibition and sympathetic activation (Kinnunen et al., 2006). The sympathetic type is understood to be characterised by an increased resting heart rate and blood pressure (Portier et al., 2001), and perhaps also involves increased sympathetic neuroendocrine activity in response to repeated stress from endurance training (Fry et al., 1991). It has been suggested to be caused by an overstimulation of the sympathetic nervous system, from high intensity training and/or inadequate rest (Winsley et al., 2005). This type of overtraining has been found in a group of elite rowers, 20d before the World Championships (Iellamo et al., 2002). Three of the seven athletes won a medal at the World Championships, which is indicative that the athletes achieved supercompensation following an overreaching training period. This evidence suggests that the sympathetic type overtraining is a positive aspect of endurance training, provided it is followed by an adequate period of reduced training and recovery.

2.4.3.2 Parasympathetic Type Overtraining

If the sympathetic type of overtraining is allowed to continue, a combination of inhibition, desensitisation, and exhaustion of the neuroendocrine system leads to a parasympathetic dominance and the parasympathetic type of overtraining (Fry et al., 1991; Lehmann et al., 1998). In the parasympathetic type, the HRV increases, the sympathetic form is inhibited and the result is a marked dominance of the parasympathetic system (Baumert et al., 2006). In contrast to the sympathetic type overtraining, a decrease in both blood pressure and heart rate has been suggested to be associated with this type of overtraining (Portier et al., 2001). The parasympathetic type of overtraining is regarded as an ultimate negative feedback

response to sustained levels of arousal (Lehmann et al., 1998). The basal urinary catecholamine excretion is normally reduced by 50-70% in an athlete suffering from the parasympathetic type of overtraining (Lehmann et al., 1998). There is a negative feedback mechanism to an increased concentration of circulating free catecholamines during prolonged heavy training sessions that cause the decrease in sympathetic intrinsic activity (Lehmann et al., 1998). This decrease in sympathetic activity could also be the cause of the decreased HR_{max} during a maximal physical effort and a decreased HR if it is not accompanied by an increase in performance capacity (Uusitalo et al., 2000).

Care needs to be taken however, as endurance training leading to enhanced endurance performance has been shown to increase parasympathetic activity and decrease sympathetic activity in the heart at rest, which can be detected by an increased HRV (Seals & Chase 1989; Uusitalo et al., 1996). This is supported by the well-established finding that endurance athletes have a lower resting HR compared with sedentary individuals (Ekblom et al., 1973) and a more rapid HR recovery following exercise due to the enhanced parasympathetic activity (Shin et al., 1995; Brenner et al., 1997). In fact, maximal ANS activity has been reported to be positively related to both VO_{2max} (Garet et al., 2004) and performance (Pichot et al., 2002; Pichot et al., 2000). In addition, large increases in aerobic power following endurance training have also shown to be related to increases in parasympathetic control of HR (Shi et al., 1995; Smith et al., 1989). In concurrence, studies to have shown only a small increase in aerobic power after training have reported that parasympathetic activity is unchanged (Ekblom et al., 1973).

2.4.4 Measures of Overtraining

2.4.4.1 Performance Tests

The gold standard of diagnosis of overtraining is the assessment of a decrement of performance (Urhausen & Kindermann 2002). An athlete cannot be diagnosed as overreached if the performance remains unchanged following an increased training load (Halson & Jeukendrup 2004). Care needs to be taken in interpreting performance results, as small performance changes are within the normal variability (Bruin et al., 1994). It has been shown previously that the normal variability in maximal work load on a cycle ergometer is 4% (Kuipers et al., 1985), and in overtrained athletes maximal work load is declined by 5 to 10% (Jeukendrup et al., 1992). Depending on the specific performance assessment, the magnitude of the decline in performance may vary widely. It has been suggested that a sports specific testing procedure that is continued until exhaustion is the most sensitive test to detect overreaching/overtraining (Urhausen & Kindermann 2002). These authors have suggested that the test needs to be a short term high intensive endurance exercise. A test which has been used successfully in the diagnosis of overtraining in controlled prospective studies is the 'stress test'. This is performed on a cycle ergometer, at an intensity 10% above the individual anaerobic threshold. Exhaustion occurs after ~15 to 40min, with reductions between 14 to 27% due to overreaching/overtraining (Urhausen et al., 1998). Time trials can also be used to indicate a state of overreaching/overtraining (Hooper et al., 1993). Overreached swimmers have been reported to increase 100m and 400m performance times by 2.4%, whereas well-trained swimmers decreased performance times by 1.1% (Hooper et al., 1993). Timing of the performance testing of potentially overreached/overtrained athletes is a major consideration. Following intensive training, there could be a degree of muscle glycogen depletion in the athlete, which would reduce both maximal performance and lactate concentration (Jeukendrup et al., 1992). Therefore, performance testing should be performed following a minimum of 2 days of reduced training or rest (Urhausen & Kindermann 2002). For the

diagnosis of overtraining, the decrease in sports-specific performance is still decreased following 2 weeks of regeneration (Budgett 1998).

2.4.4.2 Physiological Measures

The VO_{2max} or the maximal performance of overtrained athletes in an incremental graded test procedure tends to be impaired, but other studies have shown these values to be unchanged (Urhausen & Kindermann 2002). Jeukendrup et al (1992) found that 14 days of intensified training in a group of competitive cyclists led to an 8% decrease in VO_{2peak} and a 3-4% decrease in maximal power in an incremental test to exhaustion. Studies have revealed that HR_{max} is slightly, but significantly reduced in overtrained athletes (Lehmann et al., 1992; Snyder et al., 1995; Hedelin et al., 2000). An increased HR at rest has been suggested as a sign of overtraining (Stone et al., 1991). However, some studies have found this to not be the case (Fry et al., 1992; Lehmann et al., 1992; Snyder et al., 1995). It has been proposed that an increased HR at rest or during submaximal exercise might be a sign of an infectious disease or glycogen depletion (Urhausen & Kindermann 2002). Several studies have found lowered submaximal and maximal blood lactate concentration following overreaching/overtraining (Fry et al., 1992; Snyder et al., 1995; Urhausen et al., 1998). Indeed, Jeukendrup & Hesselink (1994) have shown that overtraining can lead to a right shift in the blood lactate concentration profile. This might be due to a decrease in muscle and liver glycogen stores, which causes a decrease in blood lactate concentration (Halson & Jeukendrup 2004). This is supported by studies showing a decrease in carbohydrate metabolism following overtraining, leading to an decreased respiratory exchange ratio value during both submaximal and maximal exercise (Urhausen et al., 1998; Snyder et al., 1995). Jeukendrup et al (1992) have suggested that an increased rating of perceived exertion value in relation to the exercise intensity might be used as a criterion for overtraining. Several studies have shown that overreached athletes show clear signs of psychological distress (Fry et al., 1992; Urhausen et al., 1998; Halson et al.,

2002). It has been suggested that an impaired mood state and subjective complaints are sensitive and early markers of overtraining (Snyder et al., 1993; Urhausen et al., 1998; Foster 1998). A reduced mood state occurs well in advance of the decline in performance and shows a relationship with the increased training load (Morgan et al., 1988; Urhausen et al., 1998). However, care needs to be taken, as other studies have reported increased training to lead to increased 'Profile of Mood States' scores, but to not lead to overtraining (Morgan et al., 1988).

2.4.5. Summary of the Overtraining Section

It has been suggested that an effective training-intensity distribution might be able to protect an endurance athlete from overtraining, although this is inconclusive. Research has suggested that overtraining might result from an excessive training volume or load, and/or excessive high intensity training. It has also been suggested that training programmes without adequate variation in training intensity might increase the chances of becoming overtrained, and training programmes with varied training intensities might offer a protective effect from overtraining. It is important to consider different methods to assess overtraining in a research study, but a test lasting approximately 15-40min to exhaustion, a time trial, and a mood scale seem to be the most reliable.

2.5 SENESCENT T CELLS AND EXERCISE

2.5.1 Introduction to the Human Immune System

The world contains many pathogenic and non-pathogenic microbes and a large range of toxic and allergenic substances. In order to protect the host from infection, the human immune system consists of innate and adaptive systems (Chaplin 2010). The innate system is the first line of defence against infectious pathogens and restricts the entry of microorganisms into the body using physical/structural barriers, chemical barriers and cells that kill microorganisms and/or eliminate host cells that become infected (Gleeson 2006). The adaptive system is activated by infection following failure of the innate system, and responds with a proliferation of cells that either attack the infectious agent directly, or produce antibodies, which help to restrict the pathogen from attacking the host (Gleeson 2006). The adaptive immune system is designed to prevent colonisation of pathogens and destroy invading micro-organisms (Walsh et al., 2011). The main distinction between innate and adaptive immune responses is that the adaptive responses strengthen upon repeated exposure (i.e. they possess a memory function) (Walsh et al., 2011). Adaptive responses are based mainly on the antigen-specific receptors expressed on the surfaces of B and T lymphocytes (Chaplin 2010).

2.5.2 T-cells

During development, T cells acquire a T cell receptor that fits the major histocompatibility complex (MHC) unique to the individual before entering the periphery as a fully functional naive T cell (Simpson 2011). T cell subsets can be identified using monoclonal antibodies, which recognise cell surface markers, known as cluster of differentiation (CD). All T lymphocytes express CD3, T helper cells express CD4 and most cytotoxic cells express CD8. In the blood and secondary lymphoid organs, 60% to 70% of T-cells are CD4+CD8- (CD4+) and 30% to 40% are CD4-CD8+ (CD8+) (Bruunsgaard et al., 1999). When the MHC displays the antigen on the cell surface of macrophages, T helper cells (CD4+) bind to the antigen and

stimulate the macrophage to release the cytokine, IL-1, which stimulates the T cells to grow and divide. T helper cells (CD4+) and cytotoxic cells (CD8+) are stimulated to undergo further proliferation and growth from the cytokine, IL-2, which is released from the activated T cells (Gleeson 2006). Following an immune response, most of the excess clones of effector T cells die by apoptosis, but some recirculate the tissues to protect the host against further attacks from the same infectious agent (Simpson 2011).

2.5.3. B-Cells

B cells differentiate from hematopoietic stem cells in the bone marrow and constitute approximately 15% of peripheral blood leukocytes. B cells express selectively CD19 and CD20. When a B cell leaves the bone marrow, it expresses a unique antigen-binding receptor on its membrane, called an antibody. When a naïve B cell first encounters the antigen that matches its antibody, the binding of the antigen to the antibody causes the cell to divide rapidly, and its progeny differentiate into memory B cells and effector B cells, called plasma cells (Fig. 2.10). A single plasma cell can secrete over 2000 molecules of antibody per second, and as such, antibodies are the major effector molecules of humoral immunity. Most antigens activate B cells only when the B cells are stimulated by cytokines from T-helper cells (T-cell dependent antigens), but some antigens are T-cell independent (Fig. 2.10).

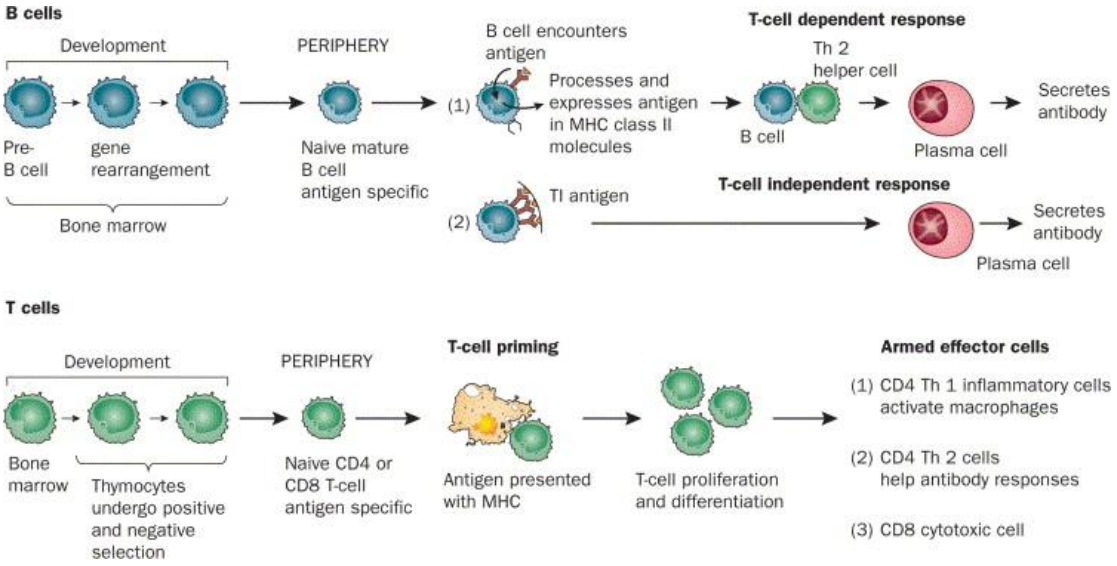

Fig. 2.10 The role of T cells and B cells in the adaptive immune system (Taken from Parkin & Cohen (2001))

2.5.4 Cellular Senescence

Cellular senescence is a state whereby cells are unable to further proliferate in response to antigenic stimuli and can be caused by excess rounds of cell division in response to repeated antigenic stimuli (Effros 2007). Under standard culture conditions, cellular proliferation ceases after a more or less fixed number of cell divisions (the 'Hayflick limit'), and is dependent upon the particular cell (von Zglinicki, 2002). Cellular senescence is therefore unaffected directly by chronological time, but is primarily affected by the replicative history of the cells (von Zglinicki, 2002). It has been reported that in human blood lymphocytes *in vitro*, CD4+ T-cells to have around 33 cellular divisions in culture before cell-cycle arrest, whereas CD8+ T-cells have around 23 (Pawelec et al., 1996; Perillo et al., 1993). Further evidence using human T cells from young donors in a long-term cell culture model has reported that repeated stimulation with antigens on nearly 100 independent cultures reach the end stage of replicative senescence, after multiple rounds of cell division (Perillo et al., 1989).

2.5.5 Mechanisms Responsible for Cellular Senescence

2.5.5.1 Telomere Shortening

Telomeres are repeated DNA sequences at the termini of linear chromosomes (Effros 2007). Due to the mechanism of DNA replication, telomeres shorten with each cell division and therefore have been suggested as a marker of replicative senescence (Effros 2007; van Baarle et al., 2005; Effros 2004). The loss of 50-100bp of telomere sequence with each cell division ultimately leads to critically short telomeres (Effros 2007). The signal for the cell cycle arrest following telomere shortening to critical lengths has been suggested to be either the shortened telomeres themselves, or due to the cell detecting DNA damage, a reduced abundance of certain telomere-binding proteins (Effros 2007). As critically short telomeres are capable of fusing with each other, potentially leading to tumorigenesis, signalling mechanisms for senescence are triggered, in order to prevent cell division (Simpson et al., 2008).

2.5.5.2 Oxidative Stress

Molecules that are capable of independent existence (free) and contain an unpaired electron (radical) are known as free radicals (Vollaard et al., 2005). Free radicals that are capable of inducing oxidative damage to biological structures are known as reactive oxygen species (ROS) (Sen 2001). An oxidative stress state occurs when there is an imbalance between free radical production and antioxidant defence (Sen 2001). In Harman's free radical theory of cellular aging, oxidative stress is thought to be responsible, at least in part, for cellular senescence (Harman 2006). Other authors also suggest that excessive exposure to oxidative stress causes immune cell senescence (Larbi et al., 2007; van Baarle et al., 2005). Indeed, increased oxidative stress has been shown to down regulate CD28 expression (Ma et al., 2003). The lack of expression of CD28 is indicative of cellular senescence (Effros 2004). Under conditions of elevated oxidative stress, the rate of telomere erosion can be accelerated (Petersen et al.,

1998). By increasing or decreasing the amount of oxidative stress, the replicative lifespan of cells can be considerably shortened or prolonged, respectively (Packer & Fuehr 1977; Balin et al., 1977). Alternatively, elevated oxidative stress might lead to premature senescence by causing single strand breaks in the chromosomal DNA, therefore independent of telomere shortening (Chen et al., 2001; von Zglinicki et al., 2000; Petersen et al., 1998).

2.5.5.3 Latent Herpesviruses

Latent herpesviruses that are prevalent among humans include herpes simplex virus (HSV-1, HSV-2), cytomegalovirus (CMV), Estein-Barr Virus (EBV) and Varicella Zoster Virus (VZV) (Simpson 2011). Primary infection with these viruses causes evident symptoms, and subsequent reactivations of the virus can occur under periods of stress. For example, a primary infection with VZV will cause chickenpox, and reactivation later in life causes shingles. Reactivation due to stress leads to T cell proliferation (Pawelec et al., 2009; Koch et al., 2007; Koch et al., 2006). The reactivation of latent viral infections is associated with a greater frequency of senescent T cells in the periphery (Koch et al., 2007). Due to persistent viral reactivation, excess T cell clones do not undergo post infection apoptosis, as they are needed for subsequent reactivations of the latent virus. Instead, the excess T cell clones become part of the memory T cell pool, and therefore take up the 'immune space', in place of antigen-virgin naive T cells, thus shrinking the naive T cell repertoire (Simpson 2011).

2.5.6 Markers of Senescence

CD57 is a cell surface glycoprotein that has been reported to be expressed on T-lymphocytes with a senescent phenotype (Brenchley et al., 2003). In healthy controls, the CD57 antigen is expressed normally only by a minority of peripheral blood CD8+ lymphocytes (Focosi et al, 2009). The transcriptional profiles of CD8+ CD57+ and CD8+ CD57- T lymphocytes differ substantially. More specifically, CD8+ CD57+ T lymphocytes have a higher cytotoxic effector potential including perforin, granzymes and granulysin, and express more adhesion molecules

and chemokine receptors (Focosi et al, 2009). In addition, CD8+ CD57+ T lymphocytes express a lower level of genes involved in cell-cycle regulation, even in response to TCR, IL-2, IL-7 and IL-15 stimulation (Focosi et al, 2009). Moreover, CD57 is expressed on T-lymphocytes with short telomeres, a long history of proliferation, an inability to proliferate in response to mitogenic stimuli (Brenchley et al., 2003) and lack cell surface expression of the co-stimulatory molecule, CD28 on the cell surface (Bruunsgaard et al., 1999). CD28 is a cell surface glycoprotein, and has been shown to be an important co-stimulatory receptor (Linsley & Ledbetter, 1993). A co-stimulatory signal is transduced through CD28 when T cells encounter an antigen presenting cell expressing either of the CD28 ligands, B7-1 or B7-2 (Boise et al, 1995). A proliferative response can occur with a lower concentration of anti-CD3 following co-stimulation *in vitro* (Gimmi et al, 1991). Moreover, the production of cytokines by helper T cells is enhanced by CD28 co-stimulation, through transcriptional and posttranscriptional regulation of gene expression (Lindsten et al, 1989). CD28 co-stimulation can also activate the cytolytic potential of cytotoxic T cells (Boise et al, 1995). Therefore, CD28 is clearly important for the activation and proliferation of naive T-cells (Ouyang et al., 2003; Labalette et al., 1999; Linsley & Ledbetter 1993). Cells lacking the expression of CD28 are known to have a lower proliferative capacity (Nociari et al., 1999). In fact, the complete and irreversible loss of CD28 has been reported following T cell replicative senescence in cell culture (Effros 2004). T cells lacking the expression of CD28 have also been shown to have shorter telomere lengths (Effros et al.,1996). It has been reported that CD28 is required for optimal upregulation in the activity of the telomere extending enzyme, telomerase (Valenzuela & Effros 2002; Weng et al., 1997). The evidence clearly shows that the expression of CD57 and the lack of expression of CD28 is indicative of a senescent phenotype. However, it has been suggested that for an even more accurate marker of a true end stage senescent cell, multiple phenotypic markers are required (Effros 2007). It has been reported that from proliferative assays and telomere analysis, the surface expression of CD57+ and CD28- T cells define the ultimate end-stage cell in the

senescent pathway (Brenchley et al., 2003). Following an antigenic stimulus, CD8+ T-lymphocytes proliferate more than CD4+ (Foulds et al., 2002). CD8+ T lymphocytes also have shorter telomeres (Bruunsgaard et al., 1999) and lack the ability to upregulate the telomere extending enzyme, telomerase (Effros 2007). For these reasons, it would therefore be expected to see a greater frequency of senescent cells among the CD8+ T cell population (Simpson et al., 2008).

2.5.7 The Impact of Cellular Senescence

Homeostatic mechanisms tightly control the proportion of naive and activated T cell populations in the peripheral T cell pool (Freitas et al., 1996; Tanchot & Rocha 1995; Rocha et al., 1989). Therefore, the proportion of T cells with a senescent phenotype in the peripheral T cell pool may increase due to a lack of apoptosis (Spaulding et al., 1999). Apoptosis is linked to proliferative status, and so the inability to enter cell cycle is a factor in the reduced apoptosis in senescent cultures (Spaulding et al., 1999). However, it is also known that non-cycling early passage cultures from the same donor undergo higher levels of apoptosis, thereby suggesting that the resistance to apoptosis is at least partially independent of these senescent cells are non-cycling. In addition, responses to several other stimuli, not linked to cycling have led to a reduced ability to undergo apoptosis (Spaulding et al., 1999). It has therefore been suggested that replicative senescence itself may involve a central block in apoptosis signalling pathways (Spaulding et al., 1999). The naive T cells are gradually replaced with T cells with a senescent phenotype, causing the naive T cell repertoire to shrink (Koch et al., 2006). As the naive T cells are vital in immune responses to novel pathogen, this change in the immune space may leave an individual at a greater risk of viral infection (Pawelec et al., 2009; Koch et al., 2007; Ouyang et al., 2003). An accumulation of senescent T cells is believed to be the cause of increased risk of infectious diseases and autoimmune disorders in the elderly (Effros 2004; Ouyang et al., 2003). More specifically, the proportion of CD28- (senescent) T cells in the elderly correlate

with mortality risk (Wikby et al., 2002), disease status (Effros 2007), disease pathogenesis in certain autoimmune disorders (Effros 2007) and a blunted response to influenza vaccines (Goronzy et al., 2001).

2.5.8 Immunosuppression Following Strenuous Exercise

A 'J shaped curve' has been used to model the relationship between exercise and susceptibility to infection (Nieman 1994). An enhanced immune function above sedentary levels might be achieved with moderate activity. Compared to a sedentary lifestyle, regular moderate exercise of about two hours per day has been reported to decrease the risk of picking up an upper respiratory tract infection (URTI) by 29% (Matthews et al., 2002). In contrast, impaired immune function might result from excessive amounts of prolonged, high-intensity exercise. There is evidence to support the suppressed immune function following strenuous exercise. The mitogen-stimulated lymphocyte proliferation has been reported to be significantly lowered at rest following just 6 days of intensified endurance training in a group of trained cyclists (Lancaster et al., 2004). These cyclists were completing ~10 hours of cycling per week before the study and the training load increased on average by 73% for the 6 days of intensive training. Following 2 weeks of reduced training (<4 hours cycling per week), the mitogen-stimulated lymphocyte proliferation returned to pre-study values. Similarly, T lymphocyte proliferation was decreased in a group of professional footballers during the season, and this returned to baseline after the season (Bury et al., 1998). Three weeks of increased training volume (35%) in a group of elite runners caused a decrease in immune function (Verde et al., 1992). After the increased training volume, the mitogen-induced lymphocyte proliferation was decreased following a 30 min run at 80% VO_{2max}, whereas it was unchanged before the increased training volume. Although speculative, it is possible that the decreased lymphocyte proliferation following chronic exercise training in these studies may have been due to an accumulation of senescent T cells in the periphery. As mentioned, the proportion of senescent

and naive T cells in the immune space is tightly controlled by homeostatic mechanisms (Freitas et al., 1996). Senescent T cells are unable to undergo apoptosis (Spaulding et al., 1999), and so it is possible that the decreased lymphocyte proliferation in these studies was due to an increased proportion of senescent T lymphocytes in the immune space, as senescent T cells are unable to proliferate (Effros 2007). Further evidence for this is the finding that regular, high-intensity exercise has been shown to cause a decrease in the proportion of CD4+ naïve T-cells expressing CD45RA (Hack et al., 1997). CD4+ and CD8+ T cells express a specific isotype of the CD45 family when emigrating from the thymus, the CD45RA antigen (Spits et al., 1995), which designates them as being immunologically naive T cells (Akbar et al., 1991). At rest, CD4/CD8 ratio has been reported to be decreased following an increased training volume (Verde et al., 1992). Likewise, the CD4/CD8 ratio was decreased at the end of the season in a group of professional footballers compared to the pre-season value (Rebelo et al., 1998) and following high intensity training for 8 weeks in previously untrained subjects (Hack et al., 1997).

In support of a reduced immune function following a strenuous training period, the incidence of URTIs has been shown to be increased following intensive exercise training. In one study (Spence et al., 2007), the incident rate ratios in URTIs were assessed over five-months of training and competition in individuals classified as; elite endurance athletes, recreationally competitive endurance athletes, or sedentary subjects. Unsurprisingly, the incident rate ratios were higher in the elite athletes and the sedentary subjects than in the recreationally competitive athletes. Furthermore, the incidence of URTIs has been reported to be greater than 40% in competitive swimmers during four weeks of intense training (MacKinnon & Hooper 1996), elite hockey players during a ten day training camp (MacKinnon 2000), and elite squash players during ten weeks of training (MacKinnon 2000). Indeed the training volume appears to be related to the risk of picking up an URTI (Heath et al., 1991) as it was reported

that compared with athletes running <778km per year, running 778-1384 and >1384km per year was associated with a 2 and a 3.5 times greater odds ratio of picking up an URTI, respectively. An increased risk of infection is a huge concern for athletes, as even minor infections can lead to an inability to sustain strenuous training regimes (Roberts 1986).

2.5.9 Mechanisms Responsible for a Decreased Immune Function Following Strenuous Training

2.5.9.1 Multifactorial:

The reason for the increased incidence of infection in athletes is likely to be multifactorial: a variety of stressors (physical, psychological, or environmental, nutritional) can suppress immune function (Gleeson 2006). Along with an increased exposure to pathogens, these effects can make the athlete more susceptible to infection (Gleeson 2007). However, likely causes appear to be elevated circulating stress hormones, particularly cortisol, and alterations in the pro/anti-inflammatory cytokine balance in response to exercise (Fig. 2.11).

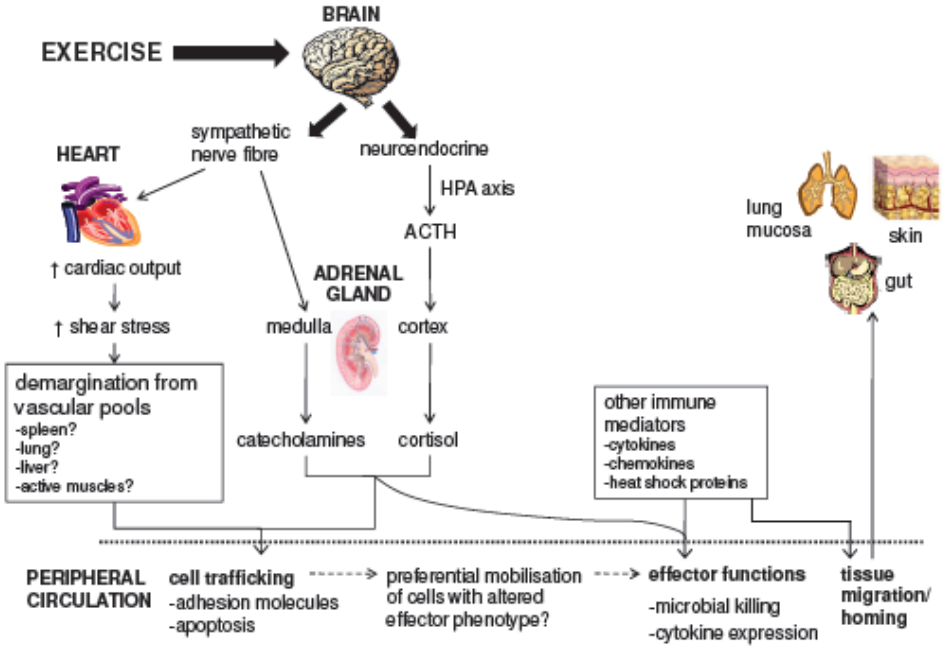

Fig. 2.11 Potential mechanisms by which acute and chronic exercise affects adaptive immunity (Taken from Walsh et al (2011)).

2.5.9.2 Senescent Cells Due to Oxidative Stress

As previously mentioned, an increase in oxidative stress may lead to cellular senescence through telomere dependent or independent mechanisms. During exercise, the oxygen uptake by the active muscle may increase over 100-fold over resting levels. This leads to an increased electron flux through the rapidly respiring mitochondria, which can cause electron leakage and consequent ROS production (Ashton et al., 1998). ROS is formed from the 2-5% of the total electron flux that 'leaks' (Boveris & Chance 1973). Research conducted over the past three decades has reported that exercise of sufficient volume, intensity and duration is capable of increasing the ROS production (Fisher-Wellman & Bloomer 2009). There is a direct relationship between ROS production and the use of aerobic metabolism (Lovlin et al., 1987). In addition, it has been reported that during anaerobic glycolysis, the production of lactic acid can transform an only slightly damaging free radical (superoxide radical) into a much more damaging free radical (perhydroxide) (Groussard et al., 2003). This response is due to the interaction of the free radical with the protons derived from the lactic acid. It seems that free radicals can also be produced during exercise by the autoxidation of catecholamines (Ramel et al., 2004) and by the release of inflammatory cytokines (McAnulty et al., 2003). An increase in ROS may lead to the oxidation of several biological molecules (lipids, proteins, nucleic acids) (Fisher-Wellman & Bloomer 2009). Following cycle exercise to exhaustion in moderately trained individuals (mean VO_{2max}: 49ml.kg.min^{-1}), a 3-fold increase in free radical production has been reported (Ashton et al., 1998). In the study by Ashton et al (1998), the cyclists that achieved a higher VO_{2max} during the test to exhaustion achieved a greater increase in free radical concentration. This demonstrates a direct effect on free radical production by oxidative flux, as previously reported (Lovlin et al., 1987). Therefore, it seems that the total amount of oxygen taken up may be an important determinant of free radical production during exercise.

Previous studies prove that oxidative stress can increase during exercise. Oxidative stress in a group of long-distance runners was increased during an eight day training camp, with increased training volume, but decreased to pre-training levels on the day after the camp was concluded (Okamura et al., 1997). This study used 8-hydroxydeoxyguanosine (8-OhdG) as a biomarker for oxidative stress. The excretion of 8-OhdG reflects the integrated rate of oxidative DNA damage and the repair of DNA in the whole body (Morillas-Ruiz et al., 2005). A study by Almar et al (2002) assessed the oxidative stress on a group of professional cyclists competing in a 4d and a 3wk cycling race. Urinary 8-OhdG excretion increased significantly in the first day and in the first week of the 4d and the 3wk race, respectively and did not show a further increase after. Maximal 8-OhdG levels were reached in parallel to longer times spent at high intensities of exercise. In a group of well trained cyclists (VO_{2max}: 63ml.kg^{-1}.min^{-1}) completing a 90 min exercise test at 70% VO_{2max}, urinary excretion of 8-OhdG significantly increased by 21% (Morillas-Ruiz et al., 2005). It seems that as exercise intensity increases, the oxidative stress increases concomitantly (Alessio et al., 1988).

Adaptations in antioxidant defence and DNA repair gene expression seem to occur as a result of chronic exercise (Sachdev & Davies 2008). Chronic exercise provides repeated exposure of the system to free radicals, which leads to an upregulation of the body's antioxidant defence system (Fatouros et al., 2004; Elosua et al., 2003). A result of an enhanced antioxidant defence system is a shift in redox balance to a more reducing environment, which causes protection from free radicals during subsequent training sessions (Fisher-Wellman & Bloomer 2009). It is therefore possible that trained athletes could have an improved resistance to oxidative stress. Indeed, total antioxidant capacity has been reported to be positively correlated with VO_{2max} in trained runners (Child et al., 1998). Indeed, it has been reported that after an incremental test to exhaustion, untrained men experience a higher DNA strand-breakage in white blood cells than trained men (Niess et al.,1996). However, an enhanced

antioxidant defence system only seems to occur if training is appropriately managed, as hormesis seems to explain the protective effect of exercise to oxidative stress. Hormesis is a dose-response phenomenon, whereby a low dose stimulates adaptation, but a high dose inhibits adaptation (Mattson 2008). Endurance training in well-trained athletes causes ROS formation, which can stimulate adaptive mechanisms, leading to reduced oxidative damage (Gomez-Cabrera et al., 2008). However, it has been reported that overloaded training compromises the antioxidant defences, leading to an increase in the exercise-induced oxidative stress (Palazzetti et al., 2003). It is also possible that individuals that exercise sporadically, and thus have not built up an antioxidant defence are at a higher risk of oxidative stress. The exercise-induced increase in free radicals has been shown to be attenuated with antioxidant supplementation. Independent or combined administration of vitamin C, vitamin E and beta-carotene have been the most commonly utilised treatment options in regards to non-eccentric aerobic exercise-induced oxidative stress (Fisher-Wellman & Bloomer 2009). Therefore, in studies that have shown an increase in oxidative stress following exercise in trained endurance athletes (Morillas-Ruiz et al., 2005; Almar et al., 2002; Okamura et al., 1997), the intensity/volume and frequency must have been adequate to overcome the already enhanced antioxidant defences.

The intensity of exercise has been reported to be an important factor in determining whether exercise induces oxidative stress. In a group of trained male cyclists, malondialdehyde was used as a marker of oxidative stress. An incremental test to exhaustion (mean duration of 16min, mean blood lactate at exhaustion: 9.8mmol.L^{-1}) caused a significant increase in malondialdehyde in plasma and erythrocytes (Munoz et al., 2010). However, in the same group of cyclists, a submaximal test (30min at 75% VO_{2max}, mean blood lactate following the 30min: 3.5mmol.L^{-1}) failed to induce a change in malondialdehyde. The authors suggest that

during the submaximal exercise test, the cyclist's antioxidant system was sufficient to defend against oxidative stress, whereas this was not the case in the incremental test to exhaustion.

2.5.9.3 Viral Reactivation

As previously mentioned, subsequent reactivations of a latent virus (HSV-1, HSV-2, CMV, EBV, VZV) can occur under periods of stress and this can lead to a greater frequency of senescent T cells in the periphery (Koch et al., 2007). Indeed, acute psychological stress mobilises CMV and EBV-specific T cells and effector T cells into the blood (Atanackovic et al.,2006) and it has been suggested that physical stress elicits the same response (Simpson & Guy 2010). It has been reported that in elite athletes, there is a significant relationship between EBV serology and URTI incidence (Gleeson et al., 2002).

2.5.10 Effect of Strenuous Exercise on Senescent T Cells

To the author's knowledge, only Cosgrove et al (2011) has investigated the effects of regular endurance training on the frequency of senescent T cells in resting, fasted blood. This study reported that 6-months of training in preparation for an ironman triathlon race led to no changes in the frequency of senescent or naive T cells in ten trained triathletes. There was a lack of physiological adaptation in the study during the training period, suggestive that the training stress was not excessive. However, blood samples were drawn three weeks after the race revealed an increased proportion of senescent T cells within the CD4+ T-cell subset, alongside a reduction in naive cells within the CD4+ T-cell subset. This suggests that the stimulus during the training period was not sufficient to induce an increase in the proportion of senescent T cells or a decrease in the proportion of naive T cells, but the ironman triathlon race itself did provide a sufficient stimulus to induce these changes in the immune space. These changes post-race, but not during the training period could be due to the so-called 'open window theory', due to an increased pathogen exposure as a consequence of lowered post-race immune surveillance or travel stress (Zurich to UK). It is therefore possible that if

the training stress were higher, there might have been an increase in the proportion of senescent T cells during the training period. Although speculative, it is possible that with regular high intensity training which is of sufficient intensity and duration to induce oxidative stress, there could be an accumulation of senescent T cells through telomere shortening or telomere independent senescence.

2.5.11 Summary of the Senescent T Cells and Exercise Section

T cell responses have been reported to be blunted following a period of endurance training with increased volume/load or intensity. Although speculative, it is possible that the blunted T cell responses could be due to an increased proportion of senescent T cells, alongside a decreased proportion of naive T cells, and therefore a change in the immune space. The result of this would be the individual being left at a higher risk of infection. If training is managed appropriately, an athlete's antioxidant defence is increased, and therefore, high intensity training can be completed with less risk of oxidative stress. However, if training is not managed appropriately, then antioxidant defences do not improve, and therefore, the athlete is left at a greater risk of oxidative stress during high intensity training. A link has been made between oxidative stress and the proportion of senescent T cells, and a link also exists between the proportion of senescent T cells and risk of infection. Therefore, a training programme needs to be appropriately managed to increase antioxidant defences, so that high intensity exercise can be completed without a risk of oxidative stress. It is therefore possible that training programmes with different intensity distributions could be more/less effective to achieve this goal and therefore it could be possible to minimise the risk of infection by manipulating the training-intensity distribution.

2.6 Conclusions to the Review of the Literature

Training intensity and its distribution is a variable in endurance training which has the potential to have a large impact on physiological adaptation and subsequent endurance performance. Descriptive studies assessing the training-intensity distribution in elite endurance athletes have reported that these athletes train with a high volume relative to the event, and spend >80% of training time at intensities lower than the lactate threshold and therefore <20% of training time at intensities higher than the lactate threshold. Experimental studies support the beneficial effects of a large proportion of training at intensities lower than the lactate threshold. As a high training volume is strongly related to endurance performance, it seems that by including a high proportion of training at a relatively low intensity (zone 1) allows for this high training volume. Of the relatively small proportion of training above the lactate threshold (zones 2 and 3), research would suggest that greater benefits in endurance performance and physiological adaptation can be gained through training in zone 3. This has been suggested to be due to greater benefits in cardiovascular adaptations and buffering capacity, but also to the finding of no difference in the acute recovery time of the autonomic nervous system to training in zone 2 and zone 3. It is possible that the manipulation of training-intensity distribution could have effects on the magnitude of physiological adaptation and endurance performance, and the risk of overtraining and picking up an infection.

CHAPTER THREE

REPRODUCIBILITY OF THE LACTATE THRESHOLDS TO DETERMINE TRAINING INTENSITY ZONES AND ADAPTATION IN SWIMMING, CYCLING AND RUNNING

3.1 ABSTRACT

Purpose: The purpose of the present study was to assess the reproducibility of the blood lactate threshold (LT) and lactate turnpoint (LTP) in swimming, cycling and running in trained endurance athletes. In addition, the reproducibility of maximal performance measures was assessed. **Methods:** Standardised incremental intensity tests took place twice for swimming (n=9), cycling (n=10) and running (n=10), separated by a minimum of two and a maximum of six complete days to assess the test-retest reproducibility. Test-retest reproducibility was assessed using the typical error of measurement (TEM). **Results:** The TEM for the LTP in swimming (2.5%), cycling (3.3%) and running (2.6%) were considered reproducible. The TEM for the LT in swimming (2.9%), cycling (3.7%) and running (3.1%) were also considered reproducible. Moreover, the TEM for the fastest 150m swim time in swimming (1.1%), 3-min peak power output in cycling (1.6%), 1-min peak power output in cycling (2.3%), 2.5-min peak running speed (0.9%) and 30-s peak running speed (1.1%) were all considered reproducible. **Conclusions:** The LTP, alongside the LT are reliable measures to demarcate training-intensity zones, assess endurance performance and monitor moderate to large adaptations to training. Practitioners should take care when deciding on the duration between testing sessions in order to test for adaptations from training. Adaptations need to be greater than these detected test-retest variations (TEM) to be considered measurably different.

Key Words: Performance testing, exercise intensity, measurement, reliability, test-retest

3.2 INTRODUCTION

It has been suggested that two typical breakpoints are passed during incremental intensity exercise (Skinner & McLellan 1980; Kindermann et al., 1979). The first breakpoint corresponds to the blood lactate threshold (LT), and has been described as the final exercise intensity before the blood lactate concentration is increased distinctly from its resting level (Aunola & Rusko 1984; Skinner & McLellan 1980; Kindermann et al., 1979). The second breakpoint corresponds to the blood lactate turnpoint (LTP) and has been described as the starting point of an accelerated increase in the blood lactate concentration (Smith & Jones 2001 ;Jones & Doust 1997; Aunola & Rusko 1984).

A recent review (Faude et al., 2009) confirms that there is a strong relationship between the LT and endurance performance. Training studies have demonstrated that training-induced increases in competitive performance significantly correlate with increases in the LT (Tanaka 1986; Tanaka & Matsuura 1984). The LT and the LTP can also be used to demarcate the boundaries between moderate and heavy exercise, and between heavy and very heavy exercise, respectively (Smith & Jones 2001). Therefore, these blood lactate thresholds can be used not only to assess endurance performance, but to demarcate training-intensity zones for exercise intensity prescription and to monitor adaptations to endurance training.

To the author's knowledge, no study exists that has assessed the reproducibility of the LTP in trained endurance athletes. Previous studies have however, assessed the reproducibility of the exercise intensity corresponding to a blood lactate concentration of 4mmol·L^{-1} in endurance trained runners. These studies found high correlation coefficients (Grant et al., 2002; Coen et al., 2001; Weltman et al., 1990), close limits of agreement (Grant et al., 2002), high intra-class correlation coefficients and small coefficients of variation (Pfitzinger & Freedson 1998). Previous studies have also assessed the test-retest reliability of the LT in

endurance trained runners, and have reported high correlation coefficients (Grant et al., 2002; Weltman et al., 1990), high intra-class correlation coefficients (Pfitzinger & Freedson 1998), close limits of agreement (LOA; Grant et al., 2002) and low coefficients of variation (Pfitzinger & Freedson 1998). To the author's knowledge, no literature exists that has assessed the reproducibility of the LT in trained endurance athletes in cycling or swimming.

If the LTP and the LT are to be used to monitor endurance performance, prescribe training intensities or track changes in training status, they must be reproducible. The researcher needs to be sure that following an intervention, any changes in these measures are outside of the day-to-day variability. The reliability of the LTP and LT is dependent on both biological and technological variability (Pfitzinger & Freedson 1998) and it is known that sampling method and site, diet and prior exercise impact upon the blood lactate concentration (Faude et al., 2009).

There are several methods available to researchers to assess the test-retest reproducibility of a particular measure. It has been suggested that the typical error of measurement (TEM; Hopkins 2000), the LOA (Atkinson & Nevill 1998) and ordinary least products regression (OLPR; Ludbrook 2010; Ludbrook 1997) should be used. The use of correlation coefficients has been discouraged as a sole method of assessing the reproducibility of a measure, as it does not give any information on systematic bias and the result can be greatly influenced by the range of values in the sample (Atkinson & Nevill 1998). Therefore, as the majority of the previous research assessing the test-retest reproducibility of blood lactate thresholds in trained endurance athletes has used correlation coefficients, this area of research is limited by inappropriate statistical analyses.

To the author's knowledge, no studies have yet assessed the reproducibility of the LTP in any mode of exercise in trained endurance athletes. In addition, no study has assessed the LT in cycling or swimming in trained endurance athletes. Therefore, the purpose of the present study was to assess the reproducibility of the LTP in swimming, cycling and running in trained endurance athletes, using standardised incremental intensity tests to exhaustion. In addition, the reproducibility of the LT and maximal performance measures were also assessed.

3.3 METHODS

3.3.1 Participants

Ten participants for the cycling and running tests and nine participants for the swimming test volunteered and provided written, informed consent to take part in the study, which was approved by the local Ethics Committee, in accordance to the Declaration of Helsinki. Participants were healthy, trained swimmers, cyclists, runners and triathletes. The mean (±SD) characteristics of the participants were: age 39 (±12) yrs, mass 77.3 (±15.6) kg, and stature 178 (±9) cm.

3.3.2 Experimental Approach to the Problem

For each discipline, the incremental test was carried out twice, with a minimum of two complete days and a maximum of six complete days between tests. Each athlete completed a training and food diary for the two days prior to the first testing session and replicated the training and diet prior to the second testing session. Participants were instructed to exercise for no more than 60 min at an easy intensity in each of the two days before the first testing session. Testing was performed at the same time of day (±1 hr) to minimise the effect of diurnal biological variation (Atkinson & Nevill 1998). For the cycling and the running tests, the laboratory ambient temperature was controlled at 18-20°C and relative humidity ranged between 30 and 50%. For the swimming test pool water temperature was maintained at 27-28°C. Participants drank water *ad libitum* throughout each test.

3.3.3 Swimming Test

The swimming test was a modified version of the protocol used by Pyne and colleagues (Pyne et al., 2001) and took place in a 25m pool, with participants wearing their own wetsuit. An incremental test took place with seven 150m stages, each 5 s quicker than the previous, with a 3-min rest period between each stage. The 4th stage had the same pace as the athletes 400m

time trial time, which was assessed within 4 weeks of the first test. The final stage was a maximal 'all out' effort to assess the fastest 150m swim time.

3.3.4 Cycling Test

The protocol for the cycling test was modified from a test previously described by Farina and colleagues (Farina et al., 2004). Participants brought their own pedals and shoes for the test. Handlebar position and saddle height of the ergometer were individualised for each participant and remained consistent for subsequent tests. A 5-min warm-up was completed at 70 W at a self-selected cadence on a Lode cycle ergometer (Excalibur Sport, Netherlands). This was immediately followed by a ramp test to volitional exhaustion, starting at a power output of 70 W and increasing by 35 W every 3-min (25 W for female) with the cadence remaining self-selected. Immediately after this test, the participants rested for 10-min before completing a shorter maximal exercise test to volitional exhaustion. The test consisted of 1-min stages, starting at the intensity of the penultimate stage of the previous test, and increased by 35 W each stage. The end time for both the 3-min and 1-min stages were used to calculate the 3-min peak power output (PPO-3) and the 1-min peak power output (PPO-1), respectively (Kuipers et al., 1985):

$PPO\text{-}3 = W_{final} + ([t/180] * PI)$

$PPO\text{-}1 = W_{final} + ([t/60] * PI)$

Where W_{final} = The power output of the final *completed* stage, t = the time achieved in the final *non-completed* stage and *PI* = the power increment.

3.3.5 Running Test

All participants were familiar with treadmill running. Participants completed a standardised warm-up for 5min at 8km·hr^{-1} on a treadmill (Powerjog, Cranlea and Co), set at a 1% incline throughout the test to simulate the energetic cost of outdoor running (Jones & Doust 1996).

This was followed by an incremental exercise test to volitional exhaustion, starting at a speed of between 8 and 10 $km \cdot hr^{-1}$ (depending on running ability) and increasing by 1 $km \cdot hr^{-1}$ every 3min (Billat et al., 2003). Thirty seconds before the end of each stage, participants supported their weight with their hands and moved their feet to the sides of the treadmill belt to allow measurements to be taken. As in the cycling test, the participants rested for 10min before completing a shorter maximal exercise test, as reported by Farina and colleagues (Farina et al., 2004). The test consisted of 30s stages, starting at the intensity of the penultimate stage of the previous test, and increasing by 0.5$km \cdot hr^{-1}$ each stage until volitional exhaustion. The end time in both the 3-min and the 30-s tests was used to calculate the 2.5-min peak running speed (PRS-2.5) and the 30-s peak running speed (PRS-30), respectively (Kuipers et al., 1985):

$PRS\text{-}2.5 = Speed_{final} + (t/150)$
$PRS\text{-}30 = Speed_{final} + ([t/30]*SI)$

Where $Speed_{final}$ = The running speed of the final *completed* stage, t = the time achieved in the final *non-completed* stage, *SI* = the speed increment.

The LTP was determined as the starting point of an accelerated blood lactate accumulation (around 3 to 6$mmol \cdot L^{-1}$), depending on the individual blood lactate profile (Aunola & Rusko 1984). The LT was determined as the final exercise intensity before the blood lactate concentration increased distinctly from its resting concentration (Aunola & Rusko 1984). In 100% of tests, the lactate concentration did not deviate by more than 0.3$mmol.L^{-1}$ in the first three stages of the test. Therefore, a line of best fit was established from these initial stages of the test. The distinct increase in lactate concentration was clear in 100% of tests, but was of an individual magnitude for each test. For example, one participant had lactate concentrations of 1.0. 1.1, 1.0, 1.0, 1.7, 2.6, 3.4, 6.6, 10.4$mmol.L^{-1}$. In this test, the distinct deviation was clear from 1.0 to 1.7$mmol.L^{-1}$. By fixing a standard magnitude of increase to the lactate concentration, such as 1.0$mmol.L^{-1}$, this increase would have been missed. The lactate

turnpoint was also clear from 3.4 to 6.6mmol.L^{-1}, due to the obvious deviation accelerated blood lactate accumulation. This was a clear deviation from the second line of best fit in 100% of the tests.

Immediately after each stage in the incremental tests, heart rate (Polar S625X, Polar Electro, Kemplete, Finland) was recorded. In addition, a 5-µL capillary blood sample was obtained and analysed for lactate concentration by micro-assay (LactatePro LT-1710, ArkRay Inc, Kyoto, Japan). The reliability and validity of this device has been previously determined(Pyne et al., 2000). The reliability was reported to have a correlation coefficient of 0.99 and a level of agreement under 1mmol·L^{-1}.

3.3.6 Statistical Analyses

Systematic bias between the test and retest scores was assessed using paired sample t-tests. Statistical significance was accepted at $P<0.05$. The assumption of homoscedasticity was confirmed before undertaking the LOA analysis. Heteroscedasticity was assessed by calculating Pearson's correlation coefficient between the individual differences from test one to test two and the individual mean value for test one and test two. Pearson's correlation coefficient was not significant ($P>0.05$) for all of the measures and the r value was ≤0.53. The LOA (Bland & Altman 1986) and the TEM along with 95% confidence intervals (Hopkins 2000) were calculated using the spreadsheet of Hopkins (Hopkins, 2000). OLPR analysis was undertaken, using the equations suggested by Ludbrook (Ludbrook 2010; Ludbrook 1997).

3.4 RESULTS

3.4.1 Absolute and Percentage Test-Retest Differences

The absolute values for the swimming times, the cycling power outputs and the running speeds associated with the LT, LTP and the maximal performance measures for test one and test two are presented in Table 3.1, along with the absolute and percentage differences in each variable from test one to test two. In addition, the absolute values for the HRs associated with these variables are also presented, along with the absolute and percentage differences from test one to test two. There were no test-retest differences for the intensity corresponding to the LT in swimming, cycling and running, with percentage differences of 1.4, 4.3 and 0.6%, respectively. Likewise, there were no test-retest differences for the LTP in swimming and cycling, with percentage differences of -0.1 and 2.5%, respectively. However, the test-retest difference for the LTP in running was significantly higher in test two (14.7km·hr^{-1}) than test one (14.1km·hr^{-1}; $P<0.05$). There were no test-retest differences for the maximal performance measures in swimming or running, or the PPO-3 in cycling. There was however, a significant test-retest difference for the PPO-1 in cycling, which was higher in test two (394W) than test one (385W; $P<0.05$). In addition, there were no test-retest differences for the HR's corresponding to the LT, LTP or the maximal HR in swimming, cycling and running (Table. 3.1).

3.4.2. Limits of Agreement, Typical Error of Measurement and Pearson's Correlation Coefficient

The absolute and percentage LOA and TEM between test one and test two are presented in Table 3.2, along with the 95% confidence intervals for the TEM. The lowest LOA values for the LT and the LTP were in swimming (8.0 and 6.9%, respectively), followed by running (8.6 and 7.2%, respectively) and cycling (10.1 and 9.2%, respectively). Similarly, the lowest TEM values for the LT and the LTP were in swimming (2.9 and 2.5%, respectively), followed by running (3.1 and 2.6%, respectively) and cycling (3.7 and 3.3%, respectively). Pearson's correlation coefficient between test one and test two are presented in Table 3.2. Every measure had a

significant correlation coefficient, with a Pearson's correlation coefficient of at least 0.89 ($P<0.05$; Table 3.2).

3.4.3 Ordinary Least Products Regression Analysis

The slope and the intercept between test one and test two using OLPR are presented in Table 3.3, along with 95% confidence intervals for both. PPO-1 had both proportional and fixed bias (Table 3.3), whereas every other measure had neither proportional nor fixed bias.

3.4.4 Bland Altman Plots

Bland Altman plots are presented in Fig 3.1, and show that all individual test-retest differences were within 1.96 standard deviations of the test-retest differences.

3.4.5 Relationship between the Magnitude of the Measure and Test-Retest Difference

The relationships between the individual differences from test one to test two and the individual mean value for test one and test two are presented in Table 3.4. Pearson's correlation coefficient was not significant ($P>0.05$) for all of the measures and the r value was ≤0.53.

Table. 3.1 Mean (±SD) values for all measures for test one and test two and the values for the difference between test one and test two.

Discipline	Measure		Test One	Test Two	Difference	Difference (%)
Swimming	**150m Swim Time (s)**	**LT**	142 (±13)	144 (±13)	2 (±6)	1.4 (±4.3)
		LTP	130 (±11)	130 (±11)	0 (±5)	-0.1 (±3.6)
		FST	123 (±13)	122 (±12)	-1 (±2)	-0.7 (±1.5)
	Heart Rate (bpm)	**LT**	127 (±11)	124 (±15)	-4 (±5)	-4.9 (±5.3)
		LTP	155 (±19)	151 (±19)	-3 (±5)	-2.1 (±3.3)
		MAX	170 (±16)	168 (±14)	-2 (±4)	-0.9 (±2.3)
Cycling	**Power Output (W)**	**LT**	181 (±39)	187 (±34)	6 (±10)	4.3 (±8.5)
		LTP	251 (±47)	256 (±41)	5 (±12)	2.4 (±5.5)
		PPO-3	332 (±47)	337 (±51)	5 (±7)	1.4 (±2.2)
		PPO-1	385 (±67)	394 (±59)*****	9 (±13)	3.0 (±4.3)
	Heart Rate (bpm)	**LT**	128 (±15)	128 (±11)	0 (±7)	0.3 (±5.4)
		LTP	151 (±15)	151 (±14)	0 (±4)	0.1 (±3.0)
		MAX	173 (±13)	175 (±13)	1 (±2)	0.7 (±1.3)
Running	**Speed (km·hr^{-1})**	**LT**	12.1 (±1.6)	12.1 (±1.8)	0.1 (±0.5)	0.6 (±4.6)
		LTP	14.1 (±1.9)	14.7 (±1.8)*****	0.5 (±0.5)	4.0 (±4.1)
		PRS-2.5	16.6 (±2.1)	16.7 (±2.1)	0.2 (±0.2)	0.9 (±1.3)
		PRS-30	17.4 (±2.3)	17.6 (±2.3)	0.2 (±0.3)	1.0 (±1.6)
	Heart Rate (bpm)	**LT**	157 (±9)	155 (±10)	-2 (±4)	-1.0 (±2.7)
		LTP	171 (±11)	174 (±9)	3 (±5)	1.8 (±2.8)
		MAX	183 (±9)	185 (±9)	1 (±4)	0.7 (±2.2)

LT: Lactate Threshold, **LTP:** Lactate Turnpoint, **FST:** Fastest 150m swim time, **PPO-3**: 3-min peak power output, **PPO-1**: 1-min peak power output, **PRS-2.5**: 2.5-min peak running speed, **PRS-30**: 30-s peak running speed. *different from test one ($P<0.05$).

Table. 3.2 Limits of agreement, typical error of the measurement with 95% confidence intervals, and Pearson's correlation coefficient for all measures.

Discipline	Measure		LOA	TEM	(95% CI)	r
Swimming	**150m Swim Time (s)**	**LT**	± 12(8.0%)	4 (2.9%)	(3, 8)	0.89*
		LTP	± 9 (6.9%)	3 (2.5%)	(2, 6)	0.92*
		FST	± 4 (2.9%)	1 (1.1%)	(1, 2)	0.99*
	Heart Rate (bpm)	**LT**	± 11 (8.1%)	4 (2.9%)	(2, 9)	0.96*
		LTP	± 10 (6.7%)	4 (2.4%)	(2, 7)	0.96*
		MAX	± 8 (4.6%)	3 (1.6%)	(2, 5)	0.98*
Cycling	**Power (W)**	**LT**	± 19 (10.1%)	7 (3.7%)	(5, 12)	0.97*
		LTP	± 23 (9.2%)	8 (3.3%)	(6, 15)	0.97*
		PPO-3	± 15 (4.3%)	5 (1.6%)	(4, 10)	0.99*
		PPO-1	± 25 (6.4%)	9 (2.3%)	(6, 17)	0.99*
	Heart Rate (bpm)	**LT**	± 14 (10.7%)	5 (3.9%)	(3, 9)	0.89*
		LTP	± 8 (5.6%)	3 (2.0%)	(2, 6)	0.96*
		MAX	± 4 (2.4%)	2 (0.9%)	(1, 3)	0.99*
Running	**Speed ($km \cdot hr^{-1}$)**	**LT**	± 1.0 (8.6%)	0.4 (3.1%)	(0.3, 0.7)	0.96*
		LTP	± 1.0 (7.2%)	0.4 (2.6%)	(0.3, 0.7)	0.96*
		PRS-2.5	± 0.4 (2.6%)	0.2 (0.9%)	(0.1, 0.3)	0.99*
		PRS-30	± 0.5 (2.9%)	0.2 (1.1%)	(0.1, 0.4)	0.99*
	Heart Rate (bpm)	**LT**	± 8 (5.3%)	3 (1.9%)	(2, 5)	0.90*
		LTP	± 9 (5.2%)	3 (1.9%)	(2, 6)	0.92*
		MAX	± 8 (4.4%)	3 (1.6%)	(2, 5)	0.89*

LT: Lactate Threshold, **LTP:** Lactate Turnpoint, **FST:** Fastest 150m swim time, **PPO-3**: 3-min peak power output, **PPO-1**: 1-min peak power output, **PRS-2.5**: 2.5-min peak running speed, **PRS-30**: 30-s peak running speed. **TEM**: Typical error of the measurement, **95% CI**: 95% Confidence Intervals, **LOA**: Limits of agreement, **r**: Pearson's correlation coefficient. *Pearson's correlation coefficient ($P<0.05$).

Table. 3.3 The slope and intercept for all measures using Ordinary Least Products Regression along with 95% confidence intervals.

Discipline	Measure		Slope	(95% CI)	Intercept	(95% CI)
Swimming	**150m Swim Time**	**LT**	0.98	(0.67, 1.45)	4	(-63, 50)
		LTP	1.04	(0.73, 1.47)	-5	(-61, 34)
		FST	0.96	(0.84, 1.08)	4	(-11, 18)
	Heart Rate (bpm)	**LT**	1.07	(0.70, 1.65)	-15	(-90, 34)
		LTP	0.98	(0.77, 1.25)	0	(-42, 32)
		MAX	0.87	(0.72, 1.05)	21	(-10, 47)
Cycling	**Power (W)**	**LT**	0.89	(0.74, 1.07)	26	(-7, 53)
		LTP	0.89	(0.73, 1.08)	33	(-15, 73)
		PPO-3	1.08	(0.98, 1.20)	-22	(-62, 13)
		PPO-1	0.87	(0.77, 0.98)**#**	60	(17, 97)**^**
	Heart Rate (bpm)	**LT**	0.75	(0.52, 1.09)	32	(-12, 61)
		LTP	0.91	(0.72, 1.14)	14	(-22, 42)
		MAX	0.99	(0.87, 1.13)	3	(-22, 24)
Running	**Speed ($km \cdot hr^{-1}$)**	**LT**	1.16	(0.93, 1.45)	-2	(-5, 1)
		LTP	0.96	(0.77 1.20)	1	(-2, 4)
		PRS-2.5	0.99	(0.91, 1.08)	0	(-1, 2)
		PRS-30	1.01	(0.90, 1.12)	0	(-2, 2)
	Heart Rate (bpm)	**LT**	1.04	(0.74, 1.47)	-8	(-75, 39)
		LTP	0.84	(0.61, 1.15)	31	(-23, 70)
		MAX	1.04	(0.72, 1.49)	-6	(-89, 52)

LT: Lactate Threshold, **LTP:** Lactate Turnpoint, **FST:** Fastest 150m swim time, **PPO-3:** 3-min peak power output, **PPO-1:** 1-min peak power output, **PRS-2.5:** 2.5-min peak running speed, **PRS-30**: 30-s peak running speed. **#** proportional bias, **^**fixed bias.

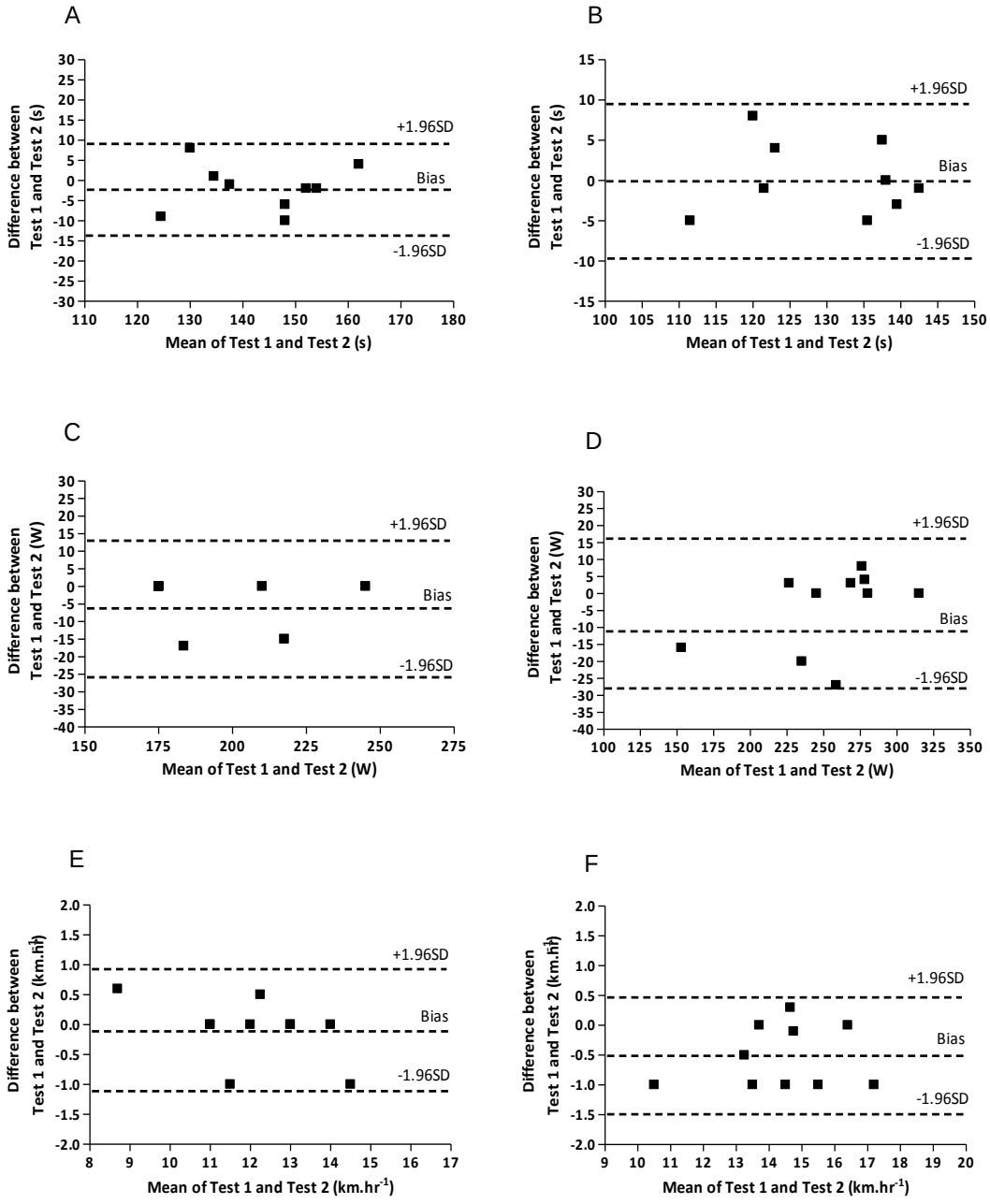

Fig. 3.1 Bland Altman plots show the relationship between the individual test-retest differences and the mean of test one and test two for the swimming LT and LTP (A and B, respectively), the cycling LT and LTP (C and D, respectively) and the running LT and LTP (E and F, respectively).

Table. 3.4 The relationship between the difference between test one and test two and the mean value from test one and test two.

Discipline	Measure		r	P
Swimming	**150m Swim Time (s)**	**LT**	0.04	0.92
		LTP	-0.10	0.81
		FST	0.30	0.43
Cycling	**Power (W)**	**LT**	0.46	0.19
		LTP	0.45	0.20
		PPO-3	-0.53	0.12
		PPO-1	0.27	0.52
Running	**Speed ($km \cdot hr^{-1}$)**	**LT**	-0.47	0.17
		LTP	0.16	0.67
		PRS-2.5	0.06	0.87
		PRS-30	-0.04	0.91

LT: Lactate Threshold, **LTP:** Lactate Turnpoint, **FST:** Fastest 150m swim time, **PPO-3:** 3-min peak power output, **PPO-1:** 1-min peak power output, **PRS-2.5:** 2.5-min peak running speed, **PRS-30**: 30-s peak running speed, **r**: Pearson's correlation coefficient, **P:** Level of significance.

3.5 DISCUSSION

The test-retest error from a reproducibility study must be assessed by the investigator to evaluate whether the value is small enough for the test to be of practical use (Atkinson & Nevill 1998). We believe that the results of the present study suggest that the LTP, along with the LT and maximal performance measures for swimming, cycling and running using these standardised incremental tests can be considered reproducible for some purposes in this population of trained endurance athletes. Indeed, the results suggest that the LTP, along with the LT, can be used by this population of trained endurance athletes not only to assess endurance performance, but also to demarcate training intensity zones for exercise intensity prescription and to monitor moderate to large adaptations to training. Exercise physiologists should take care when deciding on the duration between testing sessions to test for adaptations from training, as adaptations need to be greater than these detected test-retest variations (TEM) to be considered significant. These results therefore give exercise physiologists a value (TEM) for the day-to-day variation in these measures in a group of trained endurance athletes. Any change outside of these TEM values should be considered to be due to reasons other than day-to-day variation, such as training/detraining, nutrition, hydration, and diurnal biological variations.

We believe that the TEM values in the present study may not be sensitive enough to detect changes due to short-term training/detraining in this group of trained endurance athletes. Indeed, longer intervals between testing sessions may be required to be outside of the day-to-day variation. However, if training is drastically changed, and large adaptations are expected over a short time period, these measures should be adequately reproducible to detect these changes. In fact, peak power output has been detected to be increased by 4-5% in just 4 weeks in well-trained cyclists (Lindsay et al, 1996; Laursen et al, 2002). The TEM with confidence intervals has been suggested as the most appropriate method of analysing the

reproducibility of a measure (Hopkins 2000). TEM represents approximately 68% of the error actually present in the repeated measurement of an individual in the sample. If the TEM and the size of the confidence intervals are small, then the upper value of the confidence intervals can be used as an estimation of the lower limit for a meaningful change in a measurement with repeat testing (Hopkins 2000). When analysing test-retest data in an athletic population, LOA analysis has been suggested to be too stringent for a decision limit, and in fact, half of the LOA appears to be a more reasonable threshold (Hopkins 2000). Indeed, in the present study, the LOA for the swimming LTP (9s) and the swimming LT (12s) are large, and not sensitive enough to detect changes which might occur due to short periods of training/detraining. However, these values could be considered reproducible if halved. The Pearson's correlation coefficients in the present study are useful for comparison with previous reproducibility studies. In addition, a high correlation coefficient reflects acceptable relative reliability of that particular measurement in the population that has been investigated (Atkinson & Nevill 1998). It should be mentioned however, that it has been well established that Pearson's correlation coefficient should not be used exclusively in a reproducibility study (Hopkins 2000; Atkinson & Nevill 1998). As all of the measures in the present study had significant and high correlation coefficients ($r>0.89$), this is further evidence of the reproducibility of the measures.

Only the cycling PPO-1 had any sort of bias (fixed and proportional) between test one and test two. As previously mentioned, this is likely due to a general learning effect(Atkinson & Nevill 1998) or to motivational issues (Heitkamp et al., 1991). Every other measure taken in this study had neither fixed nor proportional bias, which is further evidence for their reproducibility. Although Pearson's correlation coefficient is indicative of the scatter of the values around the line of best fit, it is possible that the slope of the line differs from unity (proportional bias) or its intercept might differ from zero (fixed bias), and Pearson's correlation coefficient is not sensitive enough to detect these possibilities (Ludbrook 1997).

OLPR is a sensitive technique for detecting and distinguishing fixed and proportional bias between repeated measures (Ludbrook 2010; Ludbrook 1997). It was not possible to use the traditional least squares regression in this study, as this method requires that x-values are fixed by the design of the study and are not subject to error (Ludbrook 1997). However, as both the test and the retest measures in the present study are attenuated by random error, OLPR analysis was used, as suggested by Ludbrook (1997).The principle underlying the OLPR is to minimise the sum of the products of the x and y deviations from the line of best fit (Ludbrook 2010). A positive or negative fixed bias is suggestive that the measure shows an increase or decrease, respectively from test one to test two by a constant amount (Ludbrook 2010; Ludbrook 1997). Positive and negative proportional bias is indicative that test two gives values that are higher or lower, respectively than test one by an amount that is proportional to the level of the measured value.

To our knowledge, no study has assessed the reproducibility of the LTP in trained endurance athletes. However, a number of studies have assessed the reproducibility of the running speed corresponding to a blood lactate concentration of 4mmol·L^{-1} in trained runners and in agreement with the present study, found high correlation coefficients (0.89 – 0.95) (Grant et al., 2002; Weltman et al., 1990; Sjodin et al., 1982), and LOA of -0.9, 1.5km·hr^{-1} (Grant et al., 2002). Another study found an intra-class correlation of 0.99 and a coefficient of variation of 2.4% for this measure (Pfitzinger &Freedson 1998). The reproducibility of the individual anaerobic threshold in trained cyclists, as assessed by the blood lactate curve being fitted with a single exponential function has been shown to have a high correlation coefficient (r=0.98; McLellan & Jacobs 1993). Similar results to the present study have also been found for the reproducibility of the HR at the running speed corresponding to a blood lactate concentration of 4mmol·L^{-1} in trained runners , with significant correlation coefficients (r = 0.83 – 0.96)

(Grant et al., 2002; Heitkamp et al., 1991; Weltman et al., 1990) and LOA of -12, 11bpm (Grant et al., 2002).

Previous studies that have assessed the reproducibility of the LT in trained endurance athletes have reported similar values to the present study. Trained endurance runners have found high correlation coefficients (r = 0.89-0.94) (Grant et al., 2002; Weltman et al., 1990) and LOA of -0.5, 1.1km·hr^{-1} (Grant et al., 2002). Another study found a high intra-class correlation (0.99) and a low coefficient of variation (1.3%) for the reproducibility of the LT in trained runners (Pfitzinger & Freedson 1998). In agreement with the present study, good reproducibility has been found for the HR at the LT in trained runners, with high correlation coefficients (0.93-0.98) (Grant et al., 2002; Weltman et al., 1990) and LOA of -12, 7bpm (Grant et al., 2002). Also in line with the present study, the reproducibility of the maximal running speed and the maximal HR in an incremental intensity running test in trained runners have been reported to have high correlation coefficients(r = 0.96 and 0.96, respectively; Weltman et al., 1990). Care needs to be taken when comparing the results of the previous studies to those which have assessed the reproducibility of blood lactate thresholds. The pre-trial nutritional intake and exercise was not standardised in some studies, and the statistical analysis was limited (Weltman et al., 1990; Aunola & Rusko 1984). Other studies did standardise the pre-trial nutritional intake and exercise, although there were still limitations with the statistical analysis used (Pfitzinger & Freedson 1998; Heitkamp et al., 1991).

The significant increase in the cycling PPO-1 from test one to test two shows a systematic bias in this measure. This was likely due to motivational issues (Heitkamp et al., 1991) with the participants trying to better their performance from test one in test two. This could be reduced by blinding the subjects to their results throughout the study period but this is not normal practice in athlete support settings where feedback is required. There was also a

systematic bias for the running speed at the LTP. This could have been due to a general learning effect of the test procedure and/or running on the treadmill, therefore resulting in a greater running economy. Although all of the subjects were familiar with incremental exercise testing, the general learning effect could be reduced by incorporating a familiarisation trial within a week of test one. As there were no significant decreases in performance from test one to test two, it can be concluded that the time between tests and the conditions in the two days prior to a test were adequate to allow sufficient recovery from test one.

In a group of recreationally active males, it was reported that the running speed and the HR corresponding to the LT and 4mmol·L^{-1} blood lactate concentration are more reliable in individuals with a higher LT at the beginning of the study (Grant et al., 2002). This was demonstrated by tighter values for the LOA and higher correlation coefficients between test-retest values in the individuals with a higher initial LT. Similarly, it has been reported that the correlation coefficient between test-retest values of the speed and HR corresponding to the 4mmol·L^{-1} blood lactate concentration and the maximal running speed were higher in endurance trained women compared to untrained women (Heitkamp et al., 1991). In the present study, there were no statistically significant correlation coefficients between the individual differences from test one to test two and the individual mean value for test one and test two for any of the measures taken. This is likely due to the subjects in the present study being trained endurance athletes.

3.6 CONCLUSIONS

The LTP, along with the LT and maximal performance measures, assessed using the methods outlined in this study, are reproducible in this population of trained endurance athletes. These tests can therefore be used by trained endurance athletes as part of a physiological testing programme to assess not only endurance performance, but also to demarcate training

intensity zones for exercise intensity prescription and monitor moderate to large adaptations to training. Practitioners should take care when deciding on the duration between tests to test for adaptations from training, as adaptations need to be greater than these detected test-retest variations (TEM) to be considered physiologically meaningful.

CHAPTER FOUR

A 6-MONTH ANALYSIS OF TRAINING-INTENSITY DISTRIBUTION AND PHYSIOLOGICAL ADAPTATION IN IRONMAN TRIATHLETES

4.1 ABSTRACT

The present study analysed the training-intensity distribution and physiological adaptations over a 6-month period preceding an ironman triathlon race. Ten athletes; mean (±SD) age 43 (±3) yrs, mass 78.3 (±10.3) kg, and stature 179 (±5) cm, participated. The study consisted of three training periods (A-C), each of approximately two months' duration and four testing weeks. Testing consisted of incremental tests to exhaustion for swimming, cycling and running and assessments for anthropometry plus cardiovascular and pulmonary measures. The lactate threshold and the lactate turnpoint were used to demarcate three discipline-specific, exercise-intensity zones. The mean (±SD) percentage of time spent in zones one, two and three was 69 (±9), 25 (±8) and 6 (±2) % for periods A-C, combined. Only modest physiological adaptation occurred throughout the 6-month period, with small to moderate effect sizes at best (0.02 – 0.76). Relationships between the training volume/training load and the training-intensity distribution with the changes in key measures of adaptation were weak and probably reflect differences in initial training status. Our data suggest that the effects of intensity distribution are small over short-term training periods and future experimental research is needed to clarify potential impact of intensity distribution on physiological adaptation.

4.2 INTRODUCTION

Zones of exercise-intensity can be established from blood lactate concentrations at increasing exercise intensities (Kindermann et al., 1979). Intensities lower than the lactate threshold can be categorised as zone one, between the lactate threshold and the lactate turnpoint as zone two and greater than the lactate turnpoint as zone three (Skinner & McLellan 1980). Blood lactate concentration remains at or close to resting concentrations in zone one, is raised but production and removal rates re-establish equilibrium in zone two and production exceeds maximum clearance rates in zone three (Seiler & Kjerland 2006). The reproducibility of the lactate threshold, the lactate turnpoint and a maximal performance measure in swimming, cycling and running were established in chapter 3. Therefore, these thresholds and the maximal performance measure can now be used to demarcate training zones and monitor adaptations to training. Chapter 3 reveals the day-to-day variation in these measures, and therefore, only improvements greater than these day-to-day variations can be considered physiologically meaningful. The heart rate associated with specific physiological thresholds has been used by elite and sub-elite athletes to evaluate the exercise-intensity distribution during training sessions (Seiler & Kjerland 2006; Esteve-Lanao et al., 2005; Schumacher & Mueller 2002). However, training time in each of these three exercise-intensity zones for optimal physiological adaptation and avoidance of excessive stress leading to overtraining is yet to be established.

Few studies on elite-standard athletes have assessed the time spent in each exercise-intensity zone in the build up to competition (Seiler & Kjerland 2006; Schumacher & Mueller 2002; Lucia et al., 2000a; Lucia et al., 2000b). These studies have used ventilatory thresholds to demarcate three intensity zones and used the total time-in-zone approach (Seiler & Kjerland 2006) to assess the time spent in each zone. These studies reported that training time spent

in zone one was >80%, in zone two was <15% and in zone three was ≤5% of the total training time. In contrast, Esteve-Lanao and colleagues (Esteve-Lanao et al., 2005) reported that in a group of well trained, sub-elite runners, less time was spent in zone one (71%) and more time was spent in zones two (21%) and three (8%). These authors (Esteve-Lanao et al., 2005) reported a negative correlation between time spent in zone one and performance time during a cross country race (10.1 km). Thus, data to date suggest that more time spent training in zone one is beneficial for physiological adaptation and subsequent exercise performance.

Studies with elite-standard athletes (Ingham et al., 2008) and sub-elite athletes (Esteve-Lanao et al., 2007) that have examined training-intensity distribution agree that a greater percentage of training time spent in zone one is beneficial to performance and/or physiological adaptation. These studies found that with no difference in training load, a group focusing on training in zone one (≥80%) had a greater improvement in performance(Esteve-Lanao et al., 2007) and gained greater physiological adaptation (Ingham et al., 2008) than a group who spent less training time in that zone (~70%), suggesting that more training in zones two and three (~30%) is not necessarily beneficial.

Hence, there is evidence that both in elite-standard and sub-elite athletes, the greatest performance and physiological gains are achieved when training in zone one accounts for in excess of 80% of time and training in zones two and three combined accounts for less than 20%. No observations of training-intensity distribution have been made on multisport athletes in preparation for competition. Therefore, the purpose of the present study was to analyse training-intensity distribution in a group of sub-elite triathletes in three separate periods during 6-months of training preceding an ironman triathlon and to quantify effects on physiological adaptation in the three disciplines using standard incremental tests.

4.3 METHODS

4.3.1 Participants

Ten healthy participants (one female) volunteered and provided written, informed consent to take part in the study, which was approved by the local Ethics Committee, in accordance to the Declaration of Helsinki. All participants were members of the same triathlon club. The mean (±SD) characteristics of the participants at the beginning of the testing period were: age 43 (±3) yrs, mass 78.3 (±10.3) kg and stature 179 (±5) cm. Seven of the athletes had previously competed in an ironman triathlon event. All athletes had been involved in endurance training for over five years.

4.3.2 Experimental Approach to the Problem

Incremental tests to volitional exhaustion in swimming, cycling and running were used to establish three distinct, heart-rate-defined intensity zones associated with two simple reproducible values reflecting the lactate threshold and the lactate turnpoint; zone one (<lactate threshold), zone two (>lactate threshold, <lactate turnpoint) and zone three (> lactate turnpoint). The participants carried out their own training and reported all training involving swimming, cycling and running using the 'total time-in-zone approach' (Seiler & Kjerland 2006), into an online training log (www.workoutlog.com). A modified approach to the training impulse (TRIMP; (Foster et al., 2001a) was used to assess total training load. The study consisted of three training periods, Jan-Feb (A), Mar-Apr (B) and May-June (C), with the mean (±SD) duration of each period being 6.9 (±0.8), 7.6 (±0.8) and 6.7 (±0.6) weeks, respectively. There were four testing weeks (baseline, 2-months, 4-months and 6-months; Fig. 4.1) in which swimming, cycling, and running incremental tests were conducted, at least two days apart and at the same time of day. Lactate threshold and lactate turnpoint were re-established in each testing week, and the heart rate values corresponding to the updated

lactate threshold and lactate turnpoint were used to track time spent in each training zone for the forthcoming training period.

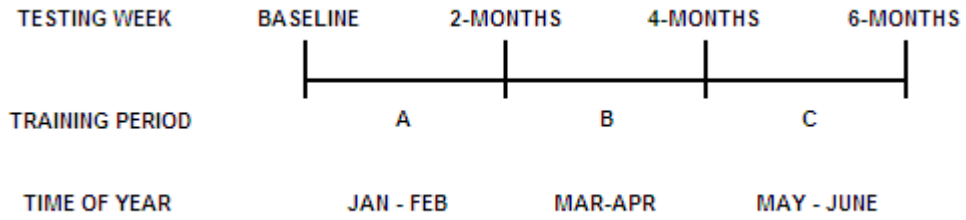

Fig. 4.1 Outline of the study period

4.3.3 Procedures

4.3.3.1 Habituation

Habituation trials took place for all testing procedures approximately two months before the study period. Training was recorded in the training logs in the period between the habituation trials and the study period to enable participants to understand the detail required in the online training log.

4.3.3.2 Swimming Test

The swimming test was a modified version of the protocol used by Pyne and colleagues (Pyne et al., 2001) and took place in a 25 m pool, with participants wearing their own wetsuit to replicate the ironman triathlon swim. An incremental test took place with seven 150-m stages, each 5 s quicker than the previous, with a 3-min rest period between each stage. The 4th stage had the same pace as the athletes' 400-m time trial time, which was assessed approximately two weeks before the start of the study. The final stage was a maximal 'all-out' effort to assess the best 150-m swim time. Stroke counts were counted for the third and sixth 25 m of each stage and the difference between stroke counts in the seven stages was used as the 'deviation in stroke counts'.

4.3.3.3 Cycling Test

The protocol for the cycling test was modified from a test previously described by Farina and colleagues (Farina et al., 2004). Participants brought their own pedals and shoes for the test. Handlebar position and saddle height of the ergometer were individualised for each participant and retained for subsequent tests. A 5-min warm-up was completed at an external intensity of 70 W at a self-selected cadence on a Lode cycle ergometer (Excalibur Sport, Netherlands). This was followed immediately by an incremental test to volitional exhaustion, starting at an intensity of 70 W and increasing by 35 W every 3 min (25 W for female) with the cadence remaining self-selected. Immediately after this test, the participants rested for 10 min before completing a shorter high-intensity exercise test. This test consisted of 1-min stages, starting at the intensity of the penultimate stage of the previous test, and increased by 35 W each stage (25 W for female). The test continued until volitional exhaustion. The end time was used to calculate peak power output (PPO) (Kuipers et al., 1985):

$$PPO = W_{final} + ([t/60] * PI)$$

Where W_{final} = The power output of the final *completed* stage, t = the time achieved in the final *non-completed* stage and *PI* = the power increment.

The PPO was also normalised to body mass using allometric scaling (PPO/BM^{b}), where BM is body mass, and b is a power exponent (Nevill et al., 1992). Allometric scaling allows for meaningful comparisons of PPO, as the effect of body mass is properly eliminated. The PPO and body mass from the baseline tests were plotted on a log-log scale and the power exponent was derived from the slope of the linear regression line (0.79).

4.3.3.4 Running Test

All participants were accustomed to treadmill running. Participants completed a standardised warm-up for 5 min at 8 $km \cdot hr^{-1}$ on a motorised treadmill (Powerjog, Cranlea and Co), set at a

1% incline throughout the test to simulate the energetic cost of outdoor running (Jones& Doust 1996). This was followed by an incremental exercise test to volitional exhaustion, starting at a speed of 9 $km \cdot hr^{-1}$ and increasing by 1 $km \cdot hr^{-1}$ every 3 min (Billat et al., 2003). Thirty seconds before the end of each stage, participants supported their weight with their hands and moved their feet to the sides of the treadmill belt to allow blood sampling. As in the cycling test, the participants rested for 10 min before completing a shorter high intensity exercise test. The test consisted of 30 s stages, starting at the intensity of the penultimate stage of the previous test, and increasing by 0.5 $km \cdot hr^{-1}$ each stage. The test continued until volitional exhaustion. The end time was used to calculate the maximal running speed ($Speed_{max}$) (Kuipers et al., 1985):

$$Speed_{max} = Speed_{final} + (t/30)*0.5$$

Where $Speed_{final}$ = The running speed of the final *completed* stage, t = the time achieved in the final *non-completed* stage.

During each stage in the incremental tests for the three disciplines, heart rate (Polar Sports Tester, Polar Electro, Kemplete, Finland) was recorded. At the end of each stage, a 5-µL capillary blood sample was obtained; from the fingertip in the cycling and the running tests and; the earlobe in the swimming test. The sample was analysed for lactate concentration by micro-assay (LactatePro LT-1710, ArkRay Inc, Kyoto, Japan). The reliability and validity of this device has been previously determined (Pyne et al., 2000).

The lactate threshold was determined as the final point before the blood lactate concentration increased distinctly from its resting concentration (Aunola & Rusko 1984). The lactate turnpoint was determined as the starting point of accelerated lactate accumulation (around 3 to 6 $mmol \cdot L^{-1}$), depending on the individual blood lactate profile (Aunola & Rusko 1984).

4.3.4 Reproducibility of the Measures

The typical error of measurement for the measures recorded in this study have been assessed in our laboratory (Chapter three) and are as follows: first, the swim speed corresponding to the lactate threshold, lactate turnpoint and best 150 m swim time were 2.9, 2.5 and 1.1%, respectively, second; the cycling power output corresponding to the lactate threshold, lactate turnpoint and the PPO were 3.8, 3.3 and 2.3%, respectively, and third; the run speed corresponding to the lactate threshold, lactate turnpoint and the $Speed_{max}$ were 3.1, 2.6 and 1.1%, respectively.

4.3.5 Anthropometry, Cardiovascular and Pulmonary Measures

Anthropometry was undertaken before either the cycling or the running test, and this remained consistent for subsequent tests. On arrival at the laboratories, participants' body mass (Balance beam scales, John White and Son, Scotland) and stature (The Leicester Height Measure, Seca, UK) (baseline test only) were recorded. Participants were then instructed to lie down and Bio-Impedance Analysis (Bodystat 1500, Bodystat Ltd, Isle of Man, UK) was performed. Participants remained lying down and breathing was controlled with a metronome (10 $breaths.min^{-1}$) for the assessment of resting heart rate and heart rate variability over a 10 minute period (Polar Electro, Kemplete, Finland), followed by a peak flow test (Mini-Wright White (standard range), Clement Clarke International, Essex, UK). A morning 12 hour fasted blood sample was taken from an antecubital vein for the assessment of haematocrit, using the micro haematocrit method and immune function markers (reported elsewhere (Cosgrove et al., 2011).

Testing was performed at the same time of day (±2 hrs) to minimise the effect of diurnal biological variation (Atkinson & Reilly 1996). The laboratory ambient temperature was controlled at 20°C and relative humidity ranged between 30 and 50%. Participants drank *ad*

libitum throughout each test. Each athlete completed a training and food diary in the two days before the initial testing week and replicated the training and diet prior to subsequent testing sessions. Participants were instructed to exercise for no more than 60 min in zone one in each of the two days before the testing sessions.

4.3.6 Statistical Analyses

A fully repeated-measures ANOVA compared the training volume/training load in each discipline/training zone across training periods. Main effects of training period (A, B, C), training discipline (swim, bike, run) and training zone (zone one, zone two, zone three) and any interaction between these with the training volume/load were reported. One-way repeated-measures ANOVA compared physiological measures across training periods. Post hoc analysis was undertaken where significance was obtained with Paired Student's t-tests using two-tailed values of *P*, with the Bonferroni method of adjustment to prevent type I error. Pearson product-moment correlation coefficients were calculated to determine associations between; training volume and time spent in each training zone, and the percentage change in physiological adaptations, both overall and for each discipline and between the between the magnitude of the key responses at baseline and the magnitude of the change in the response from baseline to 6-months. Statistical significance was accepted at $P<0.05$. All data are expressed as Mean (±SD). Effect sizes for the key responses to the incremental tests were calculated from the mean difference (baseline to 6-months) over the standard deviation of the baseline measure. These values were judged using the descriptors suggested by Cohen (Cohen 1988). Effect sizes were included to highlight the size of the training adaptations, as the P value alone does not necessarily provide this information.

4.4 RESULTS

4.4.1 Training Volume and Training-Intensity Distribution

The mean (±SD) volume of training for periods A, B and C, combined was 203 (±71), with a range of 92-266 hrs. There were main effects of training period for the mean training volume and the mean training load per week ($P<0.05$) with both greater in period B than in period A ($P<0.05$; Fig. 4.2A and 4.3A). There was also an interaction ($P<0.05$) for both mean training volume and mean training load per week by discipline, with changes over training period only for cycling. The mean cycling volume per week and the mean cycling load per week were both greater in period B than period A ($P<0.05$; Fig. 4.2B and Fig. 4.3B).

There was a main effect of training zone for the mean training volume per week ($P<0.05$), with post hoc analysis revealing that the mean time spent in zone one was greater in period B than in period A ($P<0.05$). There was an interaction ($P<0.05$) for mean training volume per week by training zone, with changes in training period observed only for zone one (Fig. 4.4). Along with the absolute training time spent in each zone, it is also important to consider the percentage of time spent in each zone. Percentage time spent in zones one, two and three across all training periods was 69 (±9), 25 (±8) and 6 (±2) %, respectively. The only difference in the percentage of time spent training in each zone for the three disciplines combined was time spent in zone two, which was greater in period A than period C ($P<0.05$; Table 4.1).

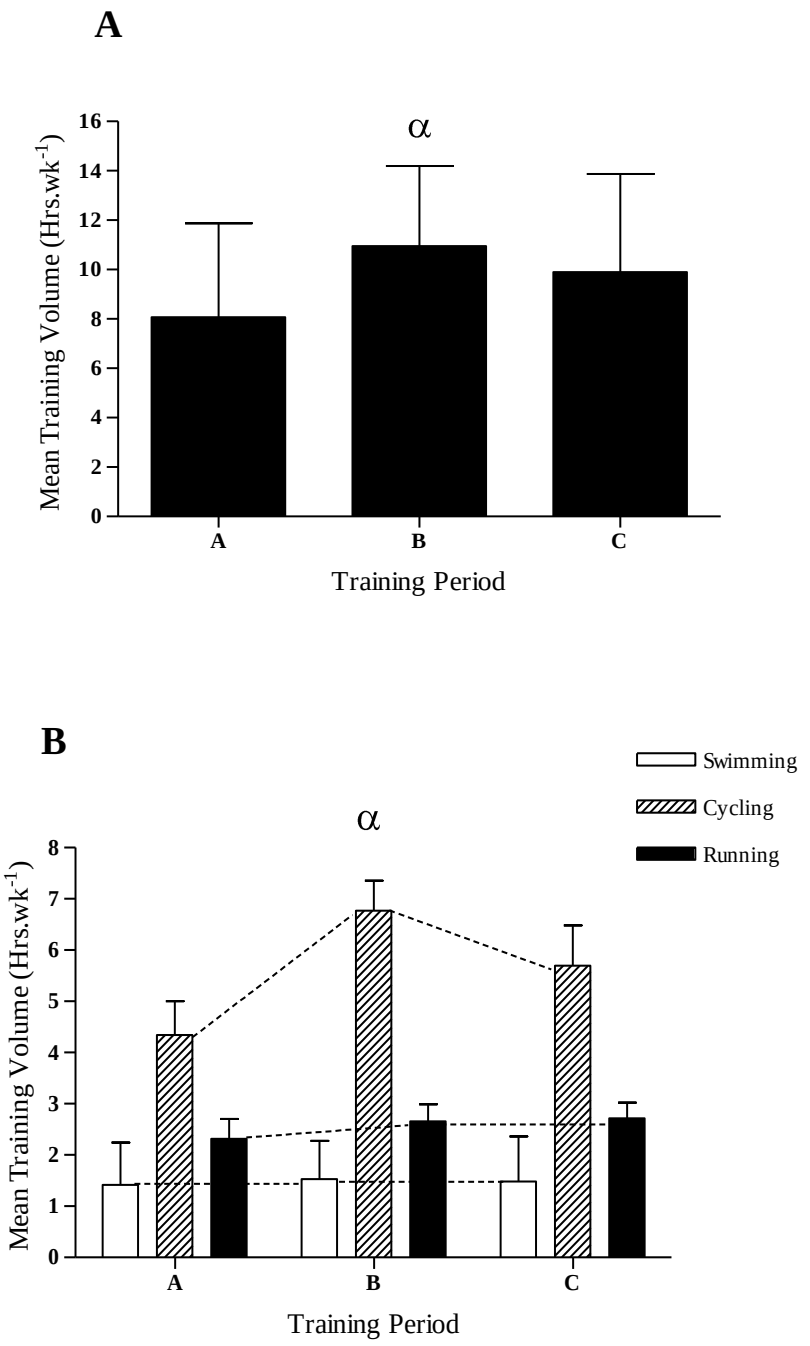

Fig. 4.2 A. Mean (±SD) training volume per week. **B.** Mean (±SD) training volume for each discipline per week. α = different from period A ($P<0.05$).

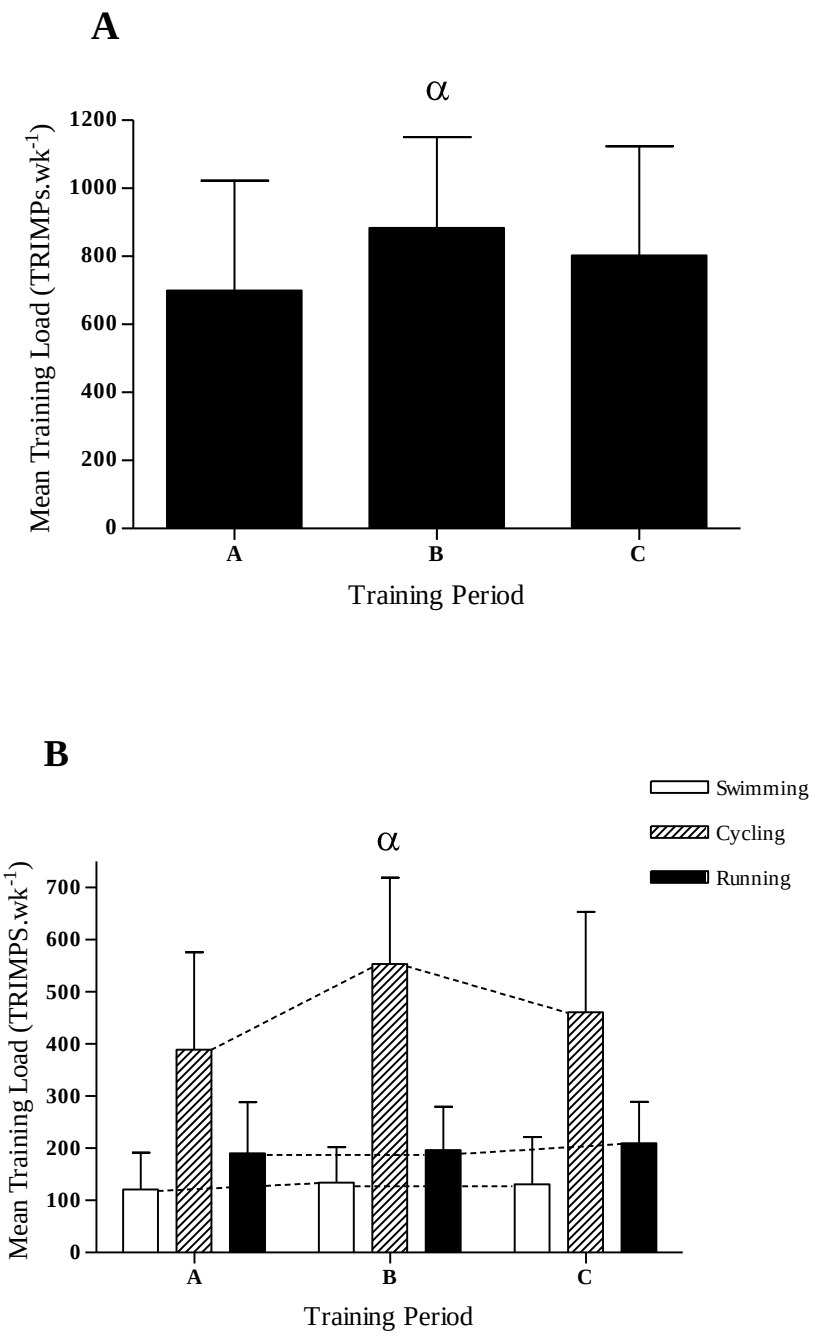

Fig. 4.3 A. Mean (±SD) training load per week. **B.** Mean (±SD) training load for each discipline per week. α = different from period A (P<0.05).

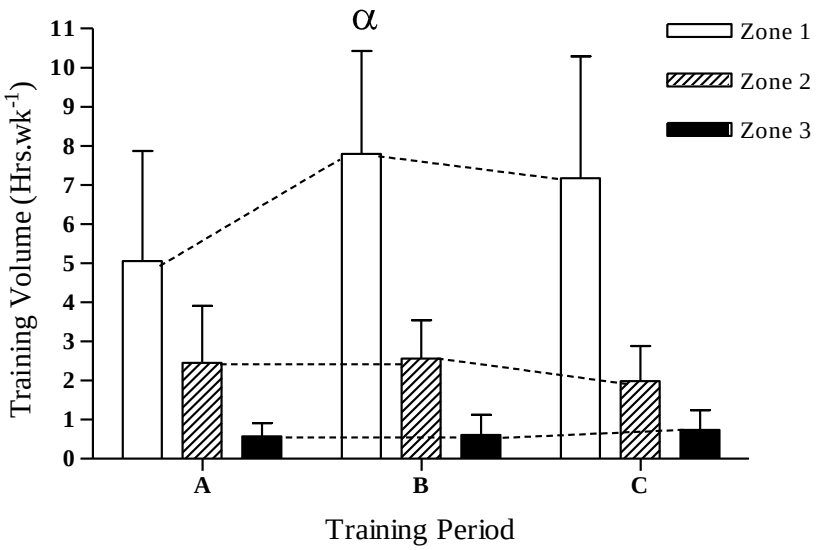

Fig. 4.4 Mean (±SD) time spent in each zone per week. α = different from period A (P<0.05).

Table 4.1 Mean (±SD) percentage of time spent in each zone during periods A-C.

	Training Period	Zone 1	Zone 2	Zone 3
Overall	A	62 ± 13	31 ± 12	7 ± 3
	B	71 ± 7	24 ± 8	5 ± 3
	C	72 ± 9	21 ± 7 **α**	7 ±4
Swimming	A	66 ± 24	26 ± 19	9 ± 11
	B	64 ± 10	27 ± 9	9 ± 6
	C	69 ± 20	24 ± 16	8 ± 11
Cycling	A	58 ± 15	34 ± 14	8 ± 5
	B	69 ± 13	26 ± 12	5 ± 4
	C	71 ± 15 **α**	22 ± 10 **α**	8 ± 6
Running	A	67 ± 22	28 ± 20	5 ± 4
	B	80 ± 12	16 ± 9	4 ± 5
	C	76 ± 14	17 ± 11	6 ± 6

α = different from period A ($P<0.05$)

4.4.2 Incremental Test Responses

There was a main effect of training period for the cycling PPO normalised to body mass using allometric scaling ($P<0.05$). Post hoc analysis revealed that this increase occurred only between the baseline and 6-month time points ($P<0.05$; Table. 4.3). There were main effects of training period for both the running speed at lactate threshold and the running speed at lactate turnpoint ($P<0.05$). The running speed at lactate threshold improved from baseline to 2 months and 6 months, and the running speed at the lactate turnpoint improved from baseline to 6 months ($P<0.05$, Table. 4.4). In both swimming and cycling, there were main effects of training period for the maximum heart rate ($P<0.05$). For both disciplines, the maximum heart rate was lower in the 4-months test than the baseline and 2-months tests ($P<0.05$), but was higher in the 6-months test than the 4-months test ($P<0.05$). The effect sizes for the key changes in the swimming, cycling and running incremental tests to exhaustion from baseline to 6 months were all trivial or small, except the running speed at lactate threshold, which was moderate (Table 4.5).

4.4.3 Anthropometry, Cardiovascular and Pulmonary Measures

There was a main effect of training period for body mass ($P<0.05$), which was less at 6 months (76.8 ± 10.0) than at baseline (78.3 ± 10.3) and 2 months (77.9 ± 9.7; $P<0.05$). The difference in body fat percentage between baseline (20.1 ± 2.5) and 6 months (17.7 ± 3.5) was large, with an effect size of 1.0, despite the lack of a main effect of training period. There was a main effect of training period for peak flow, which was higher at 6 months (621 ± 98) than at 2 months (612 ± 105) and 4 months (598 ± 106; $P<0.05$). There were no main effects of training period for resting heart rate (50 ± 6) to (50 ± 7) bpm, haematocrit (44.5 ± 2.0) to (44.6 ± 2.3) % or heart rate variability: stda (174 ± 66) to (164 ± 78)ms, and stdb: (129 ± 53) to (96 ± 63)ms.

4.4.4 Relationships

All of the significant relationships between training variables and percentage change in physiological adaptation were negative. The overall training volume (periods A-C, combined) was negatively correlated to the percentage change in swimming lactate threshold and maximum running speed ($r = -0.63$ and $r = -0.69$, respectively, $P<0.05$). Similarly, overall training volume in zone three (periods A-C, combined) was negatively correlated to the percentage change in maximum running speed ($r = -0.88$, $P<0.05$). The time spent swimming in zone two was negatively correlated to the percentage change in swimming lactate threshold ($r = -0.66$, $P<0.05$) and the time spent cycling in zone three was negatively correlated to the percentage change in cycling lactate threshold ($r = -0.79$, $P<0.05$). The percentage change in maximum running speed was negatively correlated to both the overall running volume (periods A-C, combined) ($r = -0.69$, $P<0.05$) and the running time in zone one ($r = -0.79$, $P<0.05$). Finally, the running time in zone three was negatively correlated to the percentage change in running lactate turnpoint ($r = -0.80$, $P<0.05$).

The relationship between the magnitude of the key responses at baseline and the magnitude of the change in the response from baseline to 6-months is shown in Table 4.6. There were significant relationships for the lactate turnpoint in all of the disciplines and the fastest 150m swim. The lactate threshold in swimming, the peak power output in cycling and the maximal running speed were all close to significance.

Table 4.2 Mean (±SD) responses to the swimming incremental test to exhaustion. See Fig. 4.1 for details about the timing of the testing weeks for training periods A, B and C.

	Baseline	2-months	4-months	6-months	% Δ Baseline to 6-months
150-m time at LT (s)	142 ± 16	141 ± 14	142 ± 14	141 ± 16	0.7
150-m time at LTP (s)	130 ± 18	130 ± 15	130 ± 15	127 ± 14	2.3
Best 150-m Time (s)	125 ± 18	122 ± 14	120 ± 14	121 ± 13	3.2
Max HR (bpm)	167 ± 8	164 ± 6	160 ± 8 ***α**	164 ± 9 **β**	1.8
Deviation in SC (3^{rd} 25m)	4 ± 2	3 ± 1	3 ± 2	2 ± 1	50.0
Deviation in SC (6^{th} 25m)	4 ± 3	3 ± 1	3 ± 2	3 ± 2	25.0

LT = Lactate Threshold, **LTP** = Lactate Turn Point, **HR** = Heart Rate, **RPE** = Rating of Perceived Exertion, **SC** = Stroke Count. *****different from baseline, **α** different from 2-months, **β** different from 4-months ($P<0.05$).

Table 4.3 Mean (±SD) responses to the cycling incremental test to exhaustion. See Fig. 4.1 for details about the timing of the testing weeks for training periods A, B and C.

	Baseline	2-months	4-months	6-months	% Δ Baseline to 6-months
Power at LT (W)	209 ± 33	213 ± 35	212 ± 29	216 ± 32	3.3
Power at LTP (W)	263 ± 34	263 ± 35	267 ± 29	263 ± 33	0.0
PPO (W)	373 ± 54	369 ± 52	372 ± 43	376 ± 47	0.8
$PPO/BM^{0.79}$	3.43 ± 0.25	3.42 ± 0.24	3.46 ± 0.21	3.52 ± 0.23*****	2.6
Max HR (bpm)	175 ± 10	173 ± 10	169 ± 12 *****	174 ± 11 **β**	0.6

LT = Lactate Threshold, **LTP** = Lactate Turn Point, **HR** = Heart Rate**, RPE** = Rating of Perceived Exertion, **PPO** = Peak Power Output, **BM** = Body Mass, **ln** = Natural Logarithm. *****different from baseline, **α** different from 2-months, **β** different from 4-months (P<0.05).

Table 4.4 Mean (±SD) responses to the running incremental test to exhaustion. See Fig. 4.1 for details about the timing of the testing weeks for training periods A, B and C.

	Baseline	2-months	4-months	6-months	% Δ Baseline to 6-months
Speed at LT ($km \cdot hr^{-1}$)	12.9 ± 1.2	13.6 ± 1.6*****	13.4 ± 1.5	13.9 ± 1.6*****	7.8
Speed at LTP ($km \cdot hr^{-1}$)	15.3 ± 1.4	15.3 ± 1.5	15.6 ± 1.2	15.9 ± 1.3*****	3.9
$Speed_{max}$ ($km \cdot hr^{-1}$)	17.9 ± 1.7	18.0 ± 1.6	17.9 ± 1.5	18.2 ± 1.5	1.7
Max HR (bpm)	179 ± 12	179 ± 8	177 ± 10	179 ± 9	0.0

LT = Lactate Threshold, **LTP** = Lactate Turn Point, **HR** = Heart Rate, **RPE** = Rating of Perceived Exertion. *****different from baseline, **α** different from 2-months, **β** different from 4-months (P<0.05).

Table. 4.5 Effect sizes for the key responses to the swimming, cycling and running incremental tests to exhaustion from baseline to 6 months.

Discipline	Measure	Effect Size	Descriptor*
Swimming	150-m time at LT (s)	0.06	Trivial
	150-m time at LTP (s)	0.21	Small
	Best 150-m Time (s)	0.24	Small
Cycling	Power at LT (W)	0.22	Trivial
	Power at LTP (W)	0.02	Trivial
	PPO (W)	0.06	Trivial
	$PPO/BM^{0.79}$	0.34	Small
Running	Speed at LT ($km \cdot hr^{-1}$)	0.76	Moderate
	Speed at LTP ($km \cdot hr^{-1}$)	0.42	Small
	Maximal Speed ($km \cdot hr^{1}$)	0.13	Trivial

LT = Lactate Threshold, **LTP** = Lactate Turn Point, **PPO** = Peak Power Output, **BM** = Body Mass**, ln** = Natural Logarithm, * Cohen (1988)

Table. 4.6 The relationship between the magnitude of the key responses at baseline and the magnitude of the change in the response from baseline to 6-months.

Discipline	Measure	R	p-value
Swimming	150-m time at LT (s)	-0.87	0.07
	150-m time at LTP (s)	-0.60	<0.05
	Best 150-m Time (s)	-0.77	<0.05
Cycling	Power at LT (W)	-0.60	0.38
	Power at LTP (W)	-0.31	<0.05
	PPO (W)	-0.80	0.06
	$PPO/BM^{0.79}$	-0.36	0.31
Running	Speed at LT ($km \cdot hr^{-1}$)	-0.59	0.90
	Speed at LTP ($km \cdot hr^{-1}$)	0.04	<0.05
	Maximal Speed ($km \cdot hr^{1}$)	-0.78	0.07

LT = Lactate Threshold, **LTP** = Lactate Turn Point, **PPO** = Peak Power Output, **BM** = Body Mass**,** **ln** = Natural Logarithm, **r** = Pearson's correlation coefficient.

4.5 DISCUSSION

Over three 2-month periods preceding an ironman triathlon, with a mean training volume in periods A, B and C of 8.1, 11.0 and 9.9 hrs·wk^{-1}, the percentage of training time spent in zones one, two and three was 62, 31 and 7% in period A, 71, 24 and 5% in period B and 72, 21 and 7% in period C. However, only modest physiological adaptations occurred during the entire training period, as shown by mostly trivial and small effect sizes. The statistically significant relationships between the training variables and the changes in the physiological adaptations were negative. This is probably because the participants completing the highest training volumes were already more well trained, and thus had less room for improvement in the key markers for adaptation (Wenger & Bell 1986).

Previous research has shown that it is beneficial both for elite and sub-elite endurance athletes to spend ≥80% of training time in zone one. This has been shown from descriptive studies of athletes in the lead up to competition (Seiler & Kjerland 2006; Schumacher & Mueller 2002; Lucia et al., 2000a; Lucia et al., 2000b), and in studies that have assessed differences arising from an emphasis of training in zone one rather than in zones two and three (Ingham et al., 2008; Esteve-Lanao et al., 2007). For all of the disciplines combined in the present study, mean percentage time in zone one was never ≥80%, with 62, 71 and 72% of training in zone one in periods A, B and C, respectively. Similarly, for the individual disciplines, the mean percentage of time spent in zone one ≥80% was only once for any of the three periods (80%; running in training period B). It has been suggested that less experienced athletes train too hard during low-intensity sessions and not hard enough during high-intensity sessions (Foster et al., 2001b), thus leading to a high percentage of training time in zone two. Therefore, the lack of time in zone one, and thus relatively more time in zone two, could explain the modest adaptations in the incremental test responses.

Seiler and colleagues (Seiler et al., 2007) demonstrated a delayed recovery of the autonomic nervous system immediately after training in zones two and three, compared with training in zone one. The authors suggested that the first ventilatory threshold demarcates a clear threshold for autonomic recovery. The authors further proposed that training in zone one probably induces a targeted increase or maintained stimulus for adaptation without inducing a meaningful systemic stress response. Training in zone two leads to delays in recovery, yet provides less stimulus for adaptation than training in zone three (Seiler et al., 2007). Other authors agree that zone two provides a suboptimal stimulus for eliciting further gains in endurance (Londeree 1997). Esteve-Lanao and colleagues (Esteve-Lanao et al., 2007) suggested that when training time in zone two exceeds a certain threshold (>20% of training time) at the expense of zone one training time, endurance is impaired via a down regulation of the sympathetic nervous system. In the present study, percentage time spent in zone two for all of the disciplines combined was always above 20%, i.e. 31, 24 and 21% in training periods A, B and C, respectively. Training time in zone two is comparable with the group in the study by Esteve-Lanao and colleagues (Esteve-Lanao et al., 2007), whose performance improvement was less than that of a group who spent less training time in zone two (12%).

The largest adaptations gained during the study period, determined by effect sizes were for the running lactate threshold and the running lactate turnpoint. The positive effect of zone one training has been reported (Ingham et al., 2008; Esteve-Lanao et al., 2007), and therefore these adaptations could have occurred because there was a greater percentage of training time spent in zone one for running (74%) than swimming (66%) and cycling (66%). Negative effects of excess training in zone two have been demonstrated (Esteve-Lanao et al., 2007; Londeree 1997). Adaptations in the running lactate threshold and lactate turnpoint could be attributable to a lower percentage of training time spent in zone two for running (20%) than

swimming (25%) and cycling (27%), despite these relationships not being significant. The lack of adaptation in swimming and cycling was possibly because of a small percentage of training time in zone one and a large percentage of training time in zone two. Although the relationships between the training variables and the change in the running lactate threshold and lactate turnpoint were not significant in this small group of athletes, we feel this is still a worthwhile observation.

After the increase in training volume and training load from period A to period B, the maximum heart rate in swimming and cycling was lower in the 4-months test than the baseline and 2-month tests but it increased in the 6-month test. The decreased rate in the 4-months tests could be attributable to a down regulation of the sympathetic nervous system, as previously suggested by Esteve-Lanao and colleagues (Esteve-Lanao et al., 2007). Overreaching can cause a decrease in maximum heart rate without a change in heart rate variability (Hedelin et al., 2000), as in the present study. However, formal identification of overreaching also requires a decrease in performance (Halson & Jeukendrup 2004), which was not assessed in the present study. However, it is a possibility that some of the athletes were fatigued/overreaching during period B, before recovering during period C. This is normal for endurance athletes, with performance being intentionally depressed during overreaching phases to allow for a supercompensation effect prior to key competitions (Fiskerstrand & Seiler 2004).

It is clear from the magnitude of the effect sizes that adaptations here in athletes preparing for an ironman triathlon are small. It is possible that the incremental tests conducted were not sensitive enough to detect adaptations that had occurred. In fact, these changes are in line with those observed for the day-to-day variability in chapter three, which is further suggestion for a lack of physiological adaptation in these athletes. Indeed, this has important implications

for monitoring training adaptation in athletes using these methods. Although speculative, while the lactate threshold/lactate turnpoint did not improve markedly, the percentage of the lactate threshold/lactate turnpoint at which the participants were able to race in the ironman triathlon could have been increasing throughout the study. Therefore, it is possible that performance measures such as longer time trials for each of the disciplines would have detected greater improvements. It should be noted, that we acknowledge the potential effect on the results of including mixed sex participants (Oosthuyse & Bosch, 2010). On analysis of the results, including the data from the female participant did not alter the findings of this study, and so these results were included.

4.6 CONCLUSIONS

To the best of the author's knowledge, this is the first study to have assessed training-intensity distribution in a group of multisport athletes training for an ironman triathlon. This follows on from studies of cyclists, runners, rowers and cross country skiers. Given the number of variables associated with assessing the training-intensity distribution in multisport athletes, it is not easy to draw conclusions as to the effectiveness of the training in the different disciplines on the key measures of adaptation in the different disciplines. The present study highlights the need for future research to focus on experimental manipulation of training-intensity distribution and thus improve our understanding of its impact on the training-induced adaptations in endurance athletes.

CHAPTER FIVE

THE IMPACT OF MANIPULATING TRAINING-INTENSITY DISTRIBUTION IN TRAINED CYCLISTS

5.1 ABSTRACT

Purpose: To compare the impact of a polarised training model and a threshold training model on performance and physiological adaptation in trained cyclists. **Methods:** Twelve male trained cyclists; mean (±SD) age 37 (±6) yrs, mass 76.8 (±6.6) kg, and stature 178 (±6) cm, took part in the study. The study consisted of two 6-week training interventions and used a cross-over, within-subject study design. A polarised training model (P) consisting of 80 (±4) % of training time in zone one (<lactate threshold (LT)) and 20 (±4) % in zone three (>lactate turn point (LTP)) and a threshold training model (T) consisting of 57 (±10) % of training time in zone one and 43 (±10) % in zone two (half-way between the LT and the LTP) were prescribed. A training wash out period of 4-weeks was used between each training intervention, including low intensity training of reduced volume. Performance was measured with a 40km time trial, peak power output (PPO), and time to exhaustion at 95% pre-training PPO. Physiological adaptation was measured using the LT, LTP, maximal activities of skeletal muscle oxidative enzymes and heart rate responses to the performance tests. **Results:** T had a higher weekly training volume (22%) and training load (23%) than P. The time to exhaustion at 95% of pre-training PPO improved in both P (84.9 (±44.1) %; $P<0.05$) and T (37.4 (±47.3) %; $P<0.05$) with the percentage change greater in P than T ($P<0.05$). The PPO was improved following P ($P<0.05$) but not T and the percentage change in PPO was greater in P (7.7 (±5.1) %) than T (2.7 (±4.5) %; $P<0.05$). 40km time trial mean power improved in both P (8.1 (±7.7) %) and T (4.2 (±5.7) %, $P<0.05$) with no difference in the percentage improvement between the groups. There was no difference in the maximal activity of citrate synthase from pre to post training in either P (47 (±6) to 48 (±4)) mmol·kgwet wt^{-1}·min^{-1} or T (47 (±5) to 49 (±3)) mmol·kgwet wt^{-1}·min^{-1}. Likewise, there was no difference from pre to post training in the maximal activity of beta-hydroxylacyl-CoA dehydrogenase from pre to post training in either P (15 (±2) to 15 (±2)) mmol·kgwetwt^{-1}·min^{-1} or T (15 (±2) to 15 (±1)) mmol·kgwetwt^{-1}·min^{-1}. **Conclusion:** A polarised

training model is recommended for trained cyclists wishing to improve performance and physiological adaptation over a short-term training period.

Key Words:
Endurance Training, Training Zones, Lactate Threshold, Oxidative Enzymes, Physiological Adaptation, Endurance Performance

5.2 INTRODUCTION

In chapter four, it was concluded that an accurate assessment of the effectiveness of training-intensity distribution in multisport athletes is difficult due to the number of variables involved. It was also reported in chapter four that there were only small physiological adaptations in a group of ironman triathletes over 6-months of training. It is possible, based on the findings of previous research (Esteve-Lanao et al, 2005; Esteve-Lanao et al, 2007; Ingham et al, 2008) that the small physiological adaptations were due to the athletes spending too much time in zone two at the expense of training time in zone one. This suggestion is supported in chapter four, with the finding that the largest adaptations were found in running, the discipline in which the athletes spent the greatest proportion of time in zone one and the lowest proportion of time in zone two. Therefore, in order to assess the effectiveness of training-intensity distribution with more accuracy in this chapter, we used one discipline and fixed the proportion of time spent in each training-intensity zone. Conclusions can then be made as to the effect of spending different proportions of training time in each training-intensity zone. Moreover, the reproducibility of the lactate threshold, lactate turnpoint and a maximal performance measure have been established for cycling in chapter three, so these measures can be used in the present study.

The purpose of training for an endurance athlete is to improve endurance performance and develop the components of endurance performance, whilst avoiding overtraining (Fiskerstrand & Seiler 2004). Training intensity is commonly demarcated into three distinct zones, based on two blood lactate and/or ventilatory thresholds (Skinner & McLellan 1980). Blood lactate concentration remains at or close to resting concentrations in zone one, is raised but production and removal rates re-establish equilibrium in zone two, and production exceeds maximum clearance rates in zone three (Seiler & Kjerland 2006). The percentage of time

spent training in each of these three zones is known as the training-intensity distribution. It has been suggested that two distinct training-intensity distribution models are apparent in training studies (Seiler & Kjerland 2006). A polarised training model (P) consists of a high percentage of training time at low exercise intensities (zone one; ~80%) accompanied by relatively small amounts of training time at higher exercise intensities (zones two and three, combined; ~20%). In contrast, a threshold training model (T) consists of a large proportion of training time spent in zone two.

A number of descriptive studies have assessed training-intensity distribution in elite endurance athletes from different sports, including; cross-country skiing (Sandbakk et al., 2010; Seiler & Kjerland 2006); rowing (Guellich et al., 2009); road cycling (Lucia et al., 2000a; Lucia et al., 2000b) and track cycling (Schumacher & Mueller 2002). These studies demonstrate that elite endurance athletes adopt a high training volume relative to their event, with ~80% of training time in zone one and ~20% of training time in zones two and three, combined. These studies give an indication of a successful, but not necessarily an optimal training-intensity distribution. Indeed, the positive effect of a high training volume has been reported, with a greater overall training volume being associated with better performance (Sandbakk et al., 2010; Billat et al., 2001). In addition, the positive effect of a high volume of training in zone one has been demonstrated by Esteve-Lanao and colleagues (Esteve-Lanao et al., 2005). They showed a significant relationship between performance in both long and short distance cross-country running races with time spent training in zone one, but not with time spent in zones two and three. Previous research therefore demonstrates the importance of a high total training volume, a high volume in zone one and careful management of higher intensity training (zones two and three).

Experimental studies that have manipulated the training-intensity distribution have shown that a high percentage of training time in zone one is beneficial (Ingham et al., 2008; Esteve-Lanao et al.,2007), thus supporting the descriptive data. These studies show that a high percentage of training time in zone one (≥80%) improves performance (Esteve-Lanao et al., 2007) and physiological adaptation (Ingham et al., 2008) to a greater extent than a lower percentage of time in zone one (~70%). A group of studies from South Africa (Westgarth-Taylor et al., 1997; Weston et al., 1997; Lindsay et al., 1996) found that a transition to a more polarised training approach improved performance in a group of well-trained cyclists. These athletes were training with a high training volume (~300km) and replaced ~15% of the training volume with high intensity interval training. In addition, previous studies have reported that higher performing elite runners train with more time at high intensities (zone 3) than their lower performing counterparts, with no difference in overall training volume (Billat et al., 2001) or with a lower overall training volume (Billat et al., 2003).

However, in contrast to the previously described results, some training-intensity distribution models with less time in zone one and more time in zones two and three have been reported to be more effective than training-intensity distribution models with more time in zone one (Gaskill et al., 1999; Evertsen et al., 1999). In addition, following a period of training incorporating two sessions per week at the maximal lactate steady state (MLSS) intensity (top-end of zone two), the VO_{2max}, running speed at MLSS and the duration and distance maintained at MLSS were significantly improved in well-trained runners (Billat et al., 2004). Previous studies have also reported that in moderately trained individuals, there is no difference in physiological adaptation or performance following training continuously at, or intermittently above and below the maximal lactate steady state (Philp et al., 2008), or the anaerobic threshold (Keith et al., 1992). Therefore, our understanding of an optimal

manipulation of training-intensity distribution in endurance athletes for maximal performance gains and physiological adaptation is far from complete.

Both P and T training models have been reported to be effective in trained endurance athletes, but no study has directly compared these two training models. The purpose of the present study was therefore to compare the impact of P with T, on endurance performance and physiological adaptation in trained male cyclists.

5.3. METHODS

5.3.1 Subject Information

Twelve well-trained, male cyclists were recruited from local cycling clubs and provided written, informed consent to take part in the study, which was approved by the University of Stirling, School of Sport Ethics Committee, in accordance to the Declaration of Helsinki. The mean (±SD) characteristics of the participants at the beginning of the testing period were: age 37 (±6) years, body mass 76.8 (±6.6) kg, stature 178 (±6) cm, PPO in an incremental intensity test to exhaustion with 3 min stages 359 (± 31) W.

5.3.2 Study Design

A cross-over, within-subject study design was employed. All subjects completed at least two 40km time trial (40km TT) habituation trials (CompuTrainer, RacerMate, Seattle, WA) prior to the start of the study period. Only riders who completed the 40km TT with a mean power output greater than 240W were included in the study. Subjects were pair-matched into two experimental groups (n=6), based on the mean power output of the 40km TT habituation trials.

5.3.3 Wash-Out Period

In the 4-weeks preceding each training period, subjects were instructed to not include any threshold/tempo rides, interval sessions or races, and ride only at a low intensity, whilst maintaining their normal training volume. This was to ensure that no adaptations from training above the lactate threshold (LT) were gained in the 4-weeks prior to the study period. This 4-week period was followed by one week of laboratory testing.

5.3.4 Training

For 6-weeks following the laboratory testing, subjects came into the laboratory $3d \cdot wk^{-1}$ (Mon, Wed and Fri) for training-model specific, controlled training sessions. Training intensity was

prescribed in relation to the LT and the LTP and used the session goal approach (Seiler & Kjerland 2006). When individuals exercise at an intensity relative to LT's, metabolic and cardiac stresses are similar, but can vary significantly when training at a percentage of VO_{2peak} (Baldwin et al., 2000). The aim for P was to achieve 80% of training time in zone one (284min) and 20% of training time in zone three (72min), a total of 356min·wk^{-1}, with no time in zone two. The aim for T was to achieve 60% of training time in zone one (270min) and 40% of training time in zone two (180min), a total of 450min·wk^{-1}, with no zone three time. The different total training volume results from an attempt to match the absolute time spent training in zone 1. All laboratory training was completed on a CompuTrainer, calibrated to 3.5lb following a 10-min warm up.

5.3.4.1 Polarised Training Group

P sessions consisted of 6 intervals of 4min duration with 2min rest periods. The power output of the 6 intervals was 5-10% greater than the LTP (zone 3). The average peak HR reached from each of the 6 intervals was greater than the HR corresponding to the LTP in the incremental test in all cases. Therefore, both power output and HR for the 6 intervals were in zone three, according to the session goal approach (Seiler & Kjerland 2006). During the rest periods, subjects either stopped pedalling or pedalled backwards, and this remained consistent for every training session. The minimum HR reached during the 2min recovery period was recorded. A 10-point rating of perceived exertion (RPE) developed by Foster and colleagues (Foster et al., 1996; Foster 1998) was taken within 15min of finishing each training session. If the RPE, along with the mean peak HR *and* the mean minimum HR were decreasing over two consecutive training sessions, the power output for the intervals was increased by 5-10W.

5.3.4.2 Threshold Training Group

T sessions included 60min at a power output set half-way between the LT and the LTP (zone two). The average HR for the 60min training session was recorded and the RPE was taken

within 15min of finishing each training session. If the RPE *and* the average HR were decreasing over two consecutive training sessions, the power output was increased by 5-10W.

5.3.4.3 Zone One Training

The zone one training for both groups was made up of the warm-up and cool-down for the laboratory training sessions (15-20min·session^{-1}) and low intensity cycling on the days between the interval sessions. The intensity of the zone one training was controlled with HR, and the average HR for a zone one session did not exceed the value associated with the LT, as assessed in the incremental test to exhaustion, as suggested by Seiler &Kjerland (Seiler &Kjerland 2006) when using the session goal approach. Subjects were requested to maintain a HR 5bpm below the HR corresponding to the LT at all times during a zone one training session.

5.3.5 Laboratory Testing

Before and after each training intervention, subjects reported to the laboratory between 7:00 and 9:00 in a rested, fasted state. A microbiopsy (Bard Magnum Biopsy System, Bard Peripheral Vascular Inc, AZ, USA) of the vastus lateralis muscle was performed, as described by Hayot and colleagues (Hayot et al., 2005), under local anaesthetic (Lidocaine, hydrochloride injection, Antigen Pharmaceuticals Ltd, Tipperary, Ireland; 2ml per subject). Approximately 10-20mg of tissue was collected from 1-3 samples. The tissue was immediately quenched in liquid nitrogen and stored until later analysis. Briefly, a small piece of frozen wet muscle (5-8mg) was removed from the pre- and post-training resting biopsy for the determination of two key mitochondrial oxidative enzymes; citrate synthase (CS) and beta-hydroxylacyl-CoA dehydrogenase (β-HAD) using spectrophotometric methods (ILAB Aries, Instrumention Laboratory, Italy), as described elsewhere (Bergmeyer 1974;Srere 1969). Briefly, the muscle sample was fully homogenised in 100µL of homogenising solution per mg of muscle, and put

immediately into liquid nitrogen. The homogenising solution was then put through two sets of freeze-thaw cycles before analysis.

Subjects reported to the laboratory for a second time between 15:00 and 20:00 for an incremental cycle test to exhaustion. Body mass (beam balance scales) was assessed wearing minimal clothing and stature taken (The Leicester Height Measure, Seca, UK). For the incremental test, a CompuTrainer was used in ergometer mode, fitted with the subjects own bike. Following a 10-min warm up, the CompuTrainer was calibrated to 3.5lbs, as instructed by the manufacturers for accuracy up to 500W. The test started at 100W and increased by 40W every 3-min, until volitional exhaustion, with the cadence remaining self-selected, but the speed being held above 14mph, for accurate measurements of power output. 30-s before the end of each 3-min stage, HR (S625X, Polar Electro, Kemplete, Finland) was recorded and a 5-µL capillary blood sample was obtained and analysed for lactate concentration by micro-assay (LactatePro LT-1710, ArkRay Inc., Kyoto, Japan). The reliability and validity of this device has been previously determined (Pyne et al., 2000). The LT was determined as the final point before the blood lactate concentration increased distinctly from its resting concentration (Aunola & Rusko 1984). The lactate turnpoint (LTP) was determined as the starting point of accelerated lactate accumulation (around 3 to 6mmol·L^{-1}), depending on the individual blood lactate profile (Aunola & Rusko 1984).

5.3.5.1 Peak Power Output Determination

The measure for Peak Power Output (PPO) was assessed using the following equation (Kuipers et al., 1985):

$PPO = W_{final} + ([t/180] \cdot 40)$

Where, W_{final}= the power output of the final completed stage (W), t = the time spent in the final uncompleted stage (s), 180 = the duration of each stage (s) and 40 = the increase in power output of each stage (W).

5.3.5.2 Time to Exhaustion at 95% Peak Power Output

Immediately following the incremental test to exhaustion, the power was decreased to 100W and the subject was asked to pedal at a self-selected cadence for 10 min. At 5-min, the CompuTrainer was re-calibrated to 3.5lbs. At 10-min, the power output was increased to 95% of PPO, and the subject was requested to maintain a speed above 14mph until volitional exhaustion. The time achieved was recorded to the nearest second, along with the peak HR achieved during the test.

5.3.5.3 40km Time Trial

On a separate day, but within three days of the incremental test to exhaustion, a 40kmTT was performed. Subjects brought their own bike into the laboratory and set it up on a CompuTrainer Multirider System (RacerMate, Seattle, WA). Following a 10-min warm up, the CompuTrainers were calibrated to 3.5lbs. Subjects were instructed to complete the TT as fast as possible. The only data the subjects could see was distance completed for themselves and the other riders in the group. Up to 8-riders completed this test at any one time, with the same riders for pre and post P and T. HR (Polar Team System, Polar Electro, Kemplete, Finland) was taken throughout the TT and was blind to the subject.

5.3.6 Reproducibility of the Measures

The typical error of measurement for the LT, LTP and PPO have been assessed in our laboratory, and were found to be 3.7, 3.3, 2.3% (Chapter three). In addition, the typical error of measurement for the 40km TT mean power output for the participants in the present study, in our laboratory has been found to be 2.0%. Data for the reproducibility of the 40km TT mean power are presented in a Bland Altman plot (Fig. 5.1).

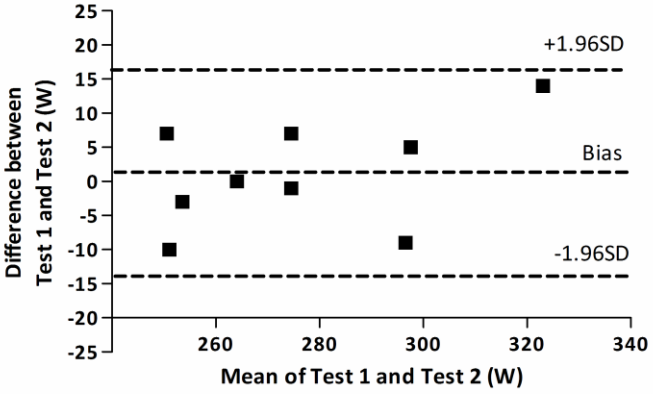

Fig. 5.1 Bland Altman plot to show the relationship between the individual test-retest differences and the mean of test one and test two for the 40km TT mean power output.

5.3.7 Mood Questionnaire

The Brunel Mood Scale (BRUMS) questionnaire (Terry et al., 2003) was collected in the laboratory every Friday during each of the 6-week training blocks. This questionnaire contains 24 simple mood descriptors with 6 subscales; anger, confusion, depression, fatigue, tension and vigour. It took 1-2 min to complete and was answered using the response time frame 'How have you felt in the past week including today'.

5.3.8 Statistical Analyses

Statistical analysis was performed using SPSS version 18 (Chicago, Il, USA). A fully repeated-measures ANOVA (2x2) compared the performance/physiological adaptation measures between training-intensity distribution models (P and T) and over time (pre to post). Main effects between training-intensity distribution models, over time and any interaction between these and the performance/physiological adaptation measures were reported. Post hoc analysis was undertaken where significance was obtained with Paired Student's t-tests using two-tailed values of *P*, with the Bonferroni method of adjustment to prevent type I error. Paired Student's t-tests using two-tailed values of P were used to compare training variables between P and T. Statistical significance was accepted at $P<0.05$. All data are expressed as Mean (±SD). Effect sizes for the key performance/physiological adaptation measures were

calculated from the mean difference (pre to post) over the standard deviation of the baseline measure. These values were judged using the descriptors suggested by Cohen (Cohen 1988). Effect sizes were included to highlight the size of the performance/physiological adaptation changes, as the P value alone does not necessarily provide this information.

5.4 RESULTS

One subject dropped out of the study, due to injury. Training adherence for the 11 remaining subjects was 96 and 97% for P and T, respectively.

5.4.1 Training

The training volume (22% higher) and the training load (23% higher) were significantly different in T than P ($P<0.05$) due to the nature of the study design. The percentage of time spent in each training intensity zone was as intended (Table. 5.1).

Table. 5.1 Mean (±SD) Details of the training for the polarised and the threshold training models.

	P	**T**
Training Volume (min·wk^{-1})	381 (±85)	458 (±120)*
Training Load (TRIMPS·wk^{-1})	517 (±90)	633 (±119)*
Zone 1 (%)	80 (±4)	57 (±9)*
Zone 2 (%)	0 (±0)	43 (±9)*
Zone 3 (%)	20 (±4)	0 (±0)*

P: Polarised Training Model; **T**: Threshold Training Model; **TRIMP**: Training Impulse. *Difference between P and T ($P<0.05$).

5.4.1.1 Polarised Training Model

There was a main effect over time for the power output sustained during each of the 4min intervals ($P<0.05$), due to an increase from week one (319 ± 33 W) to week six (340 ±34 W; $P<0.05$), an increase of 6.8 (±4.2) %. There were no differences over time for the mean peak HR reached for the 6-intervals (169 ±10 to 169 ±9bpm), the mean minimum HR during the recovery (111 ±14 to 108 ±14bpm), the RPE (7 ± 1 to 8 ±1) or the peak HR reached during the session (173 ± 10 to 171 ± 9 bpm).

5.4.1.2 Threshold Training Model

There was a main effect over time for the power output sustained during the 60min threshold sessions ($P<0.05$), due to an increase from week one (266 ± 31 W) to week six (290 ±32 W; $P<0.05$), an increase of 9.2 (±5.0) %. There were no differences over time for the mean HR during the 60min ride (158 ±12 to 159 ±9bpm), or the RPE (5 ± 1 to 6 ± 1). At the beginning of T, the power output of the training sessions corresponded to 114 (±4) % of the LT power output and 91 (±3) % of the LTP power output.

5.4.2 Endurance Performance and Physiological Adaptation

5.4.2.1 40km Time Trial

There was a main effect over time for the 40km TT mean power output and also for the mean HR ($P<0.05$; Fig. 5.2). The 40km TT mean power output was higher and the mean heart rate was lower from pre to post training in both P and T (Fig. 5.2). The percentage change in the mean power output from pre to post training was not significantly different between P and T (8 ± 8 and 4 ± 6 %, respectively; $P<0.05$). Likewise, the percentage change in the mean HR during the 40km TT was not different between P and T (-1.6 ± 1.4 and -1.3 ± 1.3 %, respectively).

5.4.2.2 Responses from the Incremental Intensity Test to Exhaustion

There was a main effect over time for the LTP and for the PPO ($P<0.05$; Fig. 5.3). There was an interaction ($P<0.05$) for the PPO by training-intensity distribution model, due to an increase from pre to post training in P ($P<0.05$) but not T. The percentage change in PPO from pre to post training was higher in P (8 (±5) %) than in T (3 (±4) %). There was a main effect over time for the maximum HR reached in the incremental test to exhaustion ($P<0.05$), due to a decrease from pre to post training in both P and T ($P<0.05$). There was no difference in the percentage change in the maximal HR between P and T (-1.5 ± 1.9 and -1.8 ± 2.3 %, respectively).

5.4.2.3 Time to Exhaustion at 95% Pre-Training Peak Power Output

There was a main effect over time for the TTE at 95% of the pre-training PPO ($P<0.05$; Fig. 5.4), with increases from pre to post training for both P and T ($P<0.05$). There was an interaction ($P<0.05$) for the TTE by training-intensity distribution model, due to a greater percentage increase from pre to post training in P (85 (±44) %; $P<0.05$) than in T (37 (±47) %; $P<0.05$). There was a main effect over time for the peak HR reached during the TTE ($P<0.05$; Fig. 5.4), due to a decrease from pre to post training in both P and T ($P<0.05$). There was no difference in the percentage change in the peak HR reached during the TTE between P and T (-2.0 ± 2.2 and -2.2 ±2.1 %, respectively).

5.4.2.4 Skeletal Muscle Oxidative Enzymes

There were no main effects over time or of training-intensity distribution model in the maximal activities of the skeletal muscle oxidative enzymes, CS or β-HAD. The maximal activity of CS from pre to post training in P and T was 47 (±6) to 48 (±4) mmol·kgwet wt^{-1}·min^{-1} and 47 (±5) to 49 (±3) mmol·kgwet wt^{-1}·min^{-1}, respectively. The maximal activity of β-HAD from pre to post training in P and T was 15 (±2) to 15 (±2) mmol·kgwetwt^{-1}·min^{-1} and 15 (±2) to 15 (±1) mmol·kgwetwt^{-1}·min^{-1}, respectively.

5.4.2.5 Brunel Mood Scale

There were no differences between P and T for anger, confusion, depression, fatigue, tension or vigour in the BRUMS questionnaire (Table. 5.3).

5.4.2.6 Body Mass

Body mass did not change from pre to post training in either P (76.5 ± 6.3 to 76.6 ± 6.2 kg) or T (77.3 ± 6.7 to 76.5 ± 6.0 kg).

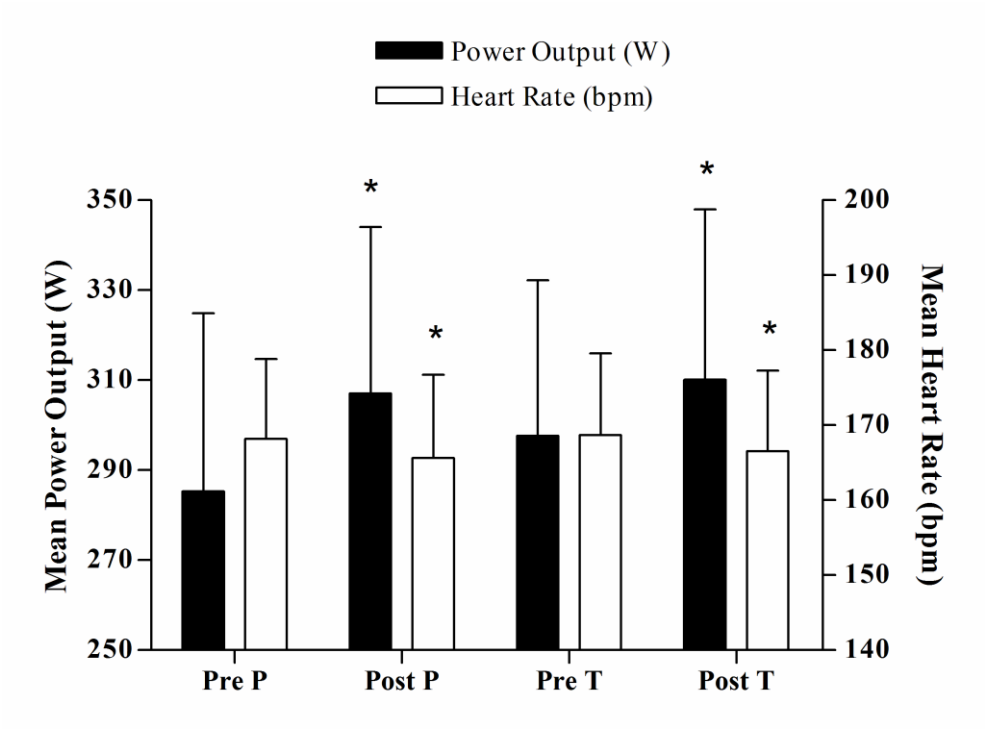

Fig 5.2 Mean (±SD) 40km time trial mean power output and mean heart rate. **P:** Polarised Training Model; **T**: Threshold Training Model. * Different from pre (P<0.05).

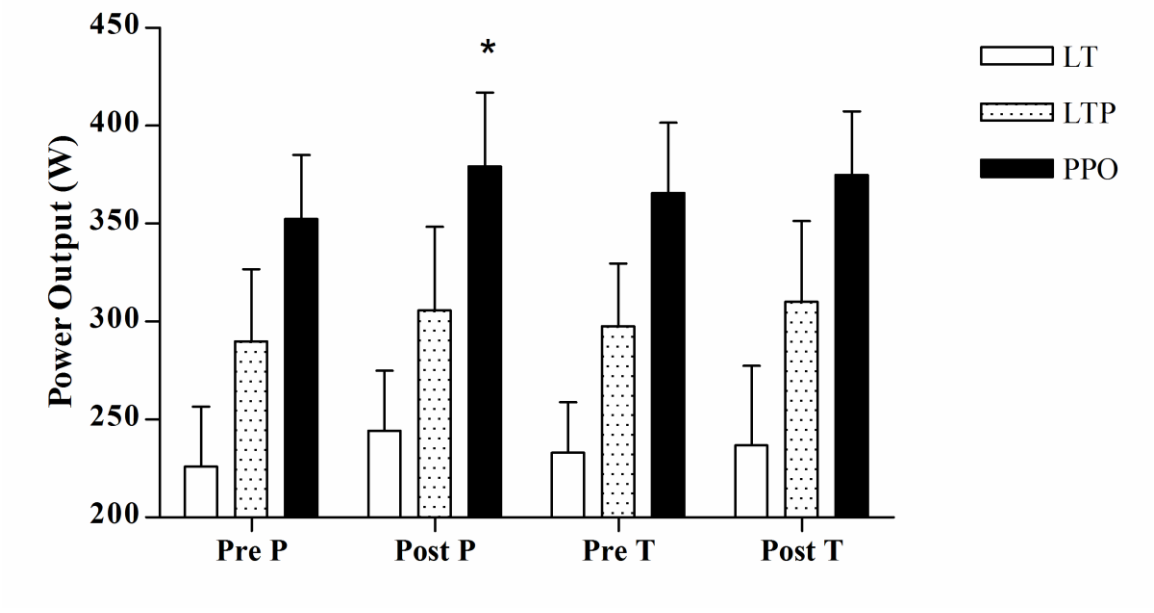

Fig. 5.3 Mean (±SD) power output corresponding to the LT, LTP and PPO.**LT:** Lactate Threshold; **LTP**: Lactate Turn Point; **PPO**: Peak Power Output; **P:** Polarised Training Model; **T**: Threshold Training Model. * Different from pre (P<0.05).

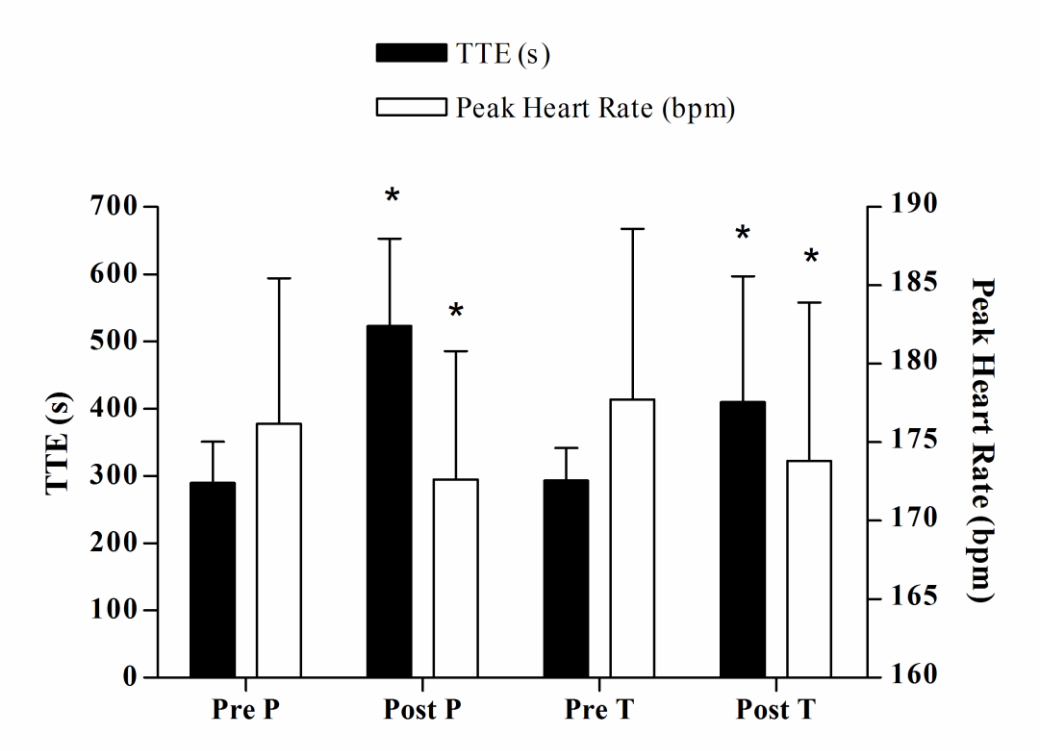

Fig. 5.4 Mean (±SD) TTE at 95% pre PPO. **TTE**: Time to Exhaustion; **PPO**: Peak Power Output. **P:** Polarised Training Model; **T**: Threshold Training Model. * Different from pre (P<0.05).

Table. 5.2 Effect sizes for the key performance measures.

Training Model	Measure	Effect Size	Descriptor*
P	40km TT MPO (W)	0.57	Moderate
	LT (W)	0.59	Moderate
	LTP (W)	0.40	Small
	PPO (W)	0.77	Moderate
	TTE (s)	2.44	Large
T	40km TT MPO (W)	0.35	Small
	LT (W)	0.11	Trivial
	LTP (W)	0.34	Small
	PPO(W)	0.26	Small
	TTE (s)	0.99	Large

P: Polarised Training Model; **T**: Threshold Training Model, **MPO** = Mean Power Output, **TT** = Time Trial; **LT** = Lactate Threshold; **LTP** = Lactate Turnpoint; **PPO** = Peak Power Output, **TTE** = Time To Exhaustion, * Cohen (1988).

Table. 5.3 Mean (±SD) responses from the BRUMS questionnaire. Data are the mean from the weekly responses.

	P	T
Anger	1.7 (±1.6)	1.0 (±1.0)
Confusion	0.8 (±1.0)	0.5 (±0.9)
Depression	0.8 (±0.9)	0.6 (±0.7)
Fatigue	3.6 (±1.7)	3.3 (±1.9)
Tension	1.2 (±1.2)	1.0 (±1.4)
Vigour	7.1 (±2.9)	8.0 (±2.9)

5.5 DISCUSSION

Despite a greater training volume and training load in T, there were greater improvements following P compared with T for the TTE at 95% pre-training PPO and the PPO. Although there was no statistically significant difference between P and T for the improvement in 40km TT from pre to post training, the effect size was much larger for P. In fact, the effect sizes for all of the key performance and physiological adaptation markers were larger for P compared with T. Elite endurance athletes have been shown to train with a high training volume and a high training load relative to their discipline, with ~80% of training at low intensities (zone one) and ~20% of training at higher intensities (zones two and three, combined) (Sandbakk et al., 2010; Guellich et al., 2009; Seiler & Kjerland 2006; Schumacher & Mueller 2002; Lucia et al., 2000a; Lucia et al., 2000b). The present study demonstrates that with a similar training-intensity distribution to the elite endurance athletes in previous studies (80% in zone one, 20% in zone three), greater improvements can be achieved compared with a different training-intensity distribution (57% in zone one, 43% in zone two). It is noteworthy that this difference exists despite a greater training volume and training load in T compared with P. This study therefore confirms the effectiveness of a polarised training model in well-trained endurance athletes even when absolute training time spent in zone one is matched.

Previous experimental studies in this area have revealed that training programmes incorporating a greater percentage of time in zone one in place of time in zone two leads to greater improvements in endurance performance (Esteve-Lanao et al., 2007) and physiological adaptation (Ingham et al., 2008). The present study is in agreement with these previous studies, as there was a significantly higher percentage of training in zone one in P compared with T. It is has been reported that zone one training is sufficient to maximise the increase in mitochondria in type I muscle fibres (Dudley et al, 1987), and this could be the explanation for its effectiveness in well-trained endurance athletes.

It has been suggested that endurance athletes might not achieve optimal gains in performance and/or physiological adaptation by spending too much time in zone two (Esteve-Lanao et al., 2007; Londeree 1997). The mechanisms responsible for these findings are not well understood, but Esteve-Lanao and colleagues (Esteve-Lanao et al., 2007) suggest that it could be due to a down-regulation of the sympathetic nervous system. Indeed, in agreement with the present study, a group of elite runners that spent more time at an intensity corresponding to the LT (zone two) had a lower performance level than a group of elite runners that spent less time in zone two and more time in zone three, despite a higher training volume (Billat et al., 2003). In addition, the recovery from the training sessions might explain the effectiveness of P compared with T. It has been reported that the acute recovery from a training session in zone one is faster than following a training session in zone two, yet the recovery following a training session in zone three is no different than following a session in zone two (Seiler et al, 2007). It has been reported that training at a higher intensity leads to larger physiological adaptation (Edge et al, 2006; Helgerud et al, 2007; Daussin et al, 2007; Driller et al, 2009). Therefore, with no difference in the recovery from the training sessions of different intensities (Seiler et al, 2007), it is possible that the training in zone three is more effective at inducing positive responses in endurance athletes than training in zone two. Moreover, as the recovery is enhanced more in zone one than zone two, it has been recommended to supplement zone three training with training in zone one (Seiler et al, 2007). In the present study, improvements in the 40km TT and the TTE for both P and T suggest that overreaching did not take place during either training intervention, and that while P appears to provide a stronger stimulus for improvement in 40km TT performance, both training models result in large gains in high-intensity exercise capacity. In addition, no differences between P and T in the mood questionnaire suggests no difference in the training stress.

The effectiveness of a training-intensity distribution containing ~80% of total training time in zone one and ~20% in zone three, as used for P in the present study have been suggested to be due to the intensity-specific adaptations and the enhanced recovery (Seiler & Kjerland, 2006). In fact, cardiovascular adaptations (Helgerud et al, 2007) and buffering capacity (Edge et al, 2006) have been reported to be enhanced more following a training period including higher intensity exercise. In the present study however, although there were decreases in the mean HR during the 40km TT, the peak HR during the TTE and the maximal HR for both P and T, there were no differences in the percentage change between P and T. This suggests that cardiovascular adaptations were not responsible for the differences in response between P and T in the present study. Although not statistically significant, the improvement in the LT and the LTP from pre to post in P were greater than in T, as shown by higher effect sizes. This may have been due to a greater decrease in lactate production (Favier et al., 1986) or a greater increase in lactate clearance (Donovan & Brooks 1983) in P compared to T. A greater decrease in lactate production would have been due to; a more improved oxygen supply and thus less need for anaerobic ATP production (Evertsen et al., 2001) or a greater increase in the capacity for pyruvate oxidation (Holloszy & Coyle 1984). Monocarboxylate transporter 1 (MCT1) is in oxidative slow twitch muscle fibres and transports lactate from blood to muscle to be oxidised (Evertsen et al., 2001). It has been reported that the expression and concentration of MCT1 is increased more at higher exercise intensities (Pilegaard et al., 1999a), and could therefore be a contributing factor in the greater increase in the LT and the LTP following P compared with T. Other contributing factors could include greater increases in buffering capacity and capillarity, which have both been reported to be increased more at higher exercise intensities (Iaia et al., 2009; Edge et al., 2006). The increase in buffering capacity is intensity-specific because the muscle tries to maintain homeostasis and therefore attempts to maintain the pH in the muscle. As the pH in the muscle is changed more during higher intensity exercise, the effects on buffering capacity are higher.

Previous studies have found comparable improvements in endurance performance in well-trained endurance athletes following a similar period of interval training as used for P in the present study (Westgarth-Taylor et al., 1997; Weston et al., 1997; Lindsay et al., 1996). Improvements in performance have been associated with reduced substrate phosphorylation and an increased fat oxidation at high intensities (Clark et al., 2004; Stepto et al., 1999; Westgarth-Taylor et al., 1997; Weston et al., 1997). In a study assessing optimal high intensity interval training programmes, the programme chosen for the present study has been reported to be very effective in already well-trained cyclists (Stepto et al., 1999). Therefore, the improvements in endurance performance and physiological adaptation reported in the present study following P are perhaps not surprising. However, no study to date has attempted to compare a purely polarised training model (no training in zone two) with a purely threshold training model (no training time in zone three) in well-trained endurance athletes. Therefore, this study confirms the effectiveness of a polarised training model in well-trained endurance athletes.

It is possible that the greater training intensity in P compared with T caused a greater increase in the activation of adenosine monophosphate activated protein kinase (AMPK), as has been reported in previous studies (Wojaszewski et al, 2000; Chen et al, 2003). Indeed, in a group of well-trained cyclists, a high intensity training session involving 8 x 5min at 85% VO_{2peak} caused an increase in the AMPK activity and phosphorylation (Clark et al, 2004). It is therefore likely that the well-trained cyclists in the present study would have experienced a similar increase in AMPK activity and phosphorylation following the training sessions in P. AMPK-signalling mechanisms are involved in the initiation of mitochondrial biogenesis, through the regulation of peroxisome proliferator receptor-γ co-activator-1α (PGC-1α) expression and activity (Puigserver & Spiegelman 2003). In the present study however, it seems unlikely that the

differences in endurance performance and physiological adaptation between P and T were due to differences in mitochondrial biogenesis, as there were no differences in the maximal activity of CS or β-HAD. It has been suggested that mitochondrial content can be estimated from the maximal activities of 'marker enzymes' such as citrate synthase, because the mitochondrial volumes estimated morphometrically parallel the changes in maximal enzyme activity (Hood et al, 2001). It is likely that the skeletal muscle of the cyclists had a high mitochondrial density and oxidative capacity at the start of the training, as demonstrated by the magnitude of the performance measures. An increase in the oxidative potential in already well-trained skeletal muscle is relatively small and may be too small to detect following a short-term training intervention (Kohn et al., 2010). Indeed, following high volume training with high intensity training included in well-trained athletes, there was no difference in the maximal activity of CS (Evertsen et al., 1999; Weston et al., 1997) and β-HAD (Weston et al., 1997), despite improvements in endurance performance. In contrast, studies using moderately trained individuals and a similar interval training programme to P in the present study, have found large increases in the maximal activities of CS and β-HAD of 20-30% (Gurd et al., 2010; Perry et al., 2008; Talanian et al., 2007). In addition, it has been documented that an increase in mitochondrial content of 50-100% is possible within six weeks, in previously untrained muscle (Fitts et al, 1975).

As exercise intensity increases, there is a greater recruitment of muscle fibres and more specifically, a greater recruitment of fast-twitch muscle fibres (Dudley et al., 1982). Adaptations in muscle are greatest in those muscle fibres that are directly activated during training (Dudley et al., 1982). As more muscle fibres would have been recruited during training in P compared with T, the specific adaptations to induce more oxidative muscle fibres would have occurred in more muscle fibres in P compared with T. It addition, it has been suggested that fast-twitch muscle fibres become more fatigue resistant following high-

intensity training, and this is likely due to more fast-twitch muscle fibres being recruited at higher exercise intensities. This could explain the greater improvements in the TTE at 95% pre-training PPO and PPO in P compared with T.

5.6 CONCLUSIONS

A polarised training model is recommended for trained cyclists wishing to maximally improve performance and physiological adaptation over a short-term training period. There is however, much still to be understood regarding the impact of endurance training periods containing different training-intensity distributions in trained endurance athletes and the mechanisms responsible for these effects. For example, the differences in endurance performance maybe due to the exercise intensity, an enhanced recovery, or due to a combination of both of these factors. Therefore, this is a fruitful area for future research, and would contribute to the optimisation of endurance training.

CHAPTER SIX – PART ONE

THE IMPACT OF TWO DIFFERENT ENDURANCE TRAINING PROGRAMMES ON THE PROPORTION OF SENESCENT BLOOD T CELLS IN TRAINED CYCLISTS

6.1 ABSTRACT

The proportion of senescent and highly differentiated T-cells may increase following intensive periods of endurance training, predisposing an individual to a greater risk of infection from new pathogens due to a shrunken naïve T-cell repertoire. **Purpose:** To implement two training interventions to induce adaptations and determine if the adaptations are at the expense of an increased proportion of senescent T cells. **Methods:** Twelve well-trained male cyclists (age 37.5 $\pm$ 6.2 yrs) provided a fasted resting blood sample in the morning before and after two 6-week training periods in a cross-over study design, with each training period preceded by a 4-week de-training period. The two training periods differed in the intensity distribution; a polarised training model consisted of 80 $\pm$ 4% of time below the lactate threshold (LT) and 20 $\pm$ 4% above the lactate turnpoint (LTP) and a threshold training model consisted of 57 $\pm$ 10% of time below the LT and 43 $\pm$ 10% of time between the LT and the LTP. Isolated blood lymphocytes were labelled with monoclonal antibodies to assess cell surface co-expression of CD57 and CD28 to identify the proportions of low differentiated (CD28+/CD57-), high differentiated (CD28+/CD57+) and senescent (CD28-/CD57+) cells within both CD4+ and CD8+ T-cell subsets using four-colour flow cytometry. Data were analysed using a linear mixed model. **Results:** Both training interventions elicited adaptation, as demonstrated by significant increases in 40km time trial mean power output and time to exhaustion at 95% of peak power output. However, no changes were found in the proportion of low or high differentiated or senescent T-cells within the CD8+ or CD4+ T-cell subsets. **Conclusion:** Both training protocols provided a sufficient stimulus to improve cycling performance without leaving the athlete at a potentially greater risk of infection due to a shrunken naïve T-cell repertoire.

6.2 INTRODUCTION

Previous research has reported that elite endurance athletes from a number of different sports train with a high training volume, and follow a polarised training model in; cross-country skiing (Sandbakk et al., 2010; Seiler & Kjerland 2006); rowing (Guellich et al., 2009); road cycling (Lucia et al., 2000a; Lucia et al., 2000b) and track cycling (Schumacher & Mueller 2002). Indeed, experimental studies support the effectiveness of a polarised training model compared with a threshold training model (Chapter 5) or other training models (Esteve-Lanaeo et al, 2007; Ingham et al, 2008). The effectiveness of the polarised training model has been suggested to be due to the effectiveness of a high training volume in zone one, the effectiveness of training in zone three in comparison to training in zone two, and an enhanced recovery. The results of chapter four can also be interpreted to support the positive effect of training in zone one and the potentially detrimental effects of excessive training time spent in zone two. Over the long term, athletes need to stay healthy in order to train consistently. If an athlete regularly picks up infections, this could have a negative effect on training, and subsequent physiological adaptation and performance. Although speculative, it is possible that the effectiveness of the polarised training model may at least in part be due to an enhanced resistance to picking up an infection. Although not reported in chapter five, it is possible that the athletes were picking up infections during the training and continuing to train with a minor infection. Indeed, a very small percentage of training was missed due to upper respiratory tract infections, although this was not reported formally.

A 'J shaped curve' has been used to model the relationship between exercise and susceptibility to infection (Nieman 1994). An enhanced immune function above sedentary levels might be achieved with moderate activity, but an impaired immune function might result from excessive amounts of prolonged, high-intensity exercise (Nieman 1994). When comparing a

group of athletes, it has been reported that the athletes with higher training volumes and training loads are at an increased risk of infection (Heath et al., 1991). Individual athletes probably have individual training thresholds, over which the risk of infection increases (Foster 1998). An increased risk of infection is a huge concern for athletes, as even minor infections can lead to an inability to sustain strenuous training regimes, which may subsequently lead to a decreased exercise performance (Roberts 1986). The precise mechanisms underlying the suppressed immune function with strenuous exercise are yet to be established.

A lowered cell-mediated response at rest can occur following a period of intensified training in already well-trained athletes, and is likely to be due to the cumulative effects of repeated bouts of intense exercise (Baj et al., 1994). The study by Baj and colleagues (Baj et al., 1994) found a diminished IL-2 production following 6-months of intensive training and racing in competitive cyclists. In addition, the proliferative capacity of T-cells has been reported to be decreased following a period of intensive training (Lancaster et al., 2004; Bury et al., 1998; Verde et al., 1992). A depressed cell-mediated response leads to an increased incidence of viral infections (Fabbri et al., 2003). It is therefore possible that the increased susceptibility to infection in endurance athletes following a period of intensive training may be due to decreases in T-cell function (Gleeson & Bishop 2005).

Cellular senescence is a state whereby cells are unable to further proliferate in response to antigenic stimuli and can be caused by excess rounds of cell division in response to repeated antigenic stimuli (Effros 2007). The co-expression of CD57+ and CD28- has been used as a marker of senescence. CD57 is a cell surface glycoprotein that has been reported to be expressed on T-lymphocytes with a senescent phenotype (Brenchley et al., 2003). More specifically, CD57 is expressed on T-lymphocytes with short telomeres, a long history of proliferation, an inability to proliferate in response to mitogenic stimuli (Brenchley et al., 2003)

and lack cell surface expression of the co-stimulatory molecule, CD28 on the cell surface (Bruunsgaard et al., 1999). Indeed, cells lacking the expression of CD28 are also known to have a lower proliferative capacity (Nociari et al., 1999). In fact, the complete and irreversible loss of CD28 has been reported following T cell replicative senescence in cell culture (Effros 2004). T cells lacking the expression of CD28 have also been shown to have shorter telomere lengths (Effros et al., 1996). It has been reported that CD28 is required for optimal upregulation in the activity of the telomere extending enzyme, telomerase (Valenzuela & Effros 2002; Weng et al., 1997). Further evidence for CD28 as a marker of senescent T cells is the finding that CD28 is important for the activation and proliferation of naive T-cells (Ouyang et al., 2003; Labalette et al., 1999; Linsley & Ledbetter 1993). The evidence clearly shows that the expression of CD57 and the lack of expression of CD28 is indicative of a senescent phenotype. However, it has been suggested that for an even more accurate marker of a true end stage senescent cell, multiple phenotypic markers are required (Effros 2007). It has been reported that from proliferative assays and telomere analysis, the surface expression of CD57 and CD28- T cells define the ultimate end-stage cell in the senescent pathway (Brenchley et al., 2003).

Homeostatic mechanisms tightly control the proportion of naive and activated T cell populations in the peripheral T cell pool (Freitas et al., 1996; Tanchot & Rocha 1995; Rocha et al., 1989). Therefore, the proportion of T cells with a senescent phenotype in the peripheral T cell pool may increase due to a lack of apoptosis (Spaulding et al., 1999). The naive T cells are gradually replaced with T cells with a senescent phenotype, causing the naive T cell repertoire to shrink (Koch et al., 2006). As the naive T cells are vital in immune responses to novel pathogen, this change in the immune space may leave an individual at a greater risk of infection (Pawelec et al., 2009; Koch et al., 2007; Ouyang et al., 2003). Although speculative, the lowered T cell response at rest following an intensive period of training could be due to an

increased proportion of senescent T cells, along with a decreased proportion of naïve T cells. Indeed, regular high intensity exercise has been shown to decrease the naive T cell repertoire (Cosgrove et al., 2011; Hack et al., 1997).

To the author's knowledge, only Cosgrove and colleagues (Cosgrove et al., 2011) has investigated the effects of regular endurance training on the proportion of senescent T cells in resting, fasted blood. This study (Cosgrove et al., 2011) reported that 6-months of training in preparation for an ironman triathlon race lead to no changes in the proportion of senescent or naive T cells in ten trained triathletes. However, only small physiological adaptations were achieved over the 6-month training period. Blood samples were also taken three weeks after the race revealed an increased proportion of senescent T cells within the CD4+ T-cell subset, alongside a reduction in naïve cells within the CD4+ T-cell subset. This suggests that the stimulus during the training period was not sufficient to induce an increase in the proportion of senescent T cells or a decrease in the proportion of naive T cells, but the ironman triathlon race did provide a sufficient stimulus to induce these changes in the immune space. Although speculative, it is possible that with regular high intensity training of sufficient intensity and duration, there could be an increase in the proportion of senescent T cells and a decrease in the proportion of naïve T cells. This mechanism could contribute to the lowered cell mediated response seen in endurance athletes following hard training periods.

Improvements in endurance performance and physiological adaptation have been reported to be different following training periods with different intensity distributions (Ingham et al., 2008; Esteve-Lanao et al., 2007). The cause of the differences in response following training periods of different training-intensity distribution are unclear, but are likely due to intensity-dependent adaptations and different rates of recovery following exercise of different intensity (Seiler et al., 2007). The aim of the present study was to induce large physiological

adaptations though intensive training and determine if the adaptations gained are at the expense of an increased proportion of senescent T cells. This was achieved with the use of two different training intervention periods, differing in their intensity distribution, as used in chapter 5.

6.3 METHODS

6.3.1 Subject Information

Twelve well-trained cyclists were recruited from local cycling clubs and provided written, informed consent to take part in the study, which was approved by the University Ethics Committee, in accordance to the Declaration of Helsinki. The mean (±SD) characteristics of the participants at the beginning of the testing period were: age 37 (±6) years, body mass 76.8 (±6.6) kg and stature 178 (±6) cm.

6.3.2 Study Design

Fasted and rested venous blood samples were drawn into K2 EDTA Vacutainer blood collection tubes (BD Biosciences, Oxford, UK) with 21 Guage ¾" BD Vacutainer Safety-Lok™ blood collection sets (BD Biosciences, Oxford, UK) between 7am and 9am in the week before and the week after two six week training periods. During the same week as the blood collection, subjects underwent a cycling 40km time-trial and an incremental cycle test to exhaustion (See chapter five for details). This was to quantify that the training had caused a sufficient stimulus for adaptation.

6.3.3 Endurance Training Programmes

The two training periods differed in their intensity distribution; one followed a polarised model (P) (80 $\pm$ 4% of time below the lactate threshold (LT) and 20 $\pm$ 4% above the lactate turnpoint (LTP)) and the other followed a threshold model (T) (57 $\pm$ 10% of time below the LT

and 43 ± 10% of time between the LT and the LTP), with no training above the LTP (See chapter four for details).

6.3.4 Isolation and Preservation of PBMCs

Peripheral Blood Mononuclear Cells (PBMCs) were isolated from whole blood using density gradient centrifugation (Lymphoprep; Axis-Shield, Oslo, Norway). Whole blood was diluted with an equal volume of 0.9% NaCl (Baxter™, Deerfield, IL USA), and 6 mL of the diluted blood was layered over 3 ml of Lymphoprep. Samples were centrifuged for 30 minutes at 800g. Following centrifugation, the distinct band formed by the PBMCs was carefully removed using a Pasteur pipette and washed twice in 0.9% NaCl for 10 minutes at 250g, then again in cell culture medium RPMI 1640 (Sigma-Aldrich, Irvine, Scotland) for 10 minutes at 250g.

The isolated PBMCs were re-suspended in 700 µL foetal bovine serum (Sigma-Aldrich, Irvine, Scotland) and 200 µL RPMI 1640, before 100 µL dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Irvine, Scotland) was added slowly to the cell mix on ice. This was immediately frozen in liquid nitrogen until later analysis. DMSO makes the cell membrane less likely to be punctured, by partially solubilising the membrane.

6.3.5 Labelling of Cell Surface Antigens

PBMCs were labelled with appropriate monoclonal antibodies (mAbs) and incubated for 1-h at room temperature. Each sample was labelled with anti-CD3 APC (Immunotools, Germany) and one of anti-CD4 (Immunotools, Germany) or anti-CD8 PECy-5 (Immunotools, Germany) mAbs to identify T lymphocyte subsets, before the addition of anti-CD57 (BD PharMingen, CA, USA) or anti- CD28 PE (BD PharMingen, CA, USA) mAbs, to identify senescent T lymphocytes. All mAbs were pre-titrated to determine optimal conditions for flow cytometric analysis.

6.3.6 Flow Cytometry

Flow cytometry was performed using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA) equipped with a 15 mW argon ion laser emitting light at a fixed wavelength of 488 nm and a 10 mW photodiode laser emitting light at 635 nm. Cell Quest Pro software (BD Biosciences, San Jose, CA, USA) was used for the cellular analysis. The forward and side light-scatter mode was used to identify and electronically gate the lymphocytes. The CD3+ T lymphocyte population was identified using the forward scatter against APC fluorescence and was gated electronically. The expression of CD4 and CD8 was used to identify T cell subsets within the CD3+ gate. Where appropriate, a further electronic gate was placed around the CD3+/CD4+ or the CD3+/CD8+ population. The expression of CD57, CD28, and the co-expression of CD57/CD28 on CD3+/CD4+ or CD3+/CD8+ cells were determined using single parameter histograms and two parameter dot plots. Proportions of low differentiated (CD28+/CD57-), high differentiated (CD28+/CD57+) and senescent (CD28-/CD57+) cells within both CD4+ and CD8+ T-cell subsets were identified. For each sample, 40,000 events were collected in the CD3+ gate for analysis. The fluorescent amplifiers of the FL1-FL4 detector filters were adjusted to ensure that the negative cell population (as defined by the lymphocytes labelled using the appropriate isotype control) appeared in the first logarithmic decade. An electronic marker was placed at the limit of the negative controls to quantify the percentage of lymphocytes and lymphocyte subsets that were positive and negative for each cell surface antigen. Electronic compensation was used to resolve any overlapping emission spectra using single-labelled tubes with each mAb.

6.3.7 Statistical Analysis

Statistical analysis was performed using SPSS version 18 (Chicago, Il, USA).A fully repeated-measures ANOVA compared the performance/physiological adaptation measures between trials (P and T) and over time (pre to post). Main effects between trials, over time and any interaction between these and the performance/physiological adaptation measures were

reported. Similarly, a fully repeated-measures ANOVA compared the percentage of T lymphocyte subsets between trials (P and T) and over time (pre to post). Main effects between trials, over time and any interaction between these and the percentage of T lymphocyte subsets were reported. Post hoc analysis was undertaken where significance was obtained with Paired Students t-tests, using two-tailed values of P. Percentage change of the performance/physiological adaptation measures over time (pre to post) were compared using Paired Students t-tests, using two-tailed values of P. Data on the proportion of senescent, high differentiated and low differentiated T-cells was analysed for changes over time (pre to post) and between trials (P and T) using separate restricted maximum likelihood linear mixed model (LMM). Subject ID was used as the within subject identifier variable, with time and trial as repeated variables. The LMM method was used to fit a covariance matrix for the residuals to account for dependency of the repeated measures, allowing different variances for each time point and different covariance's between time points. Statistical significance was accepted at $P < 0.05$.

6.4 RESULTS

As reported in Chapter 5 there was a main effect of time for the 40km TT mean power output, the 40km TT mean HR, the TTE and the peak HR achieved during the TTE ($P<0.05$: Table. 6.1). Post hoc analysis revealed that all of these variables improved from pre to post in both trials ($P<0.05$). The percentage improvement in the 40km TT was ($8.1 \pm 7.7\%$) in P and ($4.2 \pm 5.7\%$) in T, with no difference in the percentage improvement between the training periods ($P>0.05$). The percentage improvement in the TTE was higher in P ($85 \pm 43\%$) than T ($37 \pm 47\%$) ($P<0.05$). There were no main effects of time for the percentage of CD4+ and CD8+ cells among total T-cells (CD3+), or the percentage of all T-cells expressing CD57 and CD28 within the CD8+ or CD4+ T-cell subsets ($P>0.05$; Table. 6.2). There were no main effects of time in the proportion of low or high differentiated or senescent T-cells within the CD8+ or CD4+ T-cell subsets ($P>0.05$; Fig. 6.1).

Table. 6.1 Performance and heart rate responses before and after the two training interventions

Training Intervention	Time Point	40km TT MPO (W)	40km TT mean HR (bpm)	TTE at 95% PPO (s)	Peak HR during the TTE at 95% PPO
P	Pre	285 (±40)	168 (±11)	290 (±61)	176 (±9)
P	Post	307 (±37)*	166 (±11) *	523 (±130) *	173 (±8) *
T	Pre	298 (±34)	169 (±11)	293 (±49)	178 (±11)
T	Post	310 (±38) *	167 (±11) *	410 (±187) *	174 (±10) *

P: Polarised Training Model, **T**: Threshold Training Model, **TT**: Time Trial, **MPO**: Mean Power Output, **HR**: Heart Rate, **TTE**: Time To Exhaustion, **PPO**: Peak Power Output. *different from pre ($P<0.05$).

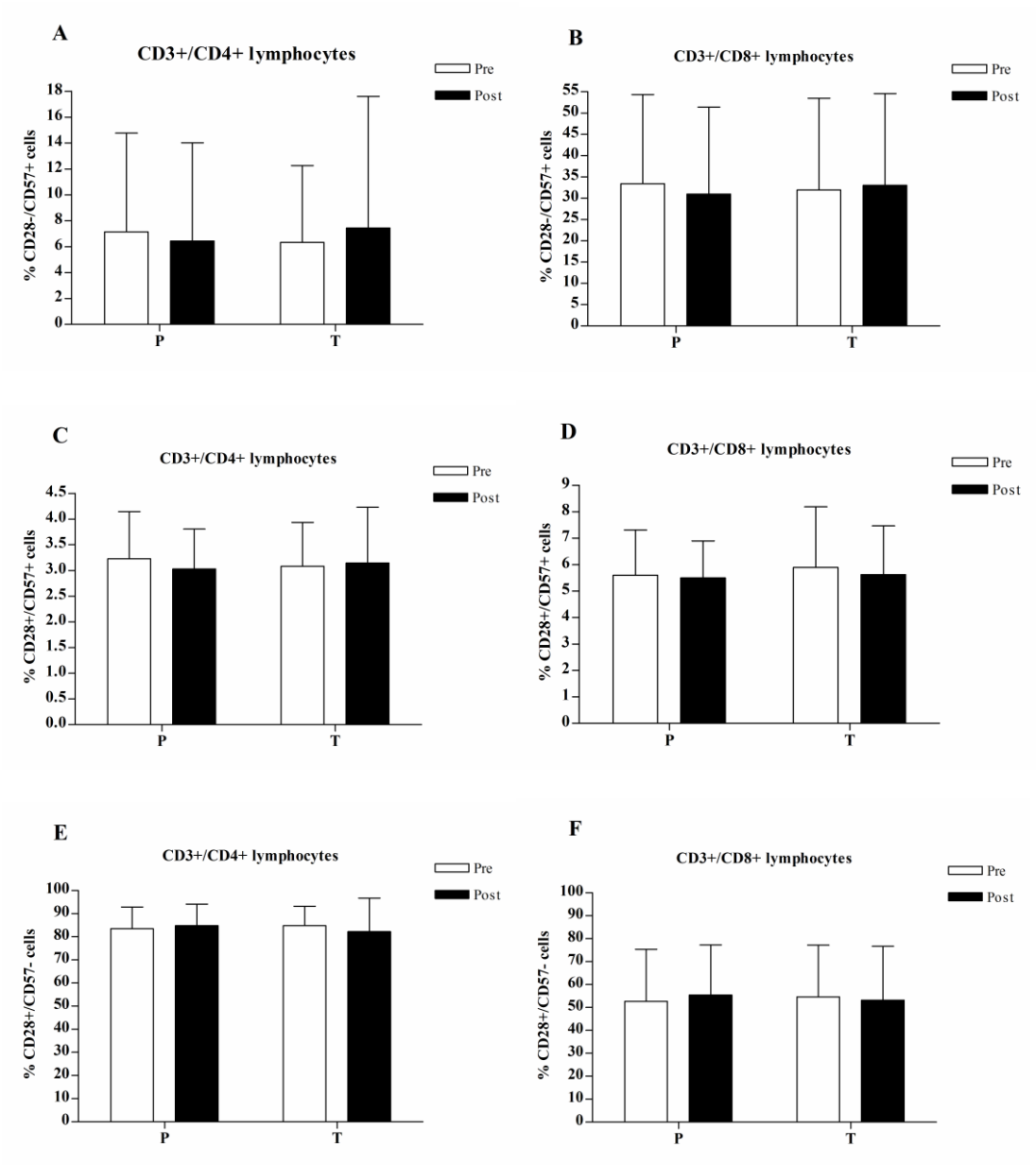

Fig. 6.1 The proportion of senescent (A, B), highly differentiated (C, D) and low differentiated (E, F) T-cells within the CD4+ (A, C, E) and CD8+ (B, D, F) T-cell subsets.

Table. 6.2 The proportions of CD4+ and CD8+ cells among total T-lymphocytes (CD3+) along with the CD4/CD8 ratio. Values are mean ± SD.

	% of T-lymphocytes (CD3+)			
	Pre P	**Post P**	**Pre T**	**Post T**
CD4+	37.9 (±7.2)	38.7 (±7.6)	38.6 (±5.7)	36.9 (±6.5)
CD8+	22.3 (±5.4)	22.3 (±7.3)	24.0 (±8.1)	22.1 (±7.0)
CD4+/CD8+	1.8 (±0.6)	1.9 (±0.8)	1.8 (±0.7)	1.8 (±0.7)

6.5 DISCUSSION

Following two different 6-week endurance training programmes, there were no differences in the proportion of senescent, highly differentiated or low differentiated T cells on CD4+ or CD8+ in rested fasting blood in trained cyclists, but endurance performance and physiological adaptation significantly improved following both training programmes.

Previous research in trained triathletes reported a significant increase in the proportion of senescent T cells and a significant decrease in the proportion of naive T cells, at rest, three weeks after an ironman triathlon race compared with three weeks before the race (Cosgrove et al., 2011). Similarly, another study reported a decrease in the proportion of CD4+ naïve T-cells expressing CD45RA following regular, high intensity exercise (Hack et al., 1997). Expression of the cell surface marker, CD45RA is indicative of a naïve T cell (Akbar et al., 1988). These studies are suggestive of a change in the immune space, which is likely to leave the athlete at a greater risk of infection, due to a shrunken naive T cell repertoire. The study by Cosgrove and colleagues (Cosgrove et al., 2011) demonstrates that changes in the proportion of senescent T cells can be significantly changed in resting blood, in trained endurance athletes, in just six weeks. However, during the 6-month build-up to the race, there was no change in the proportion of senescent T-cells, along with only modest physiological adaptation (Chapter 4). Therefore, it was hypothesised that by inducing a greater stress during training, which was likely to lead to greater physiological adaptation, would lead to an increase in the proportion of senescent T cells. It is possible that the subjects in the present study already had a higher percentage of senescent T lymphocytes (CD3+/CD4+) in resting blood than those in the study by Cosgrove and colleagues (Cosgrove et al., 2011). In the present study ~7% of CD4+ T lymphocytes were senescent (CD28-/CD57+), whereas in the 6-months training period in the study by Cosgrove and colleagues (Cosgrove et al., 2011), ~3% of CD4+ T cells were

senescent (KLRG1+/CD57+), significantly increasing to ~6% after the ironman triathlon race. The proportion of T lymphocytes with a senescent phenotype on CD8+ cells throughout the present study was comparable with those in the study by Cosgrove et al (2011) at ~30%. The largest difference between the present study and Cosgrove et al (2011) was the proportions of CD4+ and CD8+ T cells of the total T lymphocytes (CD3+). In the present study, CD4+ and CD8+ cells made up approximately 38 and 23%, respectively of the total T lymphocytes in comparison to 50-60% and 36%, respectively in the study by Cosgrove and colleagues (Cosgrove et al., 2011). In the present study, the cells were preserved with DMSO before being frozen until later analysis, whereas the cells were analysed from fresh in the study by Cosgrove and colleagues (Cosgrove et al., 2011). A strength of preserving the cells with DMSO is that all of the analysis from a single subject, at all four time points can be completed in a single day, as opposed to analysing each time point on a different day, and thus the present study had the advantage of a large intra subject reliability. However, it is possible that this preservation procedure affected the proportion of CD4+ and CD8+ T cells.

A decreased mitogen-stimulated lymphocyte proliferation and/or a decrease in the CD4/CD8 ratio are indicative of a reduced cell mediated immune response, and are sensitive to increases in training load in already well-trained athletes. A decreased mitogen-stimulated lymphocyte proliferation at rest has been reported following just six days of intensified training (73% increase in training load) in trained cyclists (Lancaster et al., 2004). The cyclists in the study by Lancaster and colleagues (Lancaster et al., 2004) also showed a significant decrease in time to exhaustion at ~74% VO_{2max} following the intensified training. A decreased CD4/CD8 has been reported following an increase in training volume of 35% for three weeks in a group of elite male distance runners (Verde et al., 1992). These studies demonstrate that cell-mediated immune response can be compromised following a period of intensified training. In the present study, there were no differences detected in the CD4/CD8 ratio. As

the subjects in the study by Lancaster and colleagues (Lancaster et al., 2004) decreased endurance performance following the intensified training period, they can be classified as being overreached. In the present study however, there were increases in endurance performance, and thus the subjects were not overreached, and this is a probable reason for a lack of change in the CD4/CD8 ratio and the proportion of T cells with a senescent phenotype.

Decreases in cell mediated immunity have also been reported in vivo, 48hr after prolonged, high intensity exercise in trained triathletes compared with non-exercising triathletes and non-exercising, moderately trained men (Bruunsgaard et al., 1999). In this study, several antigens were injected into the skin on the forearm following a half ironman race. Similarly, mitogen-stimulated T cell proliferation was decreased following both a 2.5hr run and a 2.5hr cycle at 75% VO_{2max} in a group of trained triathletes (Henson et al., 1999) and following an incremental test to exhaustion in trained male subjects (Fry et al., 1992). It is possible that repeated bouts of exercise that induce an acute reduction in T cell function eventually lead to a chronic decrease in cell mediated immune function. It has also been reported that the acute decrease in T cell proliferation is intensity-dependent, as shown by a 50% decrease following a 45min run at 80% VO_{2max} and just a 25% decrease following a 45min run at 50% VO_{2max}, 1hr following the exercise on both occasions. It is probable that there were acute decreases in T cell function following both training interventions in the present study, but this lead to no effect on the proportion of T cells with a senescent or highly differentiated phenotype.

As previously mentioned, it is possible that the decreased cell mediated immune response following intensified training could be due to an increased proportion of senescent T cells. It is also possible that an increase in senescent T cells in this situation could be largely due to oxidative stress in the athletes, as oxidative stress has been reported to lead to immune cell senescence (Larbi et al., 2007; van Baarle et al., 2005). In fact, oxidative stress has been

shown to down-regulate CD28 (Ma et al., 2003), which is indicative of cellular senescence (Effros 2004). As the proportion of CD28 was unchanged on CD4+ and CD8+ T cell subsets in the present study, it is unlikely that the two training programmes induced oxidative stress. In fact, it seems that to induce oxidative stress in well-trained athletes, the exercise needs to elicit overreaching (Almar et al., 2002; Okamura et al., 1997), which did not occur in the present study.

Another mechanism which could possibly cause an increased proportion of senescent and highly differentiated T cells following a period of intensive training is the reactivation of latent herpesviruses. This has been reported in elite athletes following a period of intensive training and was also reported to be related to the incidence of upper respiratory symptoms (Gleeson et al., 2002). Reactivation of latent herpesviruses causes T cell proliferation (Pawelec et al., 2009), and is associated with a greater frequency of senescent T cells in the periphery (Koch et al., 2007). Following persistent viral reactivation, excess T cell clones do not undergo post infection apoptosis, as they are needed for subsequent reactivations of the latent virus. Instead, they become part of the memory T cell pool, and therefore take up the so-called immune space in place of naïve T cells (Simpson 2011). This is however, theoretical and untested and therefore a pathway for future research.

Measurement of the proportion of naive T cells (CD45RA+/CD45RO-, KLRG1-/CD28+ would have strengthened this study and given more insight into the changes of the immune space. Measurement of telomere length by quantitative PCR would have also given more information about the changes in the T cells. A measure of oxidative stress would have provided information on whether there is a link between oxidative stress and senescent T cells *in vivo* and a measure of mitogen-stimulated lymphocyte proliferation would have given an insight into the function of the T lymphocytes. A measure of viral reactivation would have given

insight into the relationship between this measure and the proportion of low, high and senescent T cells. If future research assessing the effect of chronic endurance training on the proportion of senescent T cells in resting blood could incorporate these measures, our understanding would become much clearer.

6.6 CONCLUSION

Two endurance training programmes with similar volume but different distribution were sufficient to induce improvements in performance and physiological adaptation in well-trained cyclists. However, despite likely differences in recovery, both training programmes had no effect on the proportion of low or high differentiated or senescent CD8+ or CD4+ T-cells in blood. Therefore, training adaptation was achieved at no cost to this particular aspect of immune function. It is possible that a greater training stimulus (greater training load), could have an effect on the immune space and this is therefore an avenue for future research. From these results and evidence from previous studies, it seems likely that athletes need to be overreached to induce any change in immune function following a period of intensified training.

CHAPTER SIX – PART TWO

THE IMPACT OF AN IRONMAN TRIATHLON RACE ON EPSTEIN - BARR VIRUS AND VARICELLA - ZOSTER VIRUS ANTIBODY TITRES AND THE FREQUENCY OF HIGHLY DIFFERENTIATED AND SENESCENT BLOOD T-CELLS

6.7 ABSTRACT

Epstein - Barr Virus (EBV) and Varicella - Zoster Virus (VZV) are persistent herpesviruses that are maintained in a latent state after primary infection, but may reactivate in response to acute or chronic stress. The reactivation of latent viruses is indicative of a compromised immune system, which may also increase the frequency of highly differentiated and senescent T-cells. Such changes in response to a prolonged endurance race could have important implications for athlete infection risk. **Purpose:** To examine the impact of an ironman triathlon race on EBV and VZV antibody titres and the frequency of low and high differentiated and senescent blood T-cells in trained endurance athletes. **Methods:** Eight trained male triathletes (age 44 $\pm$ 5 yrs) provided a fasted resting blood sample in the morning 3-wks before an ironman triathlon race and 1, 2, 4 and 6-wks after the race. EBV and VZV IgG antibody titres were measured in plasma by ELISA. Isolated blood lymphocytes were labelled with monoclonal antibodies to assess cell surface co-expression of CD57 and CD28 to identify the proportions of low differentiated (CD28+/CD57-), high differentiated (CD28+/CD57+) and senescent (CD28-/CD57+) cells within both CD4+ and CD8+ T-cell subsets using four-colour flow cytometry. **Results:** 75% of triathletes were seropositive for EBV and 75% were seropositive for VZV. A main effect for the EBV-specific IgG titres over time ($P<0.05$) revealed higher titres 3-wks pre-race than 2, 4 and 6-wks post race and higher titres 1-wk post race than 6-wks post race. VZV-specific IgG titres did not change ($P>0.05$). Similarly, the proportion of low or high differentiated or senescent T-cells within CD8+ or CD4+ T-cells did not change. **Conclusion:** The triathlon race did not appear to elicit EBV or VZV reactivation or alter the frequency of low or highly differentiated or senescent T-cells in blood.The decrease in EBV-specific IgG titres indicates enhanced viral clearance during recovery from the race, which may have been facilitated by a substantially reduced training load.

Keywords: Latent herpesvirus, athlete health, cell surface markers, immune function

6.8 INTRODUCTION

In part one, it was reported that two endurace training programmes, with different training-intensity distributions but similar training volume volume resulted in no change in the proportion of low differentiated, high differentiated or senescent CD4+ or CD8+ T cells. The training stress induced by these training interventions was however, large enough to induce significant improvements in physiological adaptation and endurance performance. In another study (Cosgrove et al, 2011), we found that 3wks after an ironman triathlon race, there was a significant increase in the proportion of senescent T cells in resting, fasted blood, compared with 3wks before the race (Cosgrove et al, 2011). In addition, there was a significant decrease in the proportion of naive T cells 3wks after the ironman triathlon race, suggesting that there were changes taking place in the immune space, as suggested by Simpson (2011). During 6-months training for the ironman triathlon race, there were no changes in the proportions of senescent or naive T cell subsets. The physiological stress during the training however, was likely to have been minimal, as shown with a lack of physiological adaptation (chapter four). It therefore seems that the ironman triathlon race induced sufficient stress to cause changes in the 'immune space', whereas training for the event did not. We do not know however, the mechanisms responsible for these changes in the immune space 3wks after an ironman triathlon race. In addition, we do not know the time course of these changes, as in our previous study (Cosgrove et al, 2011), we only compared immune function changes from 3wks before the ironman traithlon race to 3wks following the race. It would therefore be useful to know exactly when these changes in the immune function markers took place following the race.

Epstein - Barr Virus (EBV) and Varicella - Zoster Virus (VZV) are persistent herpesviruses that are maintained in a latent state after primary infection, but may reactivate in response to acute or chronic stress. Reactivation due to stress has been reported to lead to T cell proliferation (Koch et al, 2006). Due to persistent viral reactivation, excess T cell clones do not undergo post infection apoptosis, as they are needed for subsequent reactivations of the latent virus. Instead, the excess T cell clones become part of the memory T cell pool, and therefore take up the 'immune space', in place of antigen-virgin naive T cells, thus shrinking the naive T cell repertoire (Simpson, 2011).

Reactivation of persistent herpesviruses is associated with a greater frequency of senescent T cells in the periphery (Koch et al, 2007). In response to excess rounds of cell division, cells can lose the ability to proliferate, and become senescent (Effros, 2007). The proportion of naive and activated T cell populations making up the immune space is tightly regulated by homeostatic mechanisms (Freitas et al, 1996). Senescent T cells have been shown to be resistant to apoptosis (Spaulding et al, 1999), which may cause an accumulation of cells with a senescent phenotype in the immune space and a decrease in the naive T cell repertoire. Naive T cells are vital in immune responses to novel pathogen and therefore a decrease in the naive T cell repertoire could leave an individual at a greater risk of infection (Koch et al, 2007).

The purpose of the present study was therefore to examine the impact of an ironman triathlon race on EBV and VZV antibody titres and the proportion of senescent, highly differentiated and low differentiated blood T-cells in trained endurance athletes.

6.9 METHODS

Eight trained male triathletes (age 44 ± 5 yrs) provided a fasted resting blood sample in the morning 3-wks before an ironman triathlon race and 1, 2, 4 and 6-wks after the race (Fig. 6.2). EBV and VZV IgG antibody titres were measured in plasma by ELISA.

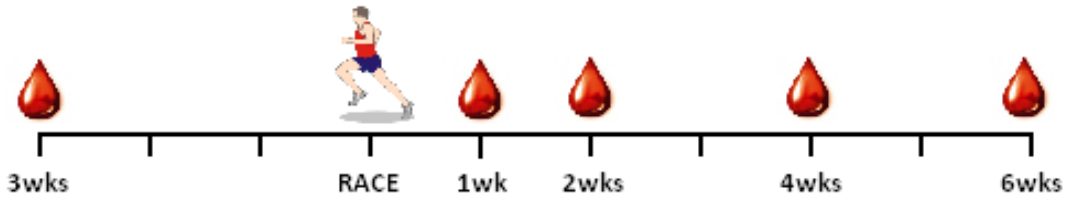

Fig. 6.2 Timeline of the study

Isolated blood lymphocytes were labelled with monoclonal antibodies to assess cell surface co-expression of CD57 and CD28 to identify the proportions of low differentiated (CD28+/CD57-), high differentiated (CD28+/CD57+) and senescent (CD28-/CD57+) cells within both CD4+ and CD8+ T-cell subsets using four-colour flow cytometry.

6.9.1 Statistical Analysis

Statistical analysis was performed using SPSS version 18 (Chicago, Il, USA). Data were analyzed for changes over time and between trials using separate maximum likelihood linear mixed model (LMM). The LMM method was used to fit a covariance matrix for the residuals to account for dependency of the repeated measures, allowing different variances for each time point and different covariances between time points. Three subjects had missing data points for the cell surface marker analysis, and therefore only five subjects were included in this analysis. Statistical significance was accepted at $p<0.05$.

6.10 RESULTS

75% of triathletes were seropositive for EBV and 75% were seropositive for VZV. A main effect for the EBV-specific IgG titres over time ($P<0.05$) revealed higher titres 3-wks pre-race than 2, 4 and 6-wks post race and higher titres 1-wk post race than 6-wks post race (Fig.6.3). VZV-specific IgG titres did not change over time ($P>0.05$; Fig. 6.4). The proportion of senescent, high or low differentiated T-cells within CD4+ or CD8+ T-cells did not change over time (Figs.6.5- 6.10). The training volume in the 3wk lead up to the race and in the 6wks after the race are shown in Fig. 6.11.

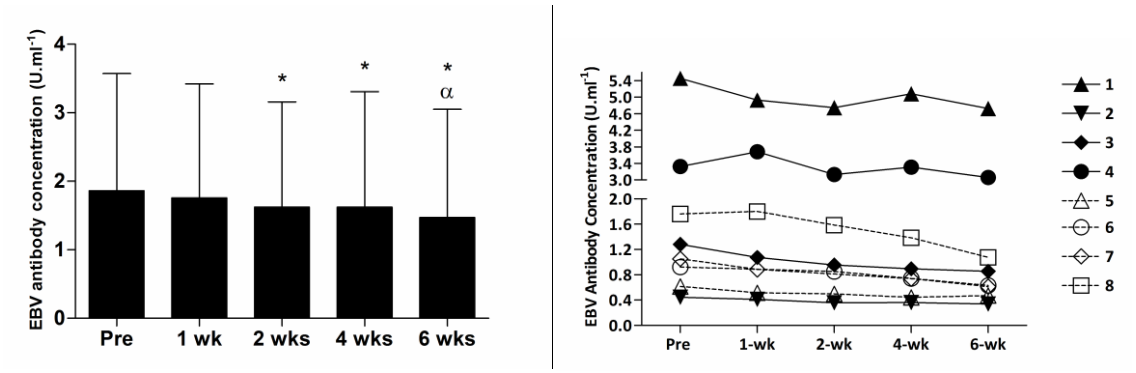

Fig. 6.3 Mean (±SD) EBV antibody concentrations at each time point (left) and the individual EBV antibody concentrations for each subject at each time point (right). * different from pre ($P<0.05$), α different from 1wk ($P<0.05$).

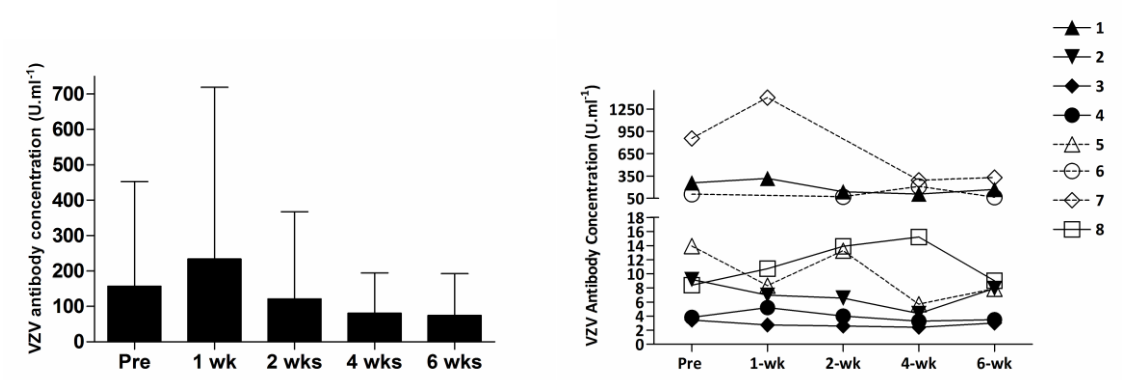

Fig. 6.4 Mean (±SD) VZV antibody concentrations at each time point (left) and the individual VZV antibody concentrations for each subject at each time point (right).

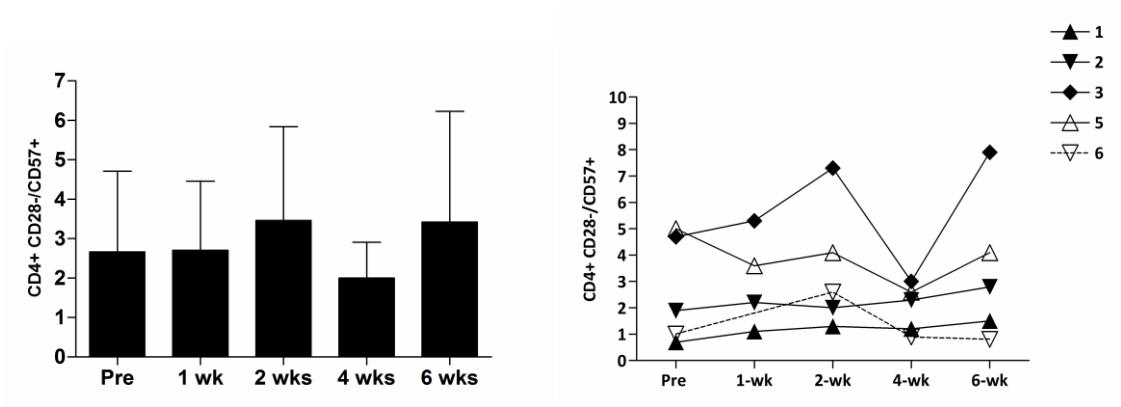

Fig. 6.5 Mean (±SD) proportion of CD28-/CD57+ on CD4+ at each time point (left) and the individual values for the proprtion of CD28-/CVD57+ on CD4+ for each subject at each time point (right)

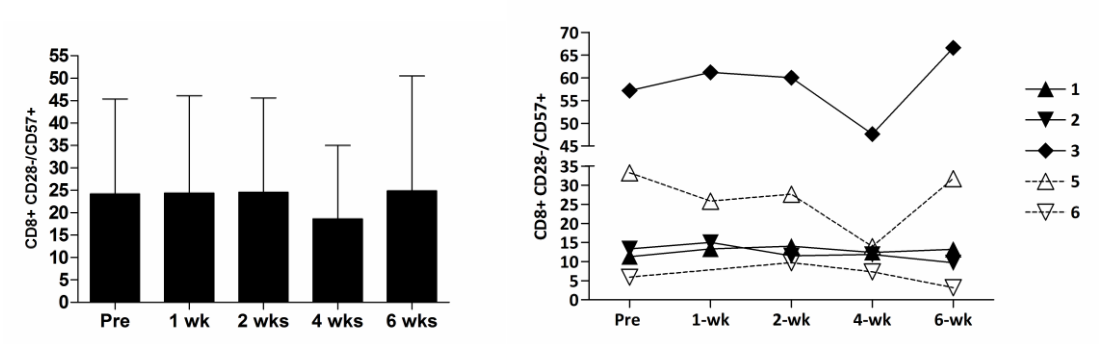

Fig. 6.6 Mean (±SD) proportion of CD28-/CVD57+ on CD8+ at each time point (left) and the individual values for the proportion of CD28-/CVD57+ on CD8+ for each subject at each time point (right)

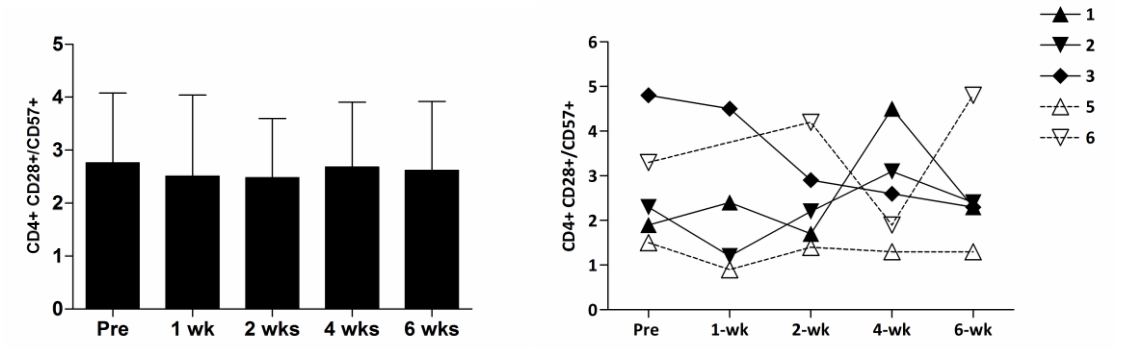

Fig. 6.7 Mean (±SD) proportion of CD28+/CVD57+ on CD4+ at each time point (left) and the individual values for the proportion of CD28+/CVD57+ on CD4+ for each subject at each time point (right)

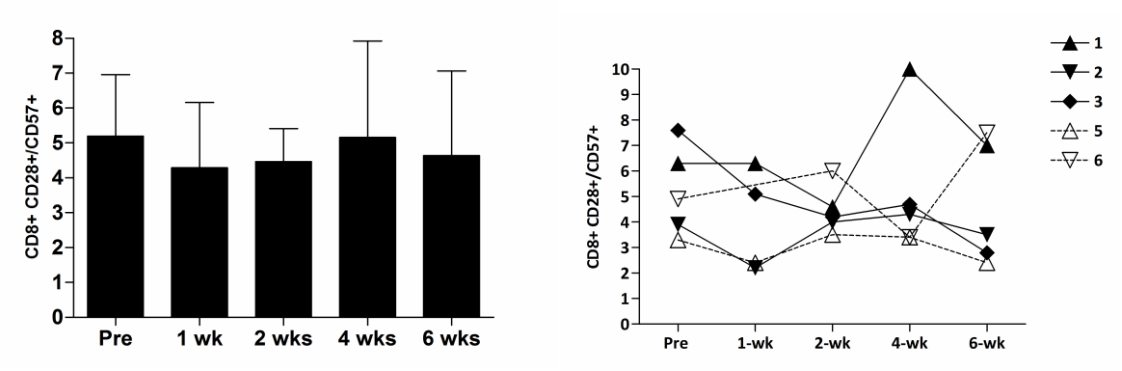

Fig. 6.8 Mean (±SD) proportion of CD28+/CVD57+ on CD8+ at each time point (left) and the individual values for the proportion of CD28+/CVD57+ on CD8+ for each subject at each time point (right)

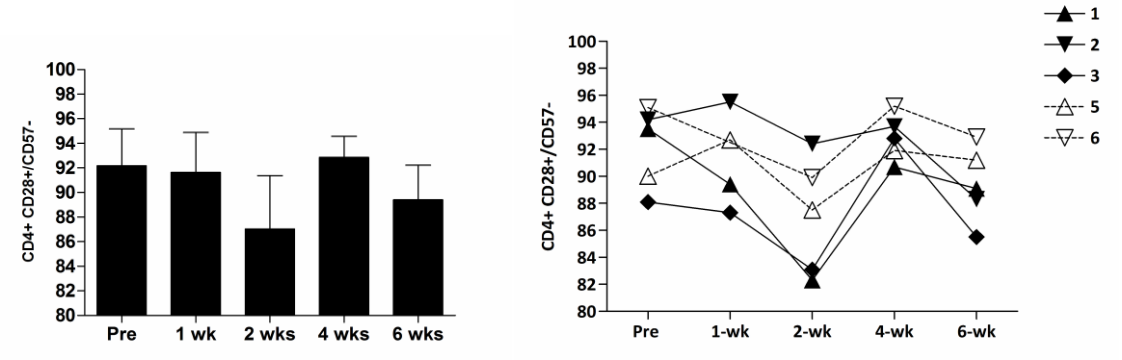

Fig. 6.9 Mean (±SD) proportion of CD28+/CVD57- on CD4+ at each time point (left) and the individual values for the proportion of CD28+/CVD57- on CD4+ for each subject at each time point (right)

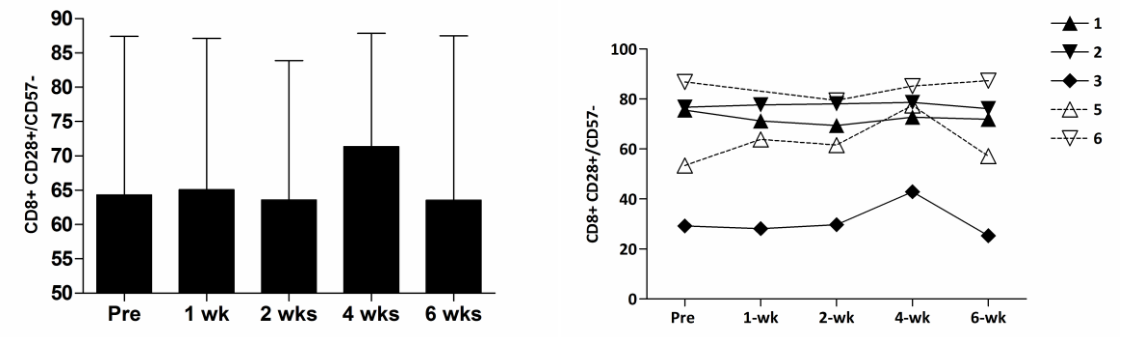

Fig. 6.10 Mean (±SD) proportion of CD28+/CVD57- on CD8+ at each time point (left) and the individual values for the proportion of CD28+/CVD57- on CD8+ for each subject at each time point (right)

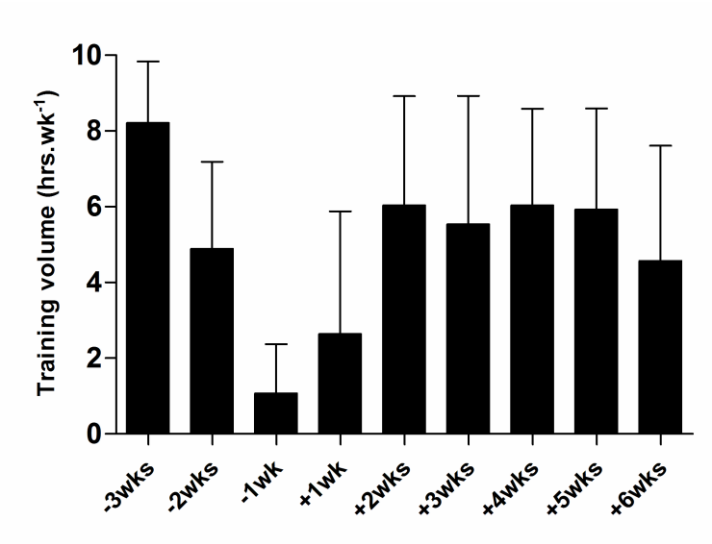

Fig. 6.11 Mean (±SD) training volume in each of the three weeks leading up to the race, and in each of the six weeks after the race.

6.11 DISCUSSION

The ironman triathlon race did not appear to elicit EBV or VZV reactivation or alter the frequency of senescent, highly differentiated or low differentiated T-cells in blood. The decrease in EBV-specific IgG titres indicates enhanced viral clearance during recovery from the race, which may have been facilitated by a reduced training load than before the study period. These results do not agree with our earlier work, showing an increase in the proportion of senescent T cells following an ironman triathlon race (Cosgrove et al, 2011).

Reactivation of latent herpesviruses has been reported to lead to T cell proliferation (Koch et al, 2006). This T cell proliferation may cause an increase in the proportion of senescent T cells in the periphery (Koch et al, 2007). As there was no change in the EBV or VZV-specific antibody concentrations over time in the present study, this would suggest that if the reactivation of latent herpesviruses is one of the factors causing an increase in the proportion of senescent T cells, then this is the reason for no change in the proportion of senescent T cells in the present study. We therefore, cannot discount a reactivation of latent herpesviruses as a potential mechanism for the increased proportion of senescent T cells in the study by Cosgrove et al (2011).

Of note in the present study, is the variability of the measures between subjects. The VZV and EBV-specific antibody concentrations range from approximately 3-900 $U.ml^{-1}$ and 0.4 – 5.4 $U.ml^{-1}$ for VZV and EBV, respectively. In addition, the proportions of senescent and low and highly differentiated T cells also showed large variation between subjects. Due to these variations, analysis will take place for individual subjects in the study:

6.11.1 Subject 7

Subject 7 was diagnosed with shingles 2wks after the ironman triathlon race. A reactivation of VZV causes singles, and accordingly, the antibody concentration for VZV was very high in this particular subject. In fact, the subject had a high VZV-specific antibody concentration 3wks before the race and 1wk after the race, and was not tested 2wks after the race due to the virus. By 6wks after the race, the VZV-specific antibody concentration had decreased to a similar magnitude to subjects 1 and 6, that did not have shingles. Unfortunately, there was a problem with the pre-race blood sample for the cell surface markers for subject 7, and so this data is unavailable. Subject 7 had a low EBV-specific antibody concentration in relation to the other subjects. The present study suggests that there is no relationship between EBV and VZV-specific antibody concentrations.

6.11.2 Subject 1

Subject 1 had high EBV and VZV-specific antibody concentrations in comparison with the other subjects, yet a low proportion of senescent T cells in comparison with the other subjects. This subject did have, however, a large increase in the proportion of highly differientiated T cells on both CD4+ and CD8+ from 2wks to 4wks, and a decrease in low differientiated T cells from 1wk post race to 2wks post race, that increased back to the 1wk post race value at 4wks post race. The subject had flu like symptoms for 10d in the 4^{th} and 5^{th} wk after the race, which caused this subject to stop training. At the time when these flu like symptoms were most severe (wk 4 after the race), the subject had the highest proportion of highly differentiated T cells, which increased by more than 2-fold from the 2wk sample for both CD4+ and CD8+. The proportion of low differientiated CD4+ T cells decreased from approximately 93% pre race to 82% 2wks after the race, and this increased back up to approximately 90% 4wks after the race. These results are suggestive that the proportion of highly differientiated CD4+ and CD8+ T cells was directly related to the subjects symptoms. It is possible that the subject was at more risk of

infection than the other subjects, as both the EBV and VZV antibody titres were high throughout the study period. The proportion of senescent T cells however, were unaffected by the infection.

6.11.3 Subject 3

Subject 3 had a high proportion of senescent CD4+ and CD8+ T cells and a low proportion of low differientiated CD8+ T cells in comparison to the other subjects. However, both VZV and EBV-specific antibody concentrations were low in comparison to the other subjects. Therefore, another mechanism other than the antibody concentration of EBV and VZV may be involved in this subject having a high proportion of senescent CD4+ and CD8+ T cells and a low proportion of low differientiated CD8+ T cells.

The subjects with the highest antibody titres 3wks before the race for EBV (subject 1) and VZV (subject 7) got infections following the race. Therefore, although speculative, it is possible that antibody titre analysis 3wks before a competition such as an ironman triathlon might have the potential to identify individuals who are at most risk of infection post race, despite being otherwise healthy. It might be that these athletes should not subject themselves to the stress of a race such as an ironman triathlon. It is possible that antibody titre analysis could have the potential to help protect endurance athletes during training, as a large training stress, or a series of large training stresses might also cause an infection. This is highly speculative, but is an exciting and worthwhile area for future research.

6.12 CONCLUSION

The mean results of the present study suggest that there is no relationship between EBV and VZV-specific antibody concentrations and the proportion of senescent, low and highly differientiated T cells. However, on analysis of individual subject data, it seems possible that subjedcts with a high antibody titre for EBV or VZV 3 wks before a competition might be more at risk of infection post race. A greater subject number would be needed in order to make a more conclusive statement about this relationship. As such a large proportion of the population have both EBV and VZV, perhaps reactivation of these specific herpesviruses has less of an impact on the proportion of senescent T cells than a herpesvirus that affects a lower proportion of the population, such as cytomegalovirus.

CHAPTER SEVEN

SUMMARY AND CONCLUSIONS

7.1 SUMMARY AND CONCLUSIONS

Elite endurance athletes have been reported to train with a large training volume relative to their event, and of the overall volume, a large proportion has been reported to be spent at a relatively low intensity (~80% of total training volume), with the remaining training volume at a relatively high intensity. However, research is lacking as to whether this represents an optimal training-intensity distribution in already well-trained endurance athletes. In fact, it has been reported that in some well-trained endurance athletes, alternative distributions in training intensity lead to larger improvements in physiological adaptation and endurance performance. Our understanding of the mechanisms responsible for these findings is thus limited. The novel data in this thesis may hopefully enhance our understanding of the responses of well-trained endurance athletes to different distributions in training intensity.

The first study assessed the reproducibility of the LT, the LTP and a maximal performance measures in swimming, cycling and running in already well-trained endurance athletes. Reliable measurement of the LT and the LTP are vital in the study of training-intensity distribution, as they demarcate three training-intensity zones. This study found that the LT, the LTP and maximal performance measures in swimming, cycling and running were all reproducible in already well-trained endurance athletes. These measures could therefore be used for the remainder of the studies in this thesis.

The second study assessed the training-intensity distribution in a group of well-trained triathletes in the 6-month build up to an ironman triathlon. Training intensity for each discipline was demarcated into three zones with the LT and the LTP and a maximal performance measure was assessed, using the methods from the first study. The HR corresponding to the LT and the LTP was used to determine the training-intensity distribution, using the TIZA. The athletes recorded every training minute into an online training log and the

amount of time spent in each of the three training intensity zones was recorded. The athletes were tested four times in the 6-month build up to the event, approximately every 2-months. This was the first study to assess the training-intensity distribution in multisport athletes. Overall, for all of the disciplines combined, the athletes spent 69, 25 and 6% of training time in zones 1, 2 and 3, respectively and only achieved modest improvements in physiological adaptation. The largest adaptations occurred in running, in which the athletes spent more time in zone one and less time in zone two than swimming and cycling. Relationships between the time spent in each zone and the magnitude of adaptations were unclear, as the initial training status of the athletes was significantly related to the magnitude of adaptation. It was clear from the second study that in order to assess the effect of training-intensity distribution on endurance performance and physiological adaptation, the training variables would need to be tightly controlled in an experimental setting.

The third study assessed the impact of manipulating the training-intensity distribution in a group of trained cyclists, using a cross-over, within subject study design. A polarised training model with a high proportion of training at a low intensity and the remaining training at a high intensity was compared with a threshold training model, with a high proportion of training at a moderately-high intensity and the remaining training time at a low intensity. Athletes in the polarised model had a significantly lower overall training volume and training load compared with the threshold training model. Although both training interventions significantly improved 40km time-trial performance, there were larger improvements in the peak power output and the time to exhaustion at 95% peak power output following the polarised training model compared with the threshold training model. In fact, all of the measures for endurance performance and physiological adaptation had higher effect sizes following the polarised model compared with the threshold model. This suggests that over a short-term training period (6 weeks), it is advisable to adopt a polarised training approach. If training time is

limited, the high intensity intervals in the polarised training model appear to compensate for the smaller overall training volume, and so a polarised approach including high intensity intervals is effective in this situation.

The fourth study assessed the impact of two different training-intensity distribution models on the proportion of low and high differentiated and senescent T cells in trained cyclists. Both training interventions caused large improvements in endurance performance, along with no effect on the proportion of low or high differentiated or senescent T cells. Therefore, the improved endurance performance did not come with an increased proportion of senescent T cells, which would probably lead to an increased susceptibility to picking up a viral infection.

The fifth study assessed the effect of an ironman triathlon race on EBV and VZV antibody titres and the proportions of low and high differentiated and senescent blood T cells. We have previously reported that an ironman triathlon race causes an increase in the proportion of senescent CD4+ T cells, alongside a decrease in the proportion of naive CD4+ T cells. In the fifth study, in comparison to 3 wks before the race, there was no difference in the proportion of low or high differentiated or senescent CD4+ or CD8+ T cells, 1, 2, 4 and 6wks after the race, in contrast to our previous work. There was no difference throughout the study period for the VZV antibody titre, but the EBV antibody titre decreased over time after the race, which could have been due to a lower training load than before the race. On analysis of the individual data, this study revealed that it might be possible to use pre race antibody titres to identify individuals that might be at higher risk of an infection post race. This is however highly speculative, although is an exciting area for future research.

The results of the studies in this thesis contribute to the understanding of the broad area of maximising endurance performance, with a focus on the variable of training-intensity distribution. Further studies are clearly needed in order to enhance our understanding of this broad area, and in particular, a greater variety of manipulation of the training-intensity distribution is needed before clearer conclusions can be made as to the effectiveness of this variable. So far, only Esteve-Lanao et al (2007), Ingham et al (2008) and chapter 5 of this thesis have tightly controlled the training-intensity distribution in well trained endurance athletes. Chapter 5 in the present thesis has been the most controlled study to date, with power output, HR and RPE being controlled for every training session above the LT. In addition, every training session above the LT took place in the laboratory. A cross-over study design was used, with the subjects acting as their own controls. This approach proved successful, with a 4-week de-training period between the 6-week intervention periods, as shown by all of the measures returning to baseline magnitudes. Chapter 5 was also the first training study with a focus of training-intensity distribution to use the LT and the LTP to demarcate three exercise intensity training zones. These zones are relatively easy and inexpensive to measure, and therefore, this method of demarcating exercise intensity training zones could be widely used by researchers and coaches in the area of endurance training. Due to the lack of controlled experimental studies exploring the effect of isolating the variable of training-intensity distribution, it is suggested that more studies are needed in manipulating this variable.

The effectiveness of training in zone one has been referred to on many occasions in this thesis. Although several studies (Fiskerstand & Seiler, 2004; Esteve-Lanao et al, 2005; Esteve-Lanao et al, 2007, Ingham et al, 2008; chapter 4 of this thesis) have found a positive outcome of more training in zone one, none of these studies focussed on this primarily. It was found in chapter 5 of this thesis that with the absolute amount of time spent in zone one matched between two

groups with different training-intensity distributions, it was the group with the higher percentage of time spent in zone one that had the greatest benefits to endurance performance and physiological adaptation. However, just as in the other published literature, this is not necessarily cause-and-effect, as there were other variables contributing to the results, with the amount of time spent in either zone two or zone three likely contributing heavily to the outcomes. Further research is therefore needed in order to isolate the variable of absolute or percentage of time spent in zone one, so that this variable can be considered important in affecting physiological adaptation and endurance performance. One such study would be to assess the training volume in zone one that causes an increase in endurance performance and physiological adaptation, as it has been suggested that training in zone one is sufficient to maximise the increase in mitochondria in type I muscle fibres (Dudley et al, 1987). Therefore, with a homogenous group of athletes, the number of minutes spent training only in zone one could be increased at regular intervals, and testing could take place at regular intervals to assess the effect of the increased training volume (Fig. 7.1). With this study design, the athletes would only increase their training volume if endurance performance had increased above the day-to-day variation. Using this approach, the training is individualised, and therefore, the effect of an increased training volume can be assessed for each individual athlete. This study would follow on from the study by Ingham et al (2008), in which zone one training in elite rowers was found to be very effective at inducing physiological adaptations, especially related to the lactate threshold.

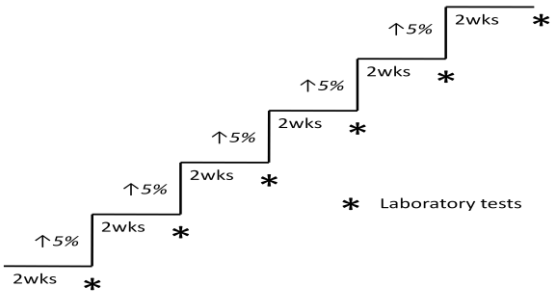

Fig. 7.1 A study design to assess the effectiveness of an increased volume of training in zone one on already well-trained endurance athletes

In this thesis, both group mean data and individual data were presented, and both of these options have advantages and disadvantages. By presenting group mean data, the overall effectiveness of an intervention can be clearly seen, yet individual responses are disregarded. By presenting individual data, the overall effectiveness of the intervention may be overshadowed by certain individual responses. In the area of training-intensity distribution, it is important to consider both of these ways of presenting the data. If training-intensity distribution and training load are fixed (as in chapter 5), then individual athletes will respond differently. It has been suggested that there are responders and non-responders (Vollaard et al, 2009) to exercise, and indeed, it can be seen from chapter 5 of this thesis that individuals respond differently to the same exercise stimulus. This is why it has been suggested that where possible, to individualise the training of the individuals for the intervention, especially the training load. Take the example of two cyclists, with mean training volumes for the 12 months prior to the study of 30 and 15 hours per week. By setting a training volume of 20 hours per week, it is possible that the cyclist entering the study with the higher training volume could decrease endurance performance, due to insufficient training volume and load. In contrast, it is also possible that the cyclist with the lower weekly training volume could decrease endurance performance due to overreaching or overtraining. Group mean data would merely show a decrease in endurance performance using the training intervention, but this can be avoided. One way of avoiding this scenario is to closely analyse the athletes

training diary for the 6 months previous to the study, and attempt to set a training load that is slightly lower than that which the athlete is accustomed to. During the study, increases in training load can be made, based on the athlete's responses to the training. If a cross-over study design is used, the training load would need to be matched for both training interventions. Another approach could be to pair-match individuals with similar training loads in the previous 6 months and match the training for these individuals.

One important issue when assessing the effectiveness of training in zone one is the intensity of exercise. In well trained endurance athletes, there is a wide range of exercise intensities corresponding to zone one. It can be assumed that there will be a relative exercise intensity at the lower end of zone one in which training becomes ineffective, but at present, this relative exercise intensity is not known. It could also be possible that training at the highest intensity in zone one (i.e. the highest exercise intensity before the blood lactate increases distinctly above resting levels (Aunola & Rusko, 1984)) is the most effective training intensity for zone one, and this is a potential study for future research. As a polarised training-intensity distribution, and more specifically, a training programme including 80, 0, 20% of training in zones one, two and three has been proposed as optimal, a potential study assessing the intensity of zone one training could use this distribution (Table 7.1). The study could have two groups, with fixed overall training load, training volume, and the intensity of zone three training. In line with chapter 5, the 72min of zone 3 training could consist of three sessions per week of 6*4min intervals, with 2min recovery. Therefore, the only difference in the two training programmes would be the intensity of zone one. It could be suggested that the two groups could include zone one intensities of 100 and 50% of LT, respectively. Therefore, if there is a difference in adaptation over a 6-week training programme, it is likely that the zone one intensity was a factor. Indeed, this study approach could also be used to assess the effectiveness of the exercise intensity used for zone two and zone three, with a fixed training-

intensity distribution. However, as zone two is relatively narrow in well-trained athletes and the range of exercise intensities in zone three that can be used for 4-min intervals also has a narrow range, these issues are likely to be less important. However, if an athlete does have a wide range of exercise intensities corresponding to zone two, there could be an effect of training at the lower and upper ends of this scale. In addition, it is possible that the zone 3 intensity used is very sensitive to overall performance gains, and so is an area worth exploring.

Table 7.1 A proposed study to assess the effectiveness of the training intensity in zone one

Group	**Training-intensity distribution (% of time in zones 1, 2 and 3)**	**Training volume (min.wk^{-1})**		**Training load (TRIMPs.wk^{-1})**	**Training intensity**	
		Zone 1	**Zone 3**		**Zone 1**	**Zone 3**
1	80, 0, 20	294	72	500	50% of LT	10% > LTP
2	80, 0, 20	294	72	500	100% of LT	10% > LTP

TRIMP: Training Impulse, **LT**: Lactate Threshold, **LTP**: Lactate Turnpoint

The results of chapters 4 and 5 suggest that an excess of training in zone two might have a detrimental effect on training, in concurrence with previous studies (Londeree, 1997; Esteve Lanao et al, 2007). However, in order to assess the effectiveness of training in zone two, studies focussed upon this factor need to be carefully designed. These studies could contain two groups, with the overall training volume load (in TRIMPs) fixed for the two groups, along with the intensity of training in the three zones. A manipulation of the percentage of time spent in zone two could be attempted. It would be useful if 0, 100, 0% of training time in zones 1, 2 and 3, respectively was compared with 50, 50, 0%. Moreover, including zone 3 in the training would be useful to observe, and so comparing 80, 0, 20% of time in zones 1, 2 and 3, respectively with 0, 80, 20% would give a clearer idea as to the overall effect of training in zone 2. It is also possible that there is an overall training load which acts as a threshold,

beyond which the effects of excess training in zone 2 become detrimental. This possibility is due to the suggestions that an excess amount of training in zone 2 causes a down regulation of the sympathetic nervous system (Esteve-Lanao et al, 2007), and a delayed recovery (Seiler et al, 2007). However, if the training load was such that recovery was possible, then the effects of zone 2 training might not be detrimental. Nevertheless, chapter 5 in this thesis, and other studies (Esteve-Lanao et al, 2005; Esteve-Lanao et a;, 2007; Londeree, 1997) still provide evidence that a polarised training model including approximately 80% of training in zone 1 and 20% of training in zone 3 is more beneficial than a threshold training model, with a large proportion of training in zone 2.

In chapter 5, it is suggested that training in zone 3 is more beneficial to improvements in endurance performance than training in zone 2. However, as chapter 5 only investigated the effectiveness of training with 20% of time in zone 3, the effectiveness on endurance performance of other manipulations of the proportion of training in zone 3 are largely unknown. Research in the area of optimising interval training suggests that 4-min intervals (Stepto et al, 1999; Seiler & Sjursen, 2004) and a two minute recovery period (Seiler & Hetlelid, 2005) are effective in well-trained endurance athletes. Esteve-Lanao et al (2007) attempted to include 15% of training in zone 3 using the total time in zone approach, and HR monitoring in a group of well trained endurance runners. It should be noted that the total time in zone approach significantly underestimates the time spent at higher exercise intensities due to the delay in HR at the start of each exercise interval. Therefore, the 15% of time attempted by the athletes in the study by Esteve-Lanao et al (2007) would certainly be much higher if the session goal method was adopted. Therefore, further manipulation of the percentage of time spent in zone three is required, using two groups, with the overall training load matched between the groups. A useful comparison would be that of 80, 0, 20% of time in zones 1, 2 and 3, respectively with 60, 0, 40%, using the session goal approach. Moreover,

introducing some training in zone 2 into the comparison would also be a worthwhile comparison, with 60, 20, 20% of training time in zones 1, 2 and 3, respectively, compared with 80, 0, 20%. As chapter 5 has reported a training-intensity distribution of 80, 0, 20% of training time in zones 1, 2 and 3, respectively to be effective at improving endurance performance in well trained cyclists, it would be useful to use this distribution as a comparison for other manipulations of the training-intensity distribution.

Once a greater understanding of the effectiveness of different training-intensity distributions has been established, then the overall training load (in TRIMPs) and training volume (in minutes) can be manipulated. In order to do this, the training-intensity distribution needs to be fixed, as does the intensity chosen within each training zone. Therefore, in two groups, with the same training-intensity distribution, a difference in the overall training load in one group, but not the other would be likely to demonstrate the effectiveness of training with a greater training load. One group could increase training load by 50 TRIMPs every two weeks, whilst keeping the same training-intensity distribution while the other group maintains the same training load throughout. Alternatively, a group of athletes could all start training at a relatively low TRIMP, which is individualised based on analysis of training diaries. Testing could take place every two weeks, and if the athlete has improved by a greater amount than the day-to-day variation in each measure of endurance performance, then the training load can be increased by 5%. The advantage with the latter approach is that individual responsiveness is taken into account, and the aim of the study, 'to assess whether a higher training load leads to a greater improvement in endurance performance with a fixed training-intensity distribution', is investigated, but on an individual athlete basis. In all of these studies, however homogenous a group of athletes are, there will still be differences in ability and rate of improvement. Therefore, where possible, it is advisable to individualise the training load to suit the individual. Therefore, chapter 5 could have been improved by individualising the

training load for each athlete, whilst keeping the training-intensity distribution fixed in each group.

More descriptive studies assessing the training-intensity distribution of truly world class endurance athletes, along with the closely controlled manipulations of training-intensity distribution previously mentioned on well trained endurance athletes would greatly improve our understanding of how to optimise endurance performance by manipulating the variable of training-intensity distribution. During the controlled experimental studies proposed previously, it is clearly important to test for the correct measures at the correct times in each study. Assessments of physiological adaptation, endurance performance, overreaching/overtraining, recovery, and athlete health need to be appropriate, and some ideas are presented below:

Physiological adaptation:

- The lactate threshold/lactate turnpoint
- Ventilatory thresholds 1 and 2
- sEMG thresholds 1 and 2
- Catecholamine thresholds 1 and 2
- Oxygen uptake throughout an incremental exercise test (exercise economy)
- Maximal oxygen uptake
- VO_2 kinetics
- Substrate metabolism (carbohydrate and fat)
- Skeletal muscle glycogen content
- Mitochondrial biogenesis (PGC1-α, AMPK, CaMK, NRF-1, Tfam)
- Oxidative and glycolytic enzymes
- Skeletal muscle fibre type
- MCT1, MCT4
- Buffering capacity
- Cardiovascular measures (heart rate, stroke volume and cardiac output)
- a-VO_{2diff}
- Capillary density
- Lung function

Endurance performance:

- A time trial (fixed distance)
- A time to exhaustion test
- A fixed time test, and assess mean intensity sustained

Measures of overreaching/overtraining:

- A time trial or a time to exhaustion test, such as the 'stress test' (Urhausen et al., 1998)
- Heart Rate Variability
- Psychological questionnaires
- Hormones (catecholamines, cortisol, prolactin, adrenocorticotrophic hormone)
- Salivary IgA
- IL-6
- CD4+/CD8+ ratio
- Mitogen-induced IgG/IgM synthesis
- Lymphocyte proliferation
- Blood leukocyte count
- Neutrophil function (oxidative burst in response to bacterial stimulant in vitro)
- Plasma glutamine
- Creatine kinase

Measures of recovery:

- HRV
- Muscle soreness
- Inflammatory markers
- Performance in one of the endurance tests described above
- Measures of overreaching/overtraining have returned to baseline

Measures of athlete health:

- Self-report of any episodes of illness
- Humoral immunity (IgA, IgG, IgM)
- Cellular immunity (T cell activation, T cell cytokine release, T cell proliferation)
- Latent viral reactivation (EBV, VZV, CMV)

In addition, the assessments of training intensity zones and training intensity during training need to be carefully thought about, and some ideas are presented below:

- A heart rate monitor
- A power meter for a cyclist (SRM or power tap)
- Lap/split times
- Global Positioning System (GPS)
- RPE
- Blood lactate

Chapters 6a and 6b assessed the effect of two different six week training interventions, and an ironman triathlon race, respectively on the proportion of low and high differentiated and senescent T cells in blood. Future studies should consider the limitations of the methodology chosen for these studies. The leukocytes in the blood were assessed for the cell surface markers that were of interest in these studies. The leukocytes in the blood are the only readily accessible leukocytes when conducting research in humans (Gleeson, 2006). This only tells us about the blood leukocytes, and not the leukocytes at other sites in the body (in the skin, lymph nodes and mucosa of the respiratory tract and gut). Moreover, at any one time, the majority of lymphocytes are not circulating in the blood and so any observed changes in peripheral blood lymphocytes may not be representative of changes that may occur in lymphocytes in other bodily tissues (Gleeson, 2006). However, many lymphocytes are in constant movement via the circulatory and lymphatic systems, and so lymphocytes in the blood are a functionally important group of cells to measure (Gleeson, 2006). Therefore, it is likely that assessing the lymphocytes in the peripheral blood is a useful measure, but a close consideration of the above theory should be used.

Immune function can be assessed in vivo or in vitro, with advantages and disadvantages of both approaches. Using the in vitro approach, the experimental conditions are highly controlled, and so detailed dose-response studies can be performed. However, in vitro systems are often highly unphysiological, using cells in isolation from other components with which they would normally react, in vivo. Therefore, care should be taken when extrapolating from in vitro studies to the whole body context. In chapter 6, it was assumed that senescent T cells were unable to undergo apoptosis, based on results of previous research in vitro (Spaulding et al, 1999; Wang, 1995). If this is indeed true in vivo, then the finding of an increased proportion of senescent cells in the blood would be irreversible. It would be useful for the area of immune function in endurance athletes if more studies were conducted like

that of Bruunsgaard et al (1997), assessing the whole body response to exercise, in vivo. This was achieved by injecting several antigens into the skin on the forearms of trained triathletes following a half ironman event. This should result in a raised red swelling, due to the stimulation of an immune response to each of the antigens. The sum of the diameters of the swelling gives an indication of the immune response. If this approach was adopted using training studies, a clearer idea of the whole body immune response at different stages of the training could be assessed.

In chapters 6a and 6b, the timing of the blood samples could have been a factor in the results collected. Indeed, a time course study was not conducted for these measures, and therefore, there is some doubt as to whether the timing of the blood samples might have been an issue. Indeed, there does not seem to be a time course study for the measures of low and high differentiated and senescent blood t cells in the literature. Our aim was to achieve a rested, fasted blood sample, with the participants not exercising or eating for 12 hours prior to the blood samples. Moreover, the blood samples were taken at the same time of day to minimise the effects of diurnal biological variation (Atkinson & Reilly, 1996). However, it is possible that taking the blood sample between 7:00 and 9:00 was not appropriate, and therefore, future research should aim at conducting a time course study for these measures before any further assessment following an intervention. Another aspect of chapters 6a and 6b that should be considered for future research is that of participant diaries to record any symptoms of illness. Although in chapter 6b, there was some evidence of illness from the participants, this was not planned formally. Therefore, future research should be sure to inform athletes to record any signs of illness, along with exact time, details of the symptoms and severity.

Chapters 6a and 6b would have been improved if the following measures would have been taken:

- The proportion of naive T cells (CD45RA+/CD45RO-, CD57-/CD28+),
- The telomere length (quantitative PCR)
- Oxidative stress (malondialdehyde, 8-hydroxydeoxyguanosine)
- Mitogen stimulated T cell proliferation
- T cell activation (CD69, CD25, CD45RO)
- T cell cytokine release (IL-2, IL-4, IL-5, IL-6, IL-13, IFN-γ)
- Serum immunoglobulins (IgA, IgG, IgM)
- Mucosal immunoglobulins (IgA)

These markers would give us a greater understanding of the effect of the training interventions on the proportion of T cell subsets. In future research wishing to analyse the effect of a training intervention on the immune space, as well as assessing the proportion of naive ad senescent T cells, it would also be useful to use another marker that has been shown to lead to cells becoming senescent, such as; telomere length and oxidative stress.

It was found that the LT and the LTP are reproducible in swimming, cycling and running (chapter 3). Therefore, future studies can use these markers to demarcate zones of exercise intensity when using these disciplines. Where possible, the session goal approach should be used (Seiler & Kjerland, 2006), as opposed to the total time in zone approach, as the latter has been shown to significantly underestimate the time spent at higher exercise intensities. In studies assessing the effect of the training intensity distribution, to under- and over estimate the amount of time spent in each zone of exercise intensity will clearly affect the responses to the training. When the aim of the research study is to assess the effectiveness of different training intensity distributions as a variable, one exercise discipline should be used. Moreover, due to the high controllability, it is recommended that cycling is used. Power taps and SRM meters can be used to continually monitor power output when riders are outside of the laboratory, along with HR monitoring. However, it is recommended that riders only complete sub-LT outside of the laboratory, due to the importance of standardising all exercise of greater

intensity than the LT. The advantage of using a power meter to measure the power output is that it is an absolute measure of exercise intensity, and is not influenced by conditions (gradient, wind, road surface), whereas speed (from a GPS) is affected by these factors. However, power output from an SRM or power tap are not influenced by temperature or humidity, and it is an advantage of a heart rate monitor that heart rate is affected by these factors. Therefore, along with HR, the power output can be used to prescribe training intensity outside of the laboratory. In addition, it has been demonstrated in this thesis that a within subject study design is possible for a training study in a group of trained endurance athletes, with a 4-week training wash-out period to diminish the adaptations gained from the previous intervention (chapter 5).

CHAPTER EIGHT

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8.1 REFERENCES

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