The influence of high intensity training on cardio-respiratory biomarkers, aerobic metabolism and time trial performance in recreationally-trained individuals

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Abstract.

The purpose of this study was to investigate the influence of high intensity sprint training, interval training and aerobic training on cardio-respiratory biomarkers, aerobic metabolism and time trial performance in recreationally trained individuals. Twenty six men (mean ± SD age 20 ± 2 yr, height 169.3 ± 8.5 cm, weight 71.3 ± 6.2 kg) were split evenly between the three training protocols, plus a non exercising control group. All subjects in the experimental groups were randomly assigned 4 weeks of either aerobic training, high intensity sprint training or high intensity interval training. Changes in 60 km time trial performance, lactate threshold, maximal oxygen uptake (VO_{2max}), resting metabolic rate (RMR), total and high density lipoprotein cholesterol (HDL), resting stroke volume (SV) and resting cardiac output (Q) were measured during the course of study pre and post intervention. Post intervention the HI training group displayed a significantly lower 60km time trial performance compared to both the aerobic group (2.9% P=0.002) and the interval training group (2.01% P=0.027), however this result was not a significant improvement within the group. No other variable changed significantly over the course of the training intervention. The implications of these results indicate that none of the tested training interventions are reliable routines to follow if the aim is to improve the aforementioned variables in a 4 week time scale, after following a pre-intervention 8 week aerobic training programme.

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Chapter 1. Introduction

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Although Government health guidelines recommend moderate intensity exercise 4-5 times a week for 30-60 minute bouts, there is a lack of conclusive evidence to determine the minimum amount of exercise needed to improve performance and/or health. Despite irrefutable evidence that regular physical training promotes a variety of cardio/respiratory benefits, in addition to be an effective preventer of chronic diseases, most 18-30 year olds fail to meet the minimum recommended physical activity guidelines (Gibala & McGee 2008).

Burgomaster, Heigenhauser and Gibala (2006) and Gibala and McGee (2008) suggest a lack of time as a considerable barrier for individuals to train or exercise; this excuse is widespread and is not limited to a specific gender, status of health, race or age group. Due to exercise participation being barred by such a universal barrier, developing training programmes that incorporate short bursts of activity could provide many individuals an opportunity to increase activity levels. High intensity (HI) training has a certain stigma associated with it, meaning that individuals find it less practical than aerobic equivalent programs and also find following a prolonged HI training program as unpractical. Nevertheless, incorporating HI training to an existing aerobic program is becoming more popular, creating an interval training regime to elicit performance and health improvements. Gibala and McGee (2008) suggests HI training 3-4 times per week yields a greater long term adherence than traditional aerobic training 4-7 times per week.

The definition of HI training is broad and encompasses sprint, interval and maximal work. For the purpose of this research, HI training will refer to repeated, maximal effort training bouts over a short period of time, performed at an intensity ≥90% of maximal oxygen uptake (VO_{2max}). Exercise bout duration relates to the set intensity of the programme and could be as little as ten seconds for an intensity at ≥100% VO_{2max}, or potentially several minutes at lower intensities. HI training has marked differences to strength training, in that strength training involves resistance against a heavy weight with an end goal to increase lean muscle mass, whereas HI training involves activities that are antonymous to muscle hypertrophy such as cycling and running with a goal to elicit performance enhancements usually associated with traditional aerobic training.

Early research conducted by Burke, Thayer and Belcamino (1994); Costill et al. (1976); Coyle et al. (1991) summarised the physiological features of typical endurance cyclists and marathon runners. These studies showed that endurance athletes have similar physical compositions that are associated with enhanced long distance cycling or running performance. Physiological markers such as VO_{2max} have been shown to be consistently high in endurance athletes irrespective of discipline (Costill et al., 1976). A high tolerance of

lactate is also synonymous with aerobic performance and has been demonstrated by a number of other authors as well, namely, Farrell, Wilmore, Coyle, Billing and Costill (1979). A few authors have also been able to show that peak power output has a positive effect on endurance performance (Hawley & Noakes, 1992). The previously mentioned performance variables have been extensively assessed as a means of improving aerobic training programs to improve endurance performance (Ekblom, 1986). However, less research has been involved with producing high intensity training programs that improve the aforementioned variables, due to lack of knowledge of the mechanisms behind HI training and an assumption that aerobic training is the optimum training method (Lindsay et al., 1996).

In 1988, Wells and Pate investigated 10,000 metre endurance athletes that were already using interval training as an element of endurance training. Wells and Pate (1988) and Daniels and Scardina (1984) define interval training as "fast paced bursts of activity coupled with a rest period or lower intensity exercise bout". Even though these athletes were incorporating interval sessions in to training regimes, a narrow understanding of the physiological benefits of interval training were apparent. Coaches, athletes and researches had limited knowledge of the actual biological mechanisms interval training was influencing or even the rate at which interval training could actually improve these mechanisms and ultimately, performance. Specifically the minimum about of training necessary to elicit performance improvements was unknown. Interestingly 10,000 metre runners and other endurance disciplines lasting less than one hour in duration are performing at intensities around and above 90% of VO_{2max} for some or all of an event and so more research in to the effects of training at such intensities needs to be explored (Coyle et al., 1991; Palmer, Hawley & Dennis, 1994).

Gibala, Little, MacDonald and Hawley (2012) have noted that irrespective of the group studied, a large percentage of interval and HI training based studies have involved short training interventions, namely, from as little as 2 sessions to short training interventions. Future work involving long term interval and HI training interventions from multiple weeks to months is desperately needed to improve knowledge on how an extended training intervention stimulates cardiorespiratory biomarkers and other markers of health and performance. More interestingly, the majority of studies have focused on acute effects on HI training on adaptations to cardiovascular fitness and intracellular signalling pathways in muscle. However, there appears to be limited understanding of the effects of a HI training and whether there is a greater effect of Hi training (either interval or sprint based) following a period of aerobic training on submaximal exercise and/or performance, as well as the implications this may have for endurance athletes.

Rationale

The basis of the study was to examine the influence of a high intensity training programme on a number of variables. Prior to the 4 week intervention, subjects completed an 8 week aerobic training intervention with the following points used as a justification for doing so.

- The initial training phase would ensure all subjects were exposed to exactly the same baseline training, going in to the high intensity training phase, ensuring a standardised programme for all participants leading in to the 4 week training interventions replicating similar patterns in real world training.

- Furthermore, discovering any potential improvements from HI training would be beneficial to athletes currently undertaking aerobic training. As individuals training aerobically experience smaller improvements the more prolonged a training intervention becomes, discovering that HI training can display improvements beyond a previous long term aerobic training intervention could be crucial to increased performance. Importantly, the initial 8 week aerobic training phase was merely a pre intervention exercise and not the focus of the current study, as such, no comparisons were made between the two training phases. For the sake of continuity however, the 4 week training intervention was referred to as weeks 8-12 rather than 0-4 to account for the initial 8 weeks of training the participants carried out.

1.1 Aim.

The broad aims of this research were:

1. To measure the effects of HI training and interval training against aerobic training on resting cardio-respiratory biomarkers and metabolic rate;

2. To measure the effects of HI training and interval training against on markers of aerobic metabolism during standardised exercise; and

3. To measure the impact of HI and interval training on 60 km cycling time trial performance compared with aerobic training.

With respect to the above aims, the following research questions were presented:

A. Does HI training have a more beneficial impact on resting cardio-respiratory biomarkers (cardiac output, stroke volume, heart rate total cholesterol and HDL cholesterol) and metabolic rate compared to both interval training and traditional aerobic training?

B. In comparison to interval training and aerobic training, does HI training result in improved power output at lactate threshold, during submaximal cycling exercise?

C. In comparison to interval training and aerobic training, does a four week HI training programme elicit an improved performance output?

1.3 Research hypotheses.

With respect to the above research questions, the following hypotheses were proposed.

H_1 HI training will significantly reduce cardiac output, stroke volume, heart rate and total cholesterol, and result in a higher resting metabolic rate and HDL cholesterol compared with both interval and aerobic training.

H_0 The results for resting cardio-respiratory biomarkers and metabolic rate will not differ between training groups.

H_2 HI training will significantly increase power (wattage) at lactate threshold during an incremental submaximal exercise test.

H_0 The results for power (wattage) at lactate threshold will not differ between groups.

H_3 HI training will significantly increase VO_{2max} and reduce time to complete a 60 km time trial in comparison to interval or aerobic training

H_0 The results for VO_{2max} and 60 km time trial performance will not differ between groups.

Chapter 2. Literature Review

Chapter 2. Literature Review.

Part 1. The effects of aerobic and high intensity training on markers of health.

2.1 The Influence of high intensity training on cardiovascular biomarkers at rest.

It is important to assess to potential benefits HI training could have on reducing coronary heart disease risk and overall improving the function of cardiovascular health in recreationally active populations. The majority of studies examining resting cardiac function, vascular and autonomic change to exercise have focused on steady state aerobic training such as regular running and cycling performed for 20-90 minutes per session. The major cardiac adaptations to aerobic exercises have included bradycardia and an increase in stroke volume due to increased left ventricular mass and improved perfusion (Heydari & Boutcher, 2012). The influence of HI training on reducing cardiovascular risk is reviewed in this section.

One study within a meta-analysis demonstrated the effect of endurance training on blood pressure and found that blood pressure decreases were most pronounced in 10 hypertensive subjects with significant decreases in systolic and diastolic blood pressure by 6.9/4.9 mmHg respectively. Although less prominent, but still significant, blood pressures reduced by 2.4/1.6 mmHg in 18 normotensive subjects (Cornelissen & Fagard, 2005), this is inconsistent with another study where ambulatory blood pressure was also measured, showing that a training intensity between 50 and 70% VO_{2max} of aerobic exercise did not decrease systolic/diastolic blood pressure in 11 sedentary men as seen in table 2.0. The sedentary group in table 2.0 indicates the period in between training programmes as the study was a cross over design (Marceau, Kouane, Lacourciere & Cleroux, 1993). Only a few studies including Marceau et al., (1993) have analyzed the effects of different intensities of exercise training on resting blood pressure (Arroll & Beaglehole, 1992) These studies also demonstrated that exercise intensity does not influence the level of blood pressure decline (Marceau et al., 1993). However, these studies did not examine exercise intensities above 90% VO_{2max} so further research is needed on the effect of HI sprint and interval training on blood pressure.

Table 2.0 Effects of two intensities on physical training on blood pressure and heart rate at rest in both supine and seated positions and during submaximal cycle ergometer exercise (100 W). Values are mean ± SEM. Train 50% and train 70% represent training at 50% and 70% of VO_{2max}, respectively and bpm represents beats per minute. *=P<0.05 vs sedentary (adapted from Marceau et al., 1993).

	Baseline	Sedentary	Train 50%	Train 70%
Supine rest				
SBP, mm Hg	138±4*	130±3	132±3	128±3
DBP, mm Hg	94±2*	87±1	90±2*	87±2
Heart rate, bpm	65±3	64±3	65±3	62±3
Seated rest				
SBP, mm Hg	156±4	152±4	153±2	155±3
DBP, mm Hg	102±2	100±3	99±3	103±2
Heart rate, bpm	80±4*	74±3	79±3*	75±3
Submaximal exercise				
SBP, mm Hg	178±6	169±8	166±6	167±6
DBP, mm Hg	97±2*	89±2	93±2	90±2
Heart rate, bpm	135±5	128±7	130±5	128±5

Safar and Lacolley, (2007); Sugawara et al., (2006); Tanaka et al., (2000) and have suggested that traditional endurance training 60 minutes 3-4 times per week at 60% VO_{2max} and also moderate intensity exercise at 65-75% VO_{2max} improves artery 'distensibility' (expandability) and reduces arterial stiffness. However, the effect of HI training on these cardiovascular markers has not yet been researched. A study investigating the differences between aerobic and HI training by Rakobowchuk et al. (2008) on peripheral arterial stiffness and flow-mediated dilation, determined HI and endurance training produce similar adaptations in moderately trained individuals. There is a regulatory effect of the distensibility of the arteries throughout the body, increases in this distensibility are positively related to cardiac improvements, which relate to improved athletic performance (Kingwell, 2002). However, a lack of distensibility in the arteries can have serious negative effect on cardiovascular health (Boutouyrie et al., 2002). There are a number of negative effects to a low distensibility, including an increase in systolic and a decrease in diastolic blood pressure, due to an earlier onset of pulse wave reflection. Stiffer arteries reposition the site of pulse

wave reflections, usually the wave intercepts the heart during the start of diastole, contributing to a greater diastole. However stiffer arteries cause a pulse wave reflection to reach the heart during systole causing more stress upon the heart.

2.2 Influence of exercise intensity and other factors on resting metabolic rate.

Resting metabolic rate (RMR) is responsible for the largest portion of an individual's energy expenditure consisting of around 60-75% of total utilization (Broeder, Burrhus, Svanevik & Wilmore, 1992). Consequently any scenario that can potentially improve RMR could have significant influence on a variety of populations. In terms of the ability of training interventions to improve resting metabolic rate, short term benefits have been noted. These immediate effects on RMR is excess post exercise oxygen consumption or EPOC. This short term effect can be divided in to two categories, the first lasting up to two hours and a minimal yet protracted effect, with a duration up to two days. There is conflicting evidence regarding the influence of exercise on RMR with some studies supporting the notion of improved RMR from training interventions and others denying any interaction between training and changes in RMR. It is interesting to investigate the potential variance different training stimuli could have on RMR. Specifically the differences, if any, HI training and aerobic training have on RMR.

The statement "to reduce weight, an individual must intake less energy than is expended" causing a negative energy balance (NEB), is universally accepted. Commonly, individuals aim to reach a NEB by following a diet that ensures an individual intakes less calories than expended. The downside to this approach is it is highly likely that previous eating habits will be restored and potentially any weight lost will be regained relatively quickly, in addition a NEB has shown to negatively influence RMR with some studies revealing a 30% reduction after exercise (Mole, 1990). Therefore, investigating methods to achieve a negative energy balance has turned to factors which influence RMR (Sims, 1989; Woo, Daniels-Kush & Horton, 1985).

Fat free mass (FFM) has a large influence on RMR although there are a lot of factors involved and a relatively large amount of variation measuring RMR. However, FFM can account for between fifty and seventy percent variation between individuals (Geliebter, et al., 1997; Heshka, Feld, Yang, Allison & Heymsfield, 1993; Westerterp, Meijer, Janssen, Saris & Ten Hoor, 1992; Zhang, et al., 2002). This has been supported by Albus et al. (1997) whereby although FFM accounts for a large percentage of RMR, there is still significant variation between individuals with similar percentages of FFM, possibly up to 3 MJ.d^{-1}.

There appear to be two separate interactions between endurance exercise and RMR. Firstly due to endurance trainings potency to improve FFM and increase lean muscle tissue, training may improve RMR through improvements in lean muscle mass. Secondly and conversely endurance training may impact the mechanisms behind residual resting metabolic rate. These changes will inevitably be a long term influence of exercise on RMR due to the length of time necessary to significantly reduce FFM as short term changes in RMR through EPOC will not influence FFM.

2.3 Effect of high intensity training on cholesterol biomarkers with focus on high density lipoprotein (HDL).

Levels of HDL are an effective measure for predicting coronary heart disease in a variety of populations (Gordon, Castelli, Hjortland, Kannel & Dawbaer, 1977). The main purpose of HDL is to transport cholesterol from bodily tissues and blood vessels to the liver to be eventually converted to bile acid. HDL also inhibits low density lipoproteins (LDL) which are removed by the LDL receptor pathway. HDL is also involved in the metabolism of other lipoproteins mainly via cholesteryl ester transfer protein. Although there is a general understanding that exercise will positively impact HDL (Leclerc, 1985), no research has determined an accurate training intensity for optimum increases in HDL.

Wood (1983) has suggested a minimum volume of training per week to induce significant improvements in HDL levels of 7-10 miles per week, divided into 3-4 sessions of low intensity endurance training. A 12 month regime of this form of training is required to elicit improvements in HDL. These findings are based on a study involving 81 recreationally active individuals aged 18-55 years. Half the cohort followed the endurance training programme while the remaining subjects acted as a control. Half of the training group that averaged the most miles per week (8 or more miles per week) increased HDL plasma levels by 4.4 mg.dl^{-1} (P<0.01). This finding is consistent with research conducted by Williams (1982) whereby volume of training appeared to be a key factor in improving HDL. This study reported a minimum of 10 miles or aerobic submaximal exercise was necessary to reduce significant improvements in HDL and that HDL remained unaffected by lower weekly volumes of exercise, this was attributed to an increased loss of body fat percentage which HDL was positively correlated to ($r = -0.47$). As such, due to the potency of HI interval training to increase whole body fat oxidation by up to 36% in 2 weeks of HI interval training as previously discussed on Talanian, Galloway, Heigenhauser, Bonen and Spriet (2007), it is especially interesting to investigate the ability of HI interval training to improve HDL cholesterol over a 4 week training period.

Volume of training appears to be the main factor when examining influence of exercise training on HDL levels. Kikkinos (1995) supports this theory and has also found moderately training track athletes improve HDL when exercising between 7-10 miles per week at submaximal intensity in 3-4 sessions per week over a long time scale. Another author which supports the claim that volume is the most important factor is Williams (1982), however intensities above 70% VO_{2max} were not investigated. Williams (1982) suggests a 1000 kcals per week energy expenditure is enough exercise stimulus to elicit significant improvements in untrained individuals, similarly Drygas, Kostka, Jegier and Kuński, (2000) found that by doubling the training volume to 2000 kcals per week, further improvements could be shown. There appears to be a strong trend to investigate volume of training, but no impetus to pursue the potential benefits of short term HI training programme.

Couillard et al. (2001) has investigated the potential benefits of a five month endurance sub maximal training programme on populations with low and high baseline HDL, also comparing the effects of low and high triglyceride levels and its potential interaction with HDL change and exercise. Results of the training intervention are shown in Figure 2.0. Interpreting the figure indicates subjects with low baseline HDL levels did not benefit from the training stimulus, only insignificantly increasing HDL by 0.4% which Couillard et al. (2001) hypothesised would be the largest beneficiary of the intervention. In contrast, subjects with a high level of TG yet low HDL significantly changed HDL ($P<0.05$) by 4.9% however this significant increase in HDL was likely due to total cholesterol actually increasing as the ratio of HDL to total cholesterol reduced by 9%.
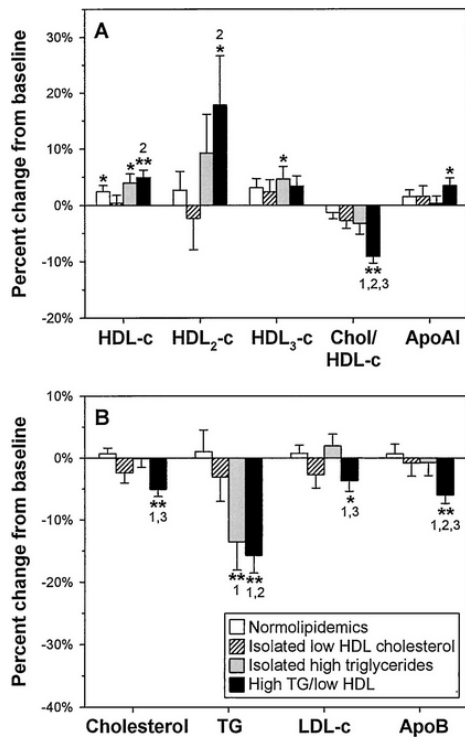
<u>Figure 2.0</u> The influence of sub maximal training on cholesterol and HDL cholesterol

A potential explanation for changes in HDLc by Durstine and Haskell, (1994) suggests exercise training can influence cholesterol metabolism at cholesterol receptor sites. Although the specific mechanisms of the influence of training on cholesterol metabolism are not currently known or what mode of training is most efficient at improving cholesterol, endurance training at least, may improve excretion of cholesterol into bile acid and therefore reduce cholesterol (Meissner et al., 2010). Low intensity training also has a role in improving the main protective function of HDL in preventing atherosclerosis by potentially up-regulating ATP-binding cassette transporters (Durstine & Haskell, 1994). Exercise is potentially not a deciding factor in altering HDL levels, there are a number of other variables that have a significant influence on HDL including diet, weight loss, enzyme and hormone activity and body fat percentage which can all confuse results when assessing the influence of just training.

Part 2. Performance and metabolic adaptations of aerobic and high intensity training.

2.4 The influence of high intensity training on performance.

By designing a HI interval training program aimed to increase VO_{2max}, it could be possible to induce an increase of VO_{2max} in middle and long-distance runners with only one session of HI interval training per week combined with a training session at lactate threshold (Jones & Carter, 2000), contributing to the suggestion by Gibala and McGee (2008), that small bouts of HI training can contribute to a wide range of aerobic benefits, thought only induced by traditional long term aerobic training programmes. The influence of incorporating HI training in addition to an existing endurance training programme from one study produces significant improvements in performance from the limited research conducted.

For example, six to eight sessions for two to four weeks of interval training at 80-150% of max power output in moderately trained cyclists has been shown by a number of authors (Lindsay et al., 1996; Stepto, Hawley & Dennis, 1998; Westgarth-Taylor et al., 1997; Weston et al., 1997) to have a significant influence on time to fatigue at 150% of peak power output. Increases between two and four percent (60.5 ± 9.3 vs 72.5 ± 7.6 s, $P<0.01$) were observed. The same authors also established significant change in peak power output and also reduced time to complete 40 km time trial performance on a stationary cycle ergometer by 8% compared to baseline results.

Smith, Davidson and Balmer (2001) demonstrated faster completion of 3000 m running performance in five well trained middle distance runners when combining sprint training and interval training in the form of eight, two to three minute sessions twice a week for a month, performed at VO_{2max} working at a work to rest ratio of 2:1. A treadmill time trial simulating a 3000 m track running performance yielded a significant difference after the training intervention. Athletes pre training 3000 m time averaged 616.6 s which then significantly changed to 599.6 s with a P value of <0.05. Prior to Smith et al. (2001), Mujuka et al. (1995) found that weekly increases in training intensity based on swimmers heart rate over a swimming season, for 18 elite swimmers positively influenced swimming performance (P<0.01), whereas frequency and volume of training had no significant influence on performance increases. Of the research examined so far, it is apparent that HI and or interval training is beneficial to at least elite level athletes when incorporating highly intense forms of training in to an existing training regime, for both endurance makers of performance (time trial performance) and also anaerobic markers of performance (peak power output) (Laursen & Jenkins, 2002).

Flynn, Pizza and Boone (1994) found that after three weeks of long duration endurance training (110 km.wk^{-1}) but with 30% of prescribed training as HI sprint training (that represented an increase of 30% in the mileage run at high intensity), eight male collegiate cross-country runners significantly increased their time to exhaustion at 110% of their preseason VO$_{2max}$ (408.3 ± 41.4 s vs 329.4 ± 31.6 s) when the HI sprint training was implemented compared to just endurance training, suggesting that including HI training into an existing aerobic training programme, can produce increased endurance performance. Similar findings have been documented by Billat (2001), whereby, to achieve victory in athletics races such as the 5000 m and 10000 m, the ability to cover the last 400 metres (the last lap) of a race well above VO$_{2max}$ is of crucial importance. The last 400 metres of a 10000 metre track event is usually completed in around 52 seconds, i.e. 27.7 km.hr^{-1}, which translates to 110% of VO$_{2max}$ for top athletes who achieve a VO$_{2max}$ at 25 km.hr^{-1}. In addition, long distance athletes must enhance their anaerobic capacity to accelerate in races. To facilitate this Billat (2001) suggested athletes can use endurance training incorporating an additional training volume of HI training for eight weeks, three sessions a week involving 130% VO$_{2max}$ effort for one minute with a work to rest ratio of 1:5, significant changes in anaerobic performance can be achieved.

Although many authors have shown a positive influence of HI and interval training, the same authors have been speculative about the underlying mechanisms responsible for these performance improvements, prevalent in both highly trained and sedentary subjects. An example of this speculation can be observed in research by Weston et al. (1997) involving elite long distance cyclists (n=6). Over a three week period twice per week subjects performed eight sets of cycling on fix placed ergometers at 80% peak power output for five minute sessions, with a work to rest ration of 5-1. The results showed a significant change (P<0.01) in 40 km time trial performance and also time to fatigue trials at 150% peak power output.

These significant changes did not influence metabolic enzyme activity of any of the six subjects. This is surprising as the cyclists previous training involved mainly aerobic exercise, promoting mostly fat metabolism, whereas the HI training carried out in the training intervention would support carbohydrate metabolism as predicted based on research by GIbala and McGee (2008) in recreationally trained individuals. This is potentially due to physiological differences between highly and recreationally trained individuals and prompt more research in to the way in which different levels of fitness respond to HI training. Although subjects in Weston et al. (1997) did not observe a shift in glcolytic activity, subjects adjusted by showing a significant (P<0.05) change in muscle buffering capacity.

HI sprint training has been introduced to moderately trained long distance runners by Iaia et al., (2009); Iaia, et al., (2008) Subjects reduced training volume by 30 km per week to 15 km per week for one month switching from aerobic training involving long distance runs 4-6 times per week up to an hour per session to HI sprint training involving 8-12 sets of 30 second sprints on a running track from three times per week at the start of the training intervention up to five times per week during the final week. Despite the dramatic loss of training volume and a shift in training intensity subjects were able to maintain 10 km treadmill running time trial times and also maintain VO_{2max}. The same subjects also improved running performance between 40-60% VO_{2max} by 23%. This study demonstrates the preservative benefits of HI sprint training, whereby a significant drop in training volume can maintain and in some areas improve endurance performance over a 4 week period.

HI interval training has shown to improve a number of physiological factors in line with increases in performance in well trained individuals such as lactate threshold (Esfarjani & Laursen, 2007) ventilatory improvements (Hoogeveen, 2000) and increased fat to carbohydrate oxidation ratio (Westgarth-Taylor et al., 1997) and an increased usage of a larger volume of muscle mass (Hoogeveen, 2000). However, these findings have not been investigated in untrained or recreationally trained individuals so research exploring the influence of HI training after a period of aerobic training is needed.

2.5 The influence of high intensity training on enzymatic changes.

Linossier et al. (1997) examined the interaction between performance and muscle enzyme activity referring specifically to improvements in 3-hydroxyacyl-CoA Dehydrogenase (HAD) and citrate synthase. Twelve untrained individuals ($\dot{V}O_{2max}$ of 3.73 ± 0.13 L.min^{-1}) followed a four week progressive HI training programme involving 4-10 sets at 30 second bouts of cycling on a fix placed cycle ergometer with 2.5-4 minutes of rest between sets. Over the month, subjects significantly improved VO_{2max}, maximum power output (wattage) and also increased the amount of distanced travelled per set. Significant improvements (P<0.05) in a number of enzymes were also discovered, with increases in hekokinase, malate dehydrogenase, phosphofructokinase (PFK) and succinate dehydrogenase. Improvements on this scale seem specific to HI training as traditional endurance training is well documented to not improve enzymatic function (Harmer et al., 2000; MacDougall, Hicks & MAcDonald, 1998; Parra et al., 2000; Rodas et al., 2000).

Endurance athletes have a better capacity to recover from HI training than recreationally trained games players (Tomlin & Wenger, 2001). In a 10 stage repeat VO_{2max} trial aerobically trained athletes with a high VO_{2max} showed greater power outputs (+10%, *P*<0.005) over the last 6 trials over recreationally trained games players due to the fact that the excess post

oxygen consumption (EPOC) was higher in the aerobically trained individuals so were able to reduce lactic acid more effectively. The aerobic athlete should be able to restore more ATP/PCr which is advantageous because the nature of HI training revolves around the effective breakdown of PCr (Tomlin & Wenger, 2001).

Following on from this Parra et al. (2000) found similar significant increases in PFK of 107% and also of aldolase at 46%, and aerobic metabolism (citrate synthase 38%, and HAD 60%) In addition, dehydrogenase, creatine kinase and pyruvate kinase increased significantly by 45, 44 and 35% respectively as a result of a short term high intensity training programme. Parra et al. (2000) suggested the more short term the training intervention, the greater the changes in glycolytic enzymes, providing a stand point to justify that having a shorter exercise programme is more beneficial than training over long periods.

Reduced muscle glycogenolysis has also been observed by Talanian et al. (2007). Muscle glycogenolysis decreased significantly (P<0.05) by 12% during 60 minutes of submaximal (60% VO_{2max}) cycling exercise directly post exercise. Muscle glycogenolysis was reduced due to decreased levels of adrenaline and the release of calcium post muscle activity causing the activation of glycogen phosphorylase in muscle. This enzyme was also stimulated via accumulation of free adenosine diphosphate (ADP) and adenosine monophosphate (AMP). A reduced adrenaline response and a reduced accumulation of the previously mentioned enzymes are typical responses to low and moderate intensity endurance training programmes (Green, Grant, bombardier & Ranney,1999). However HI training has been shown to express similar adaptations to long term aerobic training in as little as 2 weeks of HI training 3-4 times per week.

2.6 Effects of HI training on metabolic adaptations in muscle.

Various research studies have found an increase in muscle oxidative capacity (Burgomaster et al., 2006; Burgomaster et al., 2007; Gibala et al., 2006) ranging from 10% up to 40% after HI sprint training for two weeks, six sessions per week. Specifically, Gibala et al. (2006) documented that in just two weeks a group of moderately trained cyclists assigned to a HI sprint training group, with a training volume 10% of a control aerobic training group, showed comparative results in 40km time trial performance and similar changes to skeletal oxidative capacity.

As understanding of the underlying mechanisms behind HI sprint trainings ability to improve endurance performance are lacking, and some previously mentioned authors have

demonstrated an up regulation of aerobic metabolism, Gibala et al. (2012) investigated the influence of HI training on peroxisome proliferator activated receptor gamma coactivator (PGC-1α). This transcriptional coactivator is integral to the regulation of cellular energy metabolism. It has been suggested that PGC-1α is not influenced by training volume or frequency but rather the intensity of a training programme. Gibala et al. (2012) investigated six weeks of either aerobic or HI sprint training for three sessions per week involving thirty second sprints on a Wingate based cycling test, with four to five sets and four to five minutes of rest. The training intensity of each group differed by 40% with the anaerobic training group working at max effort. The anaerobic group produced a significant change ($P<0.05$) in PGC-1α which has implications for enhanced performance due to PGC-1α having an oxidative capacity. Furthermore an up regulation of PGC-1α has several implications for a better health status, as PGC-1α is positively related to anti inflammatory pathways (Gibala et al., 2012) and also aids in anti oxidant defence (Burgomaster et al., 2008).

Little, Safdar, Cermak, Tarnopolsky and Gibala (2010), found acute Wingate-based HI sprint training may produce similar increases in nuclear translocation of PGC-1α activation to endurance training. (Little, Safdar, Bishop, Tarnopolsky & Gibala 2011), found a correspondence to an increased expression of PGC-1α and increases in mRNA expression of a number of mitochondrial genes, as shown in Figure 2.1 displaying an interaction between HI training and adaptations in mitochondrial biogenesis.
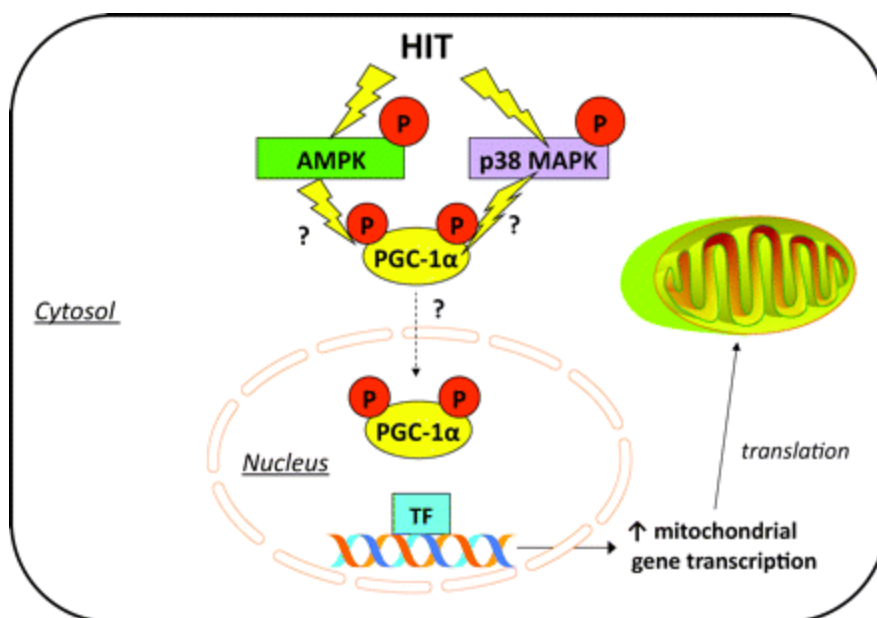
Figure 2.1 Signal mechanisms theorised to be associated with HI training. Adapted from Gibala et al. (2012)

Improvements in carbohydrate metabolism thought specific to aerobic training, have been shown by Burgomaster et al. (2006) in just twelve sessions of HI training over two weeks. The programme included four to seven sets for 30 second at max effort cycling, on a fixed place cycle ergometer with 4 minutes recovery between sets. Figure 2.2 shows an increased resting glycogen level and a lower utilization of glycogen. The study also showed a decreased lactate production during a matched wattage submaximal cycle test and finally increases in "total muscle glucose transporter 4 protein content". This has implications for improved performance, especially in team based sports such as football.

Krunstrup et al. (2006) analysed metabolites including muscle glycogen in 31 subjects during football matches with focus on sprint performance pre during and post match. Sprint performance was reduced during and post match with 30 m sprints taking 4.72 ± 0.05 s which was $2.8 \pm 0.7\%$ longer than pre game values. This loss of performance was attributed to a loss of muscle glycogen throughout the match. Muscle glycogen was 449 ± 23 mmol.kg^{-1} dry weight at rest and $42 \pm 6\%$ ($P<0.05$) less at the end of a football match. Therefore, the introduction of HI training to a team based athlete such as a footballer could increase sprint performance during the latter stages of a match.
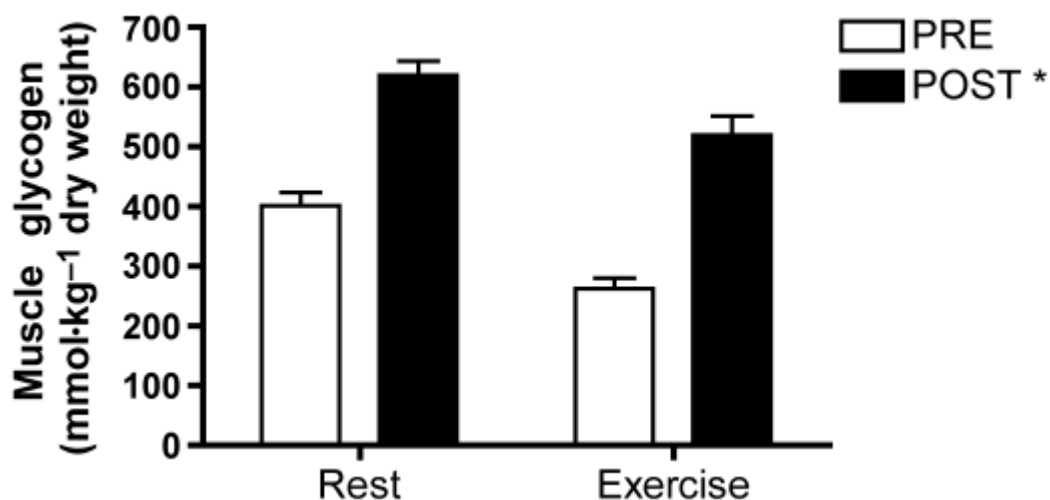
Figure 2.2 The influence of a HI training session on muscle glycogen pre and post training. Adapted from Gibala et al.,(2012)

Fat metabolism is also influenced by HI training. Whole body fat oxidation increases of 36% (from 15.0 ± 2.4 to 20.4 ± 2.5 g) post training have been observed in eight elite endurance athletes (22.1 ± 0.2 years, 65.0 ± 2.2 kg) by Talanian et al. (2007), over a two week HI running training intervention. The programme involved four minute sets at 90% VO$_{2max}$ with two minutes rest between sets four times per week. Improvements in fat metabolism of this scale are usually the outcome of a long period of endurance training (Peronnet & Massicotte,

1991). This form of training facilitated fat oxidation increases of 36% following an existing high volume endurance training programme which in itself would produce significant levels of fatty acid oxidation.

The mechanisms behind the significant increase in fat oxidation in elite athletes have also been assumed by Talanian et al. (2007). Firstly, an increase in mitochondrial volume, providing more sites for fat metabolism to occur, and a shift in the transport of fatty acids to the mitochondria, starting with lipolysis of triacylglycerol in adipose tissue. The fatty acid produced from this process is transported to the cell with the same process repeated but intramuscularly and finally fatty acid is transported to the increased number of mitochondria.

2.7 Summary.

In summary, there are many potential benefits to HI training that have been proven and many more that are yet to be explored. The purpose of the current study is to address some of the more novel benefits of HI training such as improvements in HDL cholesterol and to determine an optimal form of training for recreationally trained individuals to improve performance in endurance events and overall gaining a better understanding of the influence different types of HI training have compared with aerobic training.

Chapter 3. Method

3.0 Methodology

3.1 Pre experimental procedures

Performance testing verification

Several primary questions were addressed during the pilot phase, including assessment of an appropriate performance test ie: ascertaining the ability of recreationally trained individuals complete the proposed 60 km cycle time trial, included in this is subject familiarisation. Previous research in the human performance laboratory indicated marginal significance obtained at 20, 30 or 40 km following acute training, whereas performance differences have been observed with relatively well trained individuals. Furthermore, it could be argued that 60-90 km distances are more typical for endurance athletes and therefore more pertinent to the potential effects of HI training on endurance capabilities. A small cohort of 6 recreationally trained individuals was invited for participation for all aspects of the pilot phase under laboratory controlled conditions. The temperature was set to 20.0 ± 2ºC each test. Recreationally active was defined as participating in less than or equal to twice a week of aerobic activity for a total of 80 minutes at moderate intensity (ACSM, 2006).

To determine the suitability of 60 km distance as a time trial for recreationally trained individuals, 6 subjects carried out a 60 km time trial, using a Racermate Inc, US cycle ergometer. Ventilation, oxygen consumption, carbon dioxide production, respiratory exchange ratio, rating of perceived exertion, heart rate and time were monitored. Metalyser and metasoft software were also used during this test. Additional software used included the computrainer 3D software which comprised the virtual 60 km course that included a straight road with a simulated cyclist determining how far along the course the subject had travelled. All subjects were able to complete the time trial but more importantly, were able to maintain an average of 29 Kph ± 2 Kph, which showed the subjects were able to maintain performance throughout the trial and not fall off towards the latter stages, meaning the distance was suitable to assess performance.

3.1.2 Calibration and validity assessment of key apparatus

3.1.2.1 Calibration of the Computrainer Racemate apparatus

The reasons for standardising the Computrainer were to set the resistance at the rear wheel of the bike to match that of road resistance to allows standardisation of rolling resistance. To do this, initially, the bike was ridden for 10 minutes to warm the rear tyre of the bike. The subject was then told to cycle as fast as possible or until a speed of 28 kph was reached. At which point the bike would free wheel to a full stop where the resistance was measured by

the Computrainer. If the resistance was not within 0.05 pounds of force of 1.83 the resistance was increased or decreased to acquire the correct resistance.

3.1.2.2 Calibration of the Metalyser online gas analyser

For all tests a Metalyser 3B system online automated gas analyser (Cortex Biophysik, Leipzig Germany) was used. This system was standardised at the beginning of a testing session and involved standardising the pressure, gas and volume sensors for use in resting metabolic rate, 60km time trial and lactate threshold testing. Firstly the Metalyser was turned on and left to warm up for a minimum of 40 minutes. After which, pressure readings via a barometer were entered into Metasoft software. Two gas readings were then made, one of ambient air (0_2 20.93/ CO_2 0.03 vol%) followed by a gas cylinder, composed of O_2 15.00/ CO_2 5.00 vol% to simulate expired air. Finally using a 3 litre Hans Rudolph volume syringe the volume sensor was calibrated.

3.1.2.3 Validity of equipment

Repeatability and reliability of the Metalyser 3B system online automated gas analyser was analysed over a 6 month period by the University of Hertfordshire human physiology laboratory manager involving steady state cycling testing on the Computrainer Racemate of 6 subjects. All respiratory variables which were measured in the current study including VE, RER, VO_2 and VCO_2 were ≤ 2.1%. The strong correlations of these variables established the validity of the Metalyser 3B system (VE r=0.99, TE=1.63; RER: r=0.90, TE=0.02; (VO_2: r=0.99, TE=0.08; VCO2: r=0.99, TE=0.06).

Randox RX Monza analysed 40 patient samples for cholesterol (X) and compared the results to another commercially available method (Y) which resulted in a linear regression equation of Y= 0.971 X + 2.25 with a correlation coefficient of 0.98.

Portapress non-invasive ambulatory Finapres technology compared the technique used in the current study to rate controlled blood withdrawal phlebotomy techniques for the analysis of resting SV with 12 subjects. The coefficient of variation for SV using the Finapres was 0.88 ± 0.04 with a *P* value of 0.001). Concluding that SV measured by estimated non invasive finger pressure mirrored the blood volume drawn from rate controlled phlebotomy (Leonetti et al., 2004).

3.1.3 Control of pilot work procedures

The laboratory was set to $20.0 \pm 2^{\circ}C$. Atmospheric pressure was 1012 ± 23 Mbar each test. Each subject rode the same bicycle each test and on the same Cortex metalyser 3B and computrainer and in the same laboratory, where possible subjects were also tested at the same time as their previous test. Resting metabolic rate, lactate threshold, VO_{2max} and the 60 km time trials were all tested using the same Cortex metalyser 3B and laptop with metasoft software installed to eliminate the possibility of any variation in results between software or hardware.

In order to ensure each candidate was subjected to the same environment when under testing conditions, subjects received no encouragement at any point during any exercise test, as well, no music was permitted during any test. The only stimulus the subjects were subjected to was the 3D visualisation during the 60 km time trial test, which was the replicated for every subject. All physiological data collected during an exercise or resting test was collected without the subject being aware of what was being analysed and where possible was collected out of visual range of the subject. Additionally subjects were told to wear the same clothing for each test and where possible be tested at the same time for each subsequent test.

3.2 Main experimental procedures

3.2.1 Study design and approval

The study involved a 2 factor repeated measures, parallel intervention design with control. Ethical approval was obtained via the School of Life and Medical Sciences Ethics Committee.

Subjects were divided into 3 groups using a structured randomisation procedure, aerobic training, HI training and interval training, with 6 subjects in each group. Groups were assigned based on when the subject could attend baseline testing. A block of testing times were available and subjects chose which time to be tested. The first 6 people to sign up were unknowingly assigned to group 1 with the other 2 groups being chosen in similar fashion. In addition to the exercise groups a separate control group (n=6) was selected from individuals willing to maintain current habitual exercise levels for the duration of the study. It is noted that the more motivated subjects may have been in group 1 which may have provided testing bias.

<u>3.2.1.1 Design of training programmes</u>

Training programmes were developed in accordance to previous research by Gibala, Little, MacDonald and Hawley (2012). The authors training programmes are displayed in Table 1 below, amendments made to these studies include implementing progression over 4 weeks for the HI training rather than the original training programme lasting 6 weeks. Similarly for the initial aerobic training programme being increased to 8 weeks from 6 weeks and progression being adjusted accordingly. The interval training programme was developed solely by the current author and incorporates elements of both training programmes.

In an effort to ensure the training programmes were comparable, the weekly training volumes were proportional to the training. The aerobic programme accounted for 2250 kj per week whereas the HI training group accounted for 10% of volume at 225 kj per week. The weekly training volume for the interval training programme was not calculated but every effort was made in its design to incorporate both aerobic and HI elements and was estimated to have a weekly training volume at 50% of the aerobic training programme.

Table 1. Summary of training interventions from Gibala et al. (2012)

Variable	HI group	Endurance group
Protocol	30s x 4-6 sets, 4.5 min (4 sessions per week	40-60 min cycling (5 sessions per week)
Training intensity	'All out' maximal effort (~500 W)	65% of VO_{2peak} (150 W)
Weekly training time commitment	~10 min (~1.5 h including rest	~4.5 h
Weekly training volume	~225 kj	~2250 kj

- A separate but matched for training status control group, carrying out no training, but required to monitor habitual activity and dietary intake for consistency
- Training group 1: To undertake an aerobic training programme based on Gibala et al. (2012) at 65% VO_{2max} 40-60 minutes 4 times per week.
- Training group 2: HI training programme based on Gibala (2012) 30 second sets at 100% VO_{2max}, 4-6 repeats with 4-5 minute rests between sets (x4 per week) for the remaining 4 weeks.
- Training group 3: Interval training programme involving 20 minutes of aerobic exercise interspersed with 10 second sprints every 5 minutes for the remaining 4 weeks.

Subjects were tested at weeks 8 and 12

<u>3.2.2 Participants</u>

<u>3.2.2.1 Sample power calculations and justification</u>

As there are a multitude of variables in the current study, multiple power calculations needed to be carried out in order to ascertain a more accurate sample size. For resting measurements, Broeder et al. (1992) investigated the influence of exercise on resting metabolic rate of 18-35 year old men and detected a moderate effect size (P<0.05; η^2=0.4013). Using this effect size, an a-priori power analysis (ANOVA: Repeated measures, within-between interaction) using G*Power 3.1 software (Erdfelder, Faul and Buchner edition, 1996) was carried out, yielding a minimum sample size of 9 subjects per group to provide a power of 0.98. For cholesterol, Egan, Zhao and Axon (2010) investigated the effect of training intensity on cholesterol in twenty six men aged 18-35 and detected a high effect size (P<0.01; η^2= 0.6140) corresponding to a satisfactory sample size of six subjects per group for a power of 0.84.

For performance measures, Lindsay et al. (1996) investigated the influence of HI training in eight male cyclists on 40 km time trial performance, providing a moderate effect size (P<0.05; η^2= 0.3916) which transferred to a suggested sample size of 6 subjects with a power of 0.80. Therefore, taking in to account the power of the resting metabolic rate study was 0.98 for 9 subjects, a sample size of six per group in the current study would be sufficient to assume significance at a power of 0.80.

<u>3.2.2.2 Recruitment and subject type</u>

Subjects were all male (n= 6 per group; mean ± SD age 20 ± 2 yr, height 169.3 ± 8.5 cm, weight 71.3 ± 6.2 kg) recruited from the University of London Air Squadron (ULAS) via email and word of mouth. Subjects were also recruited from the University of Hertfordshire via similar methods. A number of subjects from ULAS also attended the University of Hertfordshire and were recruited through word of mouth and university email.

<u>3.3 Procedures</u>

<u>Timeline and overview of baseline measurements and subsequent testing sessions</u>

Initially, prior to exercise testing, subjects underwent resting metabolic rate measurements. Cardiovascular biomarkers (utilising the Finapres, Hogehilweg, Holland, Portapress non-invasive ambulatory Finapres technology) measuring arterial blood pressure, heart rate,

stroke volume, cardiac output and total peripheral resistance then followed. Succeeding resting measurements, a Racermate Inc, US cycle ergometer was calibrated. Once calibrated, subjects underwent a progressive intensity lactate threshold test using the same equipment. At 4 minute intervals, until lactate threshold exceeds 4mmol/L, blood samples were collected and analysed for lactate and glucose using capillary blood sampling.

Following on from the lactate threshold test after a 10 minute rest period, subjects underwent a VO_{2max} test utilising the same equipment as the lactate threshold test. VO_{2max} was measured using the cortex metalyser directly noted at 1 minute intervals until the subject can no longer sustain the required wattage. On a separate occasion during the same week, subjects carried out a 60 km time trial using the Racermate Inc, US, fixed place bicycle simulator. Recordings of respiratory exchange ratio (RER), ventilation (Ve), VO_2 and VCO_2 were taken at 10 km intervals. Throughout the study subjects were required to train in pairs for compliance and where possible heart rate values were relayed back to the main tester.

3.3.1 Resting Measurements – Resting metabolic rate (RMR)

Before each testing session, height (kgs), weight (kgs), age (years) and the temperature (degrees Celsius) of the laboratory were noted. Using those measurements a new test subject was selected in Metasoft software. This would be followed by starting an ambient air calibration ready for measurement. During the ambient air calibration, the subject was fitted for a oro-nasal mask, ensuring no air escaped around the nose or cheeks. The gas turbine was then inserted into the front of the mask and fitted to the subject. Once the ambient air calibration was complete the subject lay in a supine position and rested for 40 minutes until a continuous 5 minute reading of RMR was taken, defined by RMR not exceeding a 10% difference VO_2, VCO_2, this usually occurred within 40 minutes. A full list of guideline for testing RMR is listed in Appendix 2.

3.3.2 – Portapres non-invasive ambulatory Finapres technology

Subjects were then tested for cardiovascular biomarkers (utilising the Finapres, Hogehilweg, Holland, Portapres non-invasive ambulatory Finapres technology) measuring arterial systolic and diastolic blood pressures, heart rate, stroke volume and cardiac output, this was achieved by the following steps.

Firstly the frontend box was strapped to the subject's wrist. Selecting the proper sized finger cuff (large or small depending on the size of the subjects middle finger), the cuff was placed so that the LED and photodiode were symmetrically placed on each side of the fingers soft

palmer parts and had a centred position on the middle phalanx. The air hose and cuff cable was threaded between two fingers to the volar side of the hand to reach the frontend unit. The finger cuff was positioned so that the airbladder follows the curvature of the finger skin without noticeable clearance between the bladder and skin.

The hydrostatic height correction unit was also used. This was first calibrated by placing both ends of the reference and transducer parts together and pressing 'height' on the Portapress. This calibrates the height difference between each part to as without an active height correction system a hydrostatic error of 10 mmHg or even more is hard to detect and easily made, in particular when the arm is stretched and the hand is not close to the chest. During a measurement the transducer was placed at the measured finger and the compliant ending at reference level. As such the height changes of the measured finger and continuously sensed.

Once the apparatus was successfully attached to the subject the use of BeatScope software was used to obtain the results. By pressing 'start' on the menu, a drop down box would appear to enter subject details of height, weight, age and gender. Entering these values and pressing enter would start the Portapress measurement. Each subject was measured for 1 minute once 60 readings per second were being recorded by the Portapress. This information was then saved in a file for later analysis.

3.3.3 Assessment of Cholesterol using Randox RX Monza

Prior to 60km time trial testing, three capillary blood samples were taken from each subject with a combined total of 600 μl. These samples were then placed in a centrifuge for 10 minutes at 4000rpm as per Randox guidelines. The three samples of plasma were then plasma was aliquotted into a single eppendorf ensuring at least 210 μl of plasma was collected. This sample was then frozen at -80° until the end of the testing phase where all plasma samples were tested in blocks.

3.3.4 Total cholesterol (CHOL)

Using fresh distilled water (ddH$_2$O) a new gain calibration in cuvette mode was performed. 'CHOL' was selected in the Run Test screen on the Randox RX Monza and as instructed a water blank was carried out. Once the water blank calibration was complete the cuvettes were prepared as shown in the following table.

	Reagent Blank S0	Standard S1	Sample
ddH$_2$O	5µl	-	-
Standard	-	5µl	-
Sample	-	-	5µl
Reagent	500 µl	500µl	500µl

The cuvettes were then mixed and incubated for 5 minutes at 37ºC in a dry block thermostat for incubating cuvettes.. At this point the samples were ready to be read by the Randox and were inserted into the RX Monza flowcell holder and results read within 60 minutes of incubation.

3.3.5 HDL-Cholesterol (HDL)

 Firstly 200µl of sample, 200µl of standard and 500µl of diluted precipitant were pipetted into centrifuge tubes, these were then mixed and allowed to sit at room temperature for 10 minutes, then centrifuged for 2 minutes at 12,000 rpm as per Randox guidelines. The supernatant was then separated within two hours of centrifugation and the cholesterol determined by the CHOD-PAP method.

Similar to CHOL determination using fresh ddH$_2$O a new Gain calibration in cuvette mode was performed. 'HDL' in the Run Test screen was selected and as instructed a water blank was carried out. Once the water blank calibration was complete the cuvettes were prepared as shown in the following table.

	Reagent Blank S0	Standard S1	Sample
ddH$_2$O	50µl	-	-
Standard	-	50µl	-
Sample	-	-	50µl
Reagent	500 µl	500µl	500µl

The cuvettes were then mixed and placed for 5 minutes at 37ºC in a dry block thermostat for incubating cuvettes. Optimised for use with RX monza assays. At this point the samples were ready to be read by the Randox and were inserted into the RX Monza flowcell holder and read within 60 minutes of incubation.

<u>3.3.6 Lactate threshold and VO$_{2max}$</u>

Following resting metabolic rate analysis, exercise testing of lactate threshold and VO$_{2max}$ was carried out using a Racermate Inc, US cycle ergometer. Once calibration of the Computrainer was completed the subject was fitted with a Polar hear rate monitor and the bike seat set to the correct height for that subject. As ambient air calibration was being carried out by the Metalyser, the subject warmed up for 5-10 minutes. After the warm up phase the rear wheel resistance was then calibrated to road resistance ready to start the test and the subject fitted with a facemask for gas analysis.

Using the computer with Computrainer software installed, the appropriate test intensity was selected, based on a specific wattage the lactate threshold test would start on. Most subjects began the test on 120 or 140 watts depending on the time taken to achieve lactate threshold in familiarisation phase. The subjects that took longer than six stages to achieve lactate threshold began at 140 watts for the main intervention. The lactate threshold test involved subjects cycling, matching the wattage set by the Computrainer until lactate threshold was attained, defined by blood lactate exceeding mmol.L^{-1}. The test was divided into four minute stages with wattage increasing by 20 watts each stage. Measurements of Ve, VO$_2$, VCO$_2$ and RER comprised the respiratory variables. Other measurements included HR and RPE.

Following on from the lactate threshold test after a 10 minute rest period, subjects underwent a VO$_{2max}$ test utilising the same equipment as the lactate threshold test. VO$_{2max}$ was measured using the cortex Metalyser. HR and RPE directly noted at 1 minute intervals until the subject could no longer sustain the required wattage. Metasoft provided an absolute and relative value of VO$_{2max}$, which was assumed when the subject could no longer maintain the required wattage for a stage and was measured over a thirty second period around the peak of VO$_{2max}$.

<u>3.3.7 60km Cycle Time Trial</u>

On a separate occasion in the same testing week to lactate threshold and VO$_{2max}$ but using the same Racermate Inc, US cycle ergometer, subjects underwent a 60km time trial. Firstly subjects were fitted with a Polar heart rate monitor and the same procedure of calibration of the bike made. Metalyser and Metasoft software were also used during this test. Additional software used included the Computrainer 3D software which comprised the virtual 60 km

course that included a straight road with a simulated cyclist determining how far along the course the subject had travelled. This feature was mostly aesthetic and mainly served to act as stimulus for subjects to focus on as cycling for 2 hours in laboratory conditions can be monotonous and cause tedium. Recordings of respiratory exchange ratio (RER), ventilation (Ve), VO_2 and VCO_2 were taken at 10 km intervals.

3.4 Additional control of data in the experimental procedure

The laboratory was set to 20.0 ± 2ºC. Atmospheric pressure was 1012 ± 23 Mbar each test. Each subject rode the same bicycle each test and on the same Metalyser and Computrainer and in the same laboratory, where possible subjects were also tested at the same time as their last test.

Resting metabolic rate, Lactate threshold, VO_{2max} and the 60 km time trials were all tested using the same Cortex Metalyser and laptop with Metasoft software installed to eliminate the possibility of any variation in results between software or hardware.

3.5 Statistics

Primarily the use of a mixed design ANOVA within-between interaction with Bonferonni post hoc test was conducted. The use of multiple One-way ANOVAs with Tukeys post-hoc test if significance was shown was used in this research study with an alpha level of $P<0.05$ to accept significance. If measurements were not normally distributed a non-parametric Kruskal Wallis ANOVA was used. Data was inputted into IBM SPSS statistics 20 and was presented as mean ± SD.

Chapter 4. Results

Chapter 4. Results

For all figures presented, the red column represents baseline data after eight weeks of aerobic training which coincides with the beginning of the training interventions and the green column represents the final testing session after four weeks of prescribed training.

The pilot work 60 km time trial performance is demonstrated in figure 4.1. Mean time for completion of the time trial was 122.30 ± 2.22 mins, with all participants completing the trial.
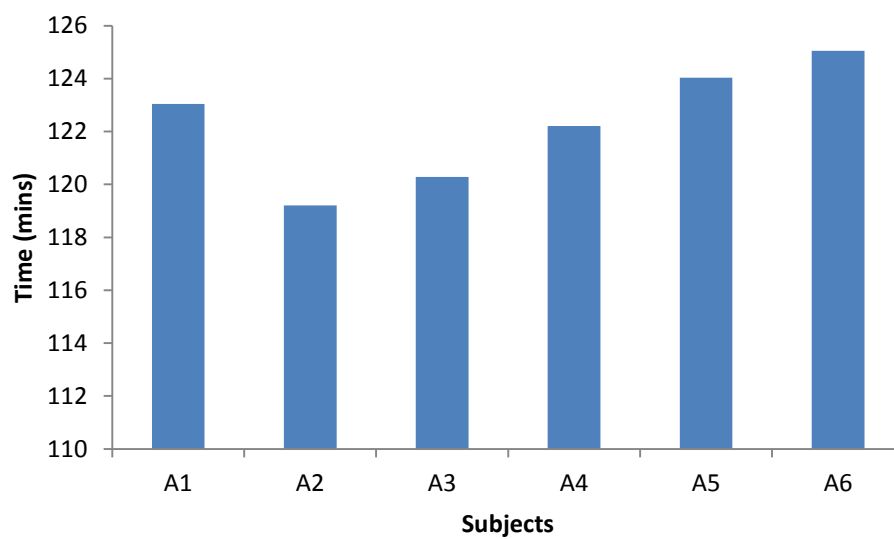
Figure 4.1 60 km time trial results for all subjects over pre experimental period.

All groups showed statistically insignificant differences as determined by a mixed design ANOVA within-between interaction at baseline (2180 ± 155 kcal.d^{-1}) for resting metabolic rate and no group significantly changed resting metabolic rate (RMR) over the research study between groups (2180 ± 155 kcal.d^{-1}) to (2219 ± 138 kcal.d^{-1}) (figure 4.2).

Figure 4.2 RMR values from all groups at baseline (week 8) and concluding the training interventions at week 12. Data shown as mean ± SD. No significant changes observed ($P>0.05$)

No significant difference was observed between groups determined by a mixed design ANOVA within-between interaction at baseline (75 ± 6.81 ml) or at the completion of the training intervention (75 ± 8.10 ml) in regards to resting stroke volume measurements (figure 4.3).

Figure 4.3 Resting stroke volume data for all groups at baseline (week 8) and concluding the training interventions at week 12. Data shown as mean ± SD. No significant changes observed ($P>0.05$)

Figure 4.4 demonstrates resting cardiac output (Q) results at baseline (5.14 ± 0.62 L.min^{-1}) and at the completion training intervention (5.12 ± 0.53 L.min^{-1}) between groups. All groups showed no significant difference determined by a mixed design ANOVA within-between interaction at baseline between or within groups over the training intervention.

Figure 4.4 Resting cardiac output data for all groups at baseline (week 8) and concluding the training interventions at week 12. Data shown as mean ± SD. No significant changes observed (*P*>0.05)

The baseline and training comparisons between HI training, aerobic training and interval training for total cholesterol are expressed in figure 4.5. All groups demonstrated no significant differences determined by a mixed design ANOVA within-between interaction at baseline (3.90 ± 0.62 mmol.L^{-1}) and did not significantly change total cholesterol at the completion of the training intervention (3.93 ± 0.56 mmol.L^{-1}).

<u>Figure 4.5</u> Total cholesterol data for all groups at baseline (week 8) and concluding the training interventions at week 12. Data shown as mean ± SD. No significant changes observed (P>0.05)

The baseline and training comparisons between HI training, aerobic training and interval training for High density lipoprotein (HDL) cholesterol are expressed in figure 4.6. All groups demonstrated no significant differences determined by a mixed design ANOVA within-between interaction at baseline (1.15 ± 0.42 mmol.L^{-1}) and did not significantly change total cholesterol (1.14 ± 0.40 mmol.L^{-1}) by the completion of the study.
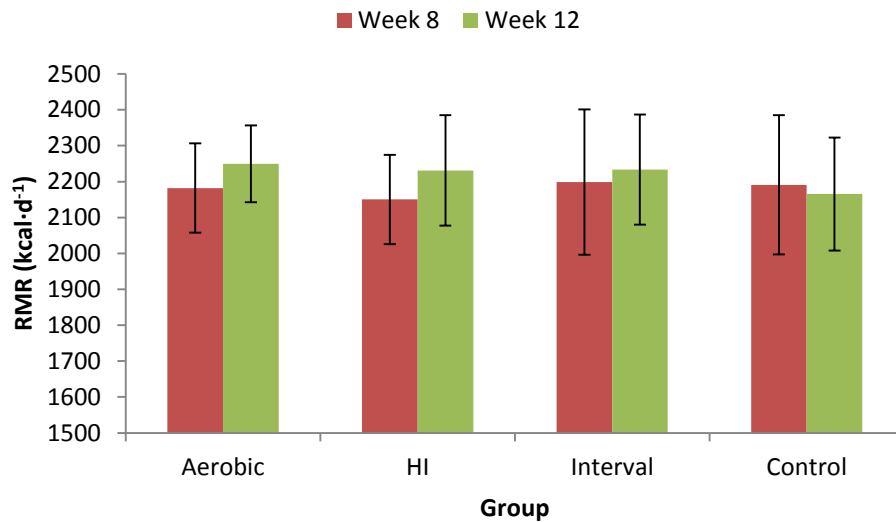
Figure 4.6 HDL cholesterol data for all groups at baseline (week 8) and concluding the training interventions at week 12. Data shown as mean ± SD. No significant changes observed (*P*>0.05)

The baseline and training comparisons between HI training, aerobic training and interval training for completion of the lactate threshold test are expressed in figure 4.7. All groups displayed no significant differences determined by a mixed design ANOVA within-between interaction at baseline (159.33 ± 19.12) and did not significantly change wattage at lactate threshold by the end of the training intervention (163.5 ± 20.54 W).
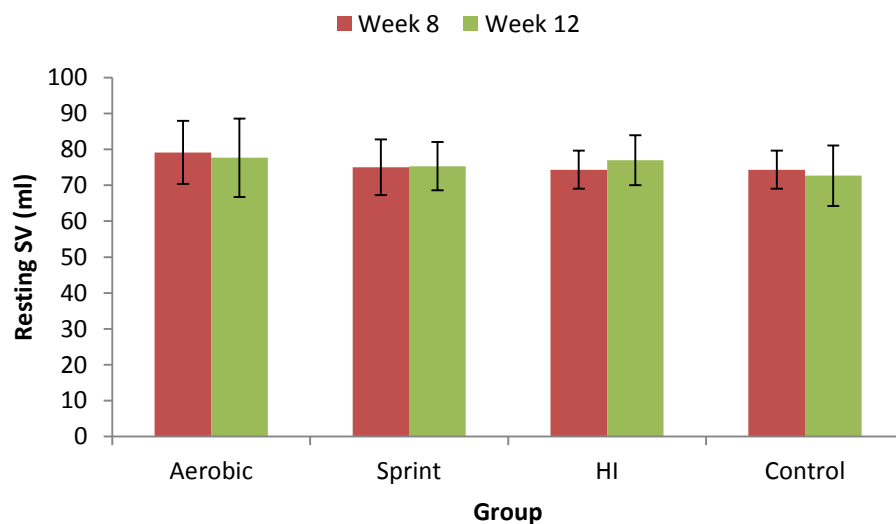

Figure 4.7 Wattage at lactate threshold for all groups at baseline (week 8) and concluding the training interventions at week 12. Data shown as mean ± SD. No significant changes observed (*P*>0.05)

The baseline and training comparisons between HI training, aerobic training and interval training for completion of the VO_{2max} test are demonstrated in figure 4.8. No group significantly changed VO_{2max} throughout the training intervention. All groups displayed no significant differences as determined by a mixed design ANOVA within-between interaction at baseline (52.5 ± 3.10 ml.kg^{-1}.min^{-1}) and did not significantly change VO_{2max} by the end of the training intervention (54.9 ± 4.12 ml.kg^{-1}.min^{-1}).
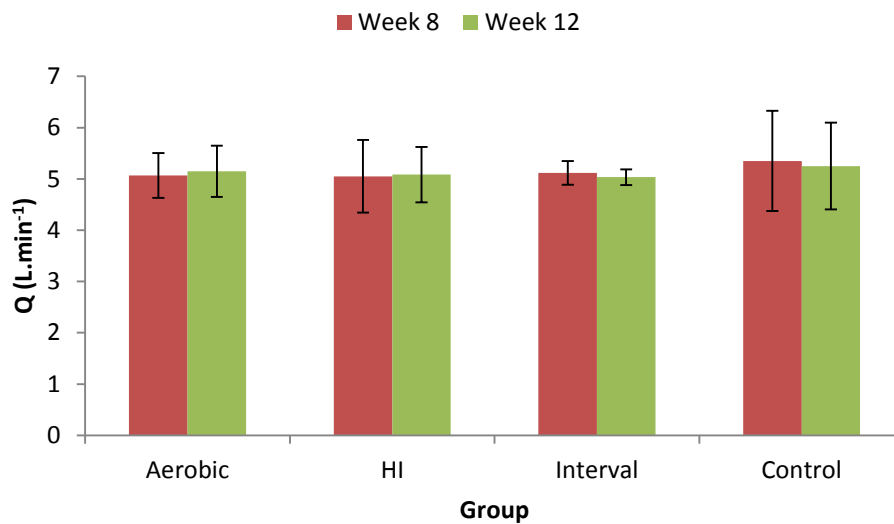

Figure 4.8   Relative $VO2_{max}$ data for all groups at baseline (week 8) and concluding the training interventions at week 12. Data shown as mean ± SD.

The baseline and training comparisons between HI training, aerobic training and interval training for completion of the 60km time trial test are demonstrated in figure 4.9. All groups showed no significant differences at baseline. At week 12 the HI training group displayed a significantly different 60km time trial completion time (*) compared to both the aerobic group (2.9% *P*=0.002) and the interval training group (2.01% *P*=0.027) however no group significantly changed the time to complete a 60 km time trial over the study.

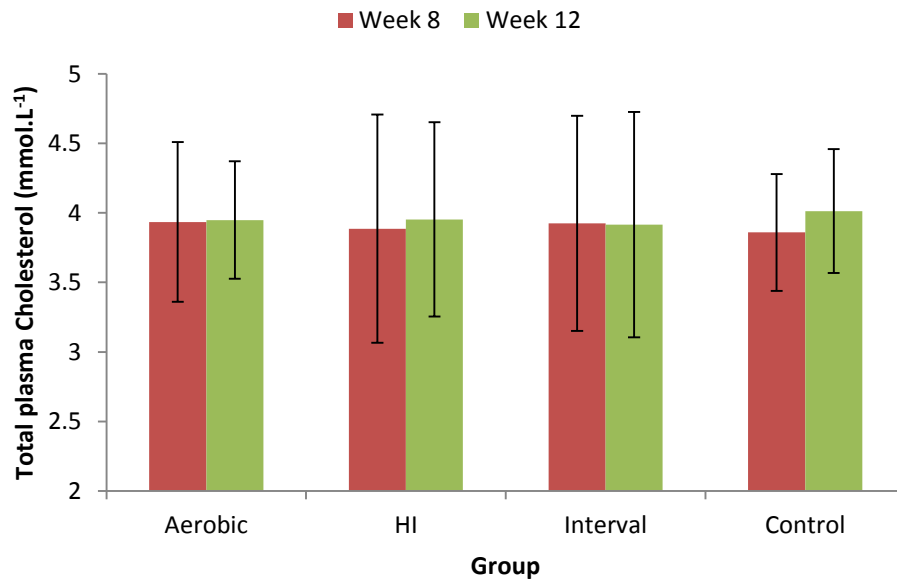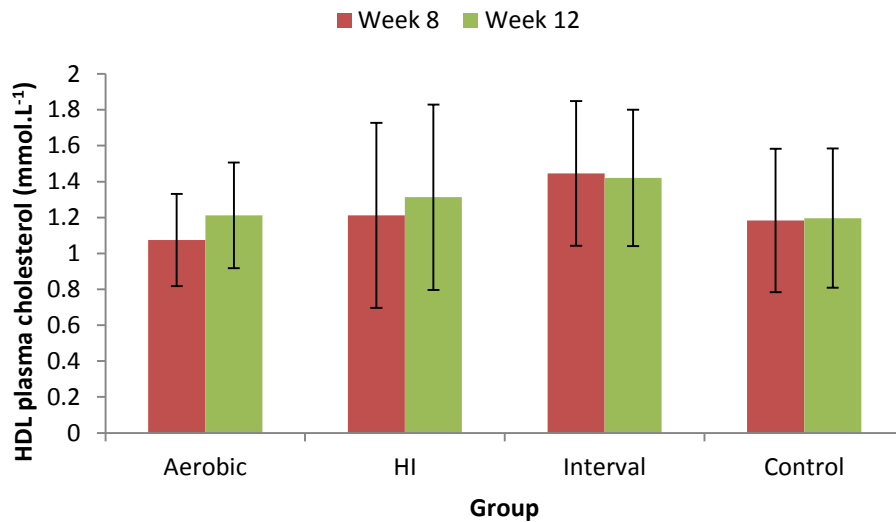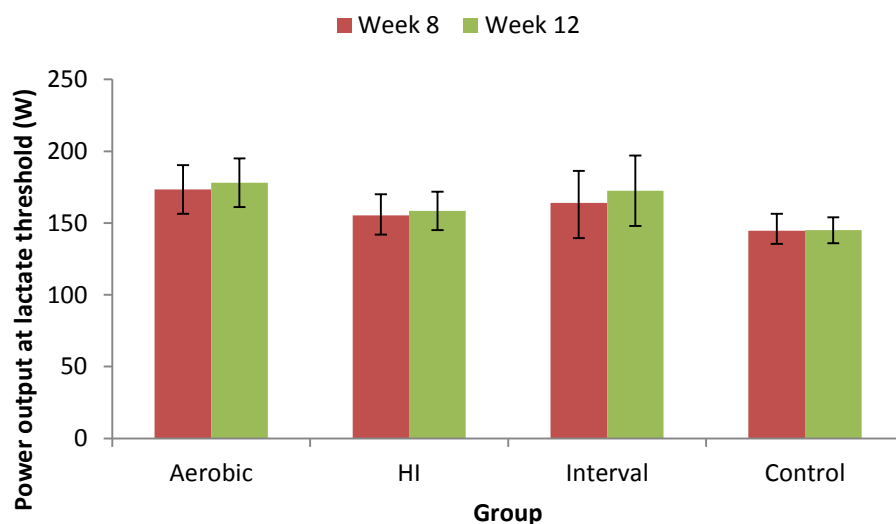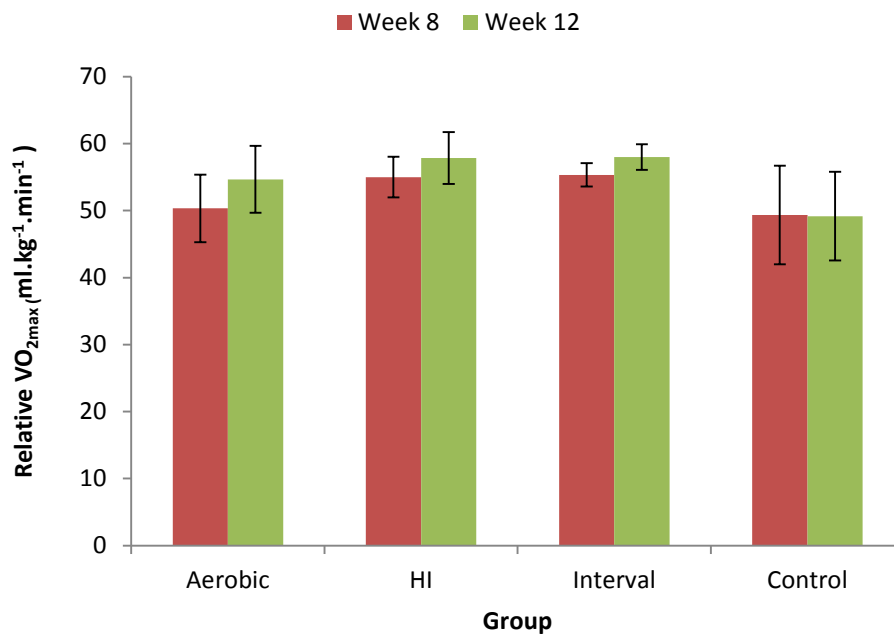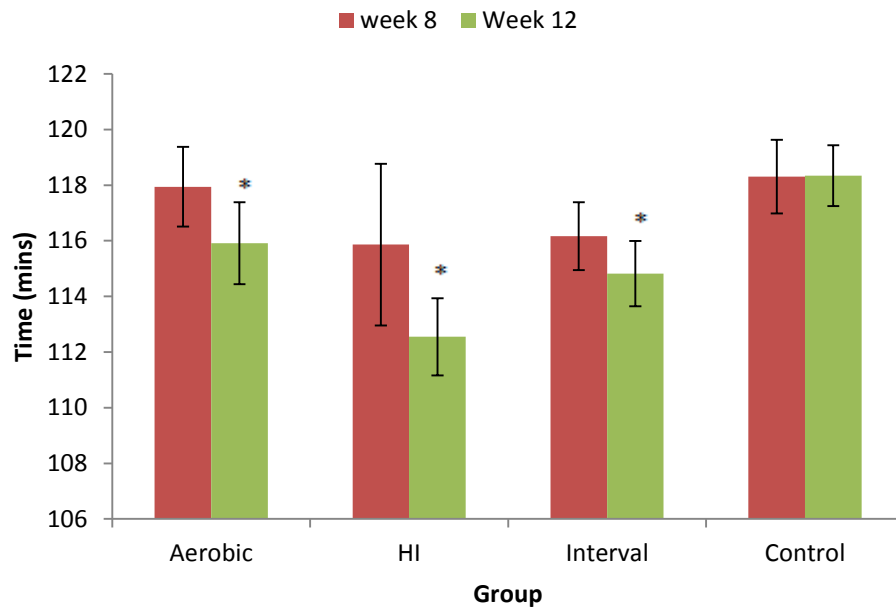Figure 4.9 60 km time trial performance results for all groups at baseline, week 8 and concluding the training interventions at week 12. Data shown as mean ± SD. (P<0.05)

Chapter 5. Discussion

Chapter 5 Discussion

The purpose of this research was to determine which of three training methods improved 60km time trial performance, measures of aerobic metabolism and measures of cardio-respiratory biomarkers most effectively. Twenty six of the original forty subjects (14 subjects dropped out of the study after baseline testing or realised they could not adhere to a twelve week training programme) completed the whole training and testing programme resulting in four evenly distributed groups, with six subjects in each training protocol and six subjects acting as a non training control group..

The strategy for using three different training protocols was formulated in an effort to determine the influence of HI training on recreationally trained individuals and how HI training in comparison to comparable modes of training. Various measures were recorded before and during exercise, these measures were used to determine adaptations, if any, experienced as a result of either training protocol that could be identified as having a significant influence on 60km time trial performance, aerobic metabolism or measures of cardio respiratory biomarkers.

5.1 Resting metabolic rate

All groups including control began the research study with similar RMR levels and neither group reduced or increased RMR at any point during the programme. The results of the current research study agree with other studies showing that when exercise training was the primary determinant in producing a modification in RMR, RMR remained unchanged after an extended period of training.

Many studies have examined the effect of training on RMR. In general, however, these studies have focussed on the relationship between exercise and restrictive calorie intake and any related effects on weight loss. The very nature of modifying the diet and introducing an exercise programme therefore confuses the interpretation of the recorded result in RMR. Despite this, research has been conducted focussing on the effect exercise training has on RMR yielding conflicting results.

Sharp, Reed, Sun, Abumrad and Hill (1992) investigated the effect of HI training and endurance aerobic training on RMR and found a significant increase in RMR in both training varieties. However the HI training in this instance differed to that of the current study as subjects were training at 85% VO_{2max} as opposed to 100% maximum effort. The aerobic training programmes were comparable however, involving similar durations and intensities.

Conversely, Van Etten, Westerterp & Verstappen (1995), was unable to replicate the same findings, showing neither and increase nor decrease in RMR regardless of training followed. This analysis better reflects the current study as the forms of HI training used were very similar, both involving maximum effort sets with similar rest periods. Although the reasons for the variation in results is not clearly defined, a seemingly defining factor in increased or decreased expressions of RMR appears to be the relation between when the measurement of RMR was taken and the last exercise bout. Referring back to the effect of long term EPOC which may influence RMR thirty-six to forty-eight hours post training. During this time frame, an interpretation of RMR may be confused with an influence of EPOC. Meaning any assumptions of improved RMR from training will be influenced by EPOC in this time frame.

Interestingly, studies which leave more than 24 hours before measuring RMR from the last training session, suggest there is no correlation between training and changes in RMR. However those studies which measure RMR within 24 hours of the previous training session have found a significant correlation between exercise and changes in RMR as seen in Table 5. Byrne and Wilmore (2001) conducted a study which accounted for the effects of EPOC and measured RMR 72 hours post exercise (table 5.2). The study comprised of a 9 week training intervention involving a HI training group training 4 times per week at an intensity of 90% VO_{2max}. A combined HI and endurance aerobic training group also involving 4 sessions per week but at mixed intensity ranging from 60-90% VO_{2max} and also a non exercise control group. Lean tissue mass significantly increased ($P<0.01$) in both exercise groups by 1.9 kg with the control group eliciting no change in lean mass. The study also observed a significant ($P<0.05$) 3% change in RMR in the HI only training group but did not see the same improvements with the mixed intensity training group which although insignificant, reduced RMR by 3.8% after 9 weeks of training as seen in table 5.2. Westerterp et al. (1992) found similar results from a 44 week aerobic training half marathon intervention. This training increased energy demands on the subjects by 30% compared to baseline. Subjects responded to the training by losing on average 3.8 kg of fat mass and gained on average 1.6 kg of fat free mass. Despite these improvements RMR declined.

This finding indicates that all the increase in RMR in this treatment could be attributed to changes in fat free mass. The most striking results, however, were those that did both HI and aerobic training, in which RMR actually declined by 3.8% and therefore when expressed relative to fat free mass the difference was increased to 7.4%. This data are more simply expressed as RMR against body mass for the HI trained group at baseline compared with post training (figure 5.1). Another study by Westerterp et al. (1992) found similar results from a 44-week training intervention for a half-marathon. This training increased energy demands on the subjects by 30%. Subjects responded to the training by losing fat mass (3.8kg), and

gained fat free mass (1.6kg). Despite these improvements RMR declined. Therefore, it can be assumed that improvements in fat free mass are positively attributed to changes in RMR after a period of HI training.

The reason for decline in RMR from aerobic and mixed intensity exercise is speculative. Speakman & Selman (2003) suggests a reduction of Uncoupling Protein-3 (UCP3) in muscle which enhances muscle mechanical efficiency during exercise when up regulated. However Schrauwen, Saris and Hesselink (2001) suggests a reduction in UCP3 would influence RMR through thermogenesis and also the uncoupling mechanism of UCP3 which is not evident in the previously mentioned study, nevertheless neither author can identify a reason as to why a change on UCP3 would influence aerobic training any differently that HI training.

Table 5.1 Comparison between baseline and EPOC values in a multitude of studies with reference to the time interval before measurement. Adapted from Speakman and Selman (2003)

Reference	Magnitude of EPOC (% above non-exercised control)	Time interval from completion of exercise (h)
Binzen et al. (2001)	18·6	2
Melby et al. (1992)	7·0	2
Melby et al. (1993)	11·7	2
	4·7–9·4	15
Osterberg & Melby (2000)	4·2	15
Dolezal et al. (2000)		
Trained subjects	11·0	24
	6·4	48
	0	72
Untrained subjects	24·7	24
	16·8	48
	0	72

Table 5.2 Comparisons between high intensity (HI) and mixed intensity interval training (HI Ae) with a control (C) on fat free mass (FFM) and resting metabolic rate (RMR) pre and post training. Adapted from Speakman and Selman (2003).

Treatment group	HI	HI Ae	C
FFM (kg)			
Pretreatment	49·5	45·6	43·9
Post treatment	51·4	47·5	44·4
Difference	+1·9	+1·9	+0·5
RMR (MJ/d)			
Pretreatment	6·07	5·81	5·89
Post treatment	6·25	5·59	5·97
Difference: MJ/d	+0·18	−0·22	+0·08
%	+3·0	−3·8	+1·35
RMR/FFM (kJ/kg per d)			
Pretreatment	123	127·6	134·7
Post treatment	122·2	118·0	135·1
Difference: kJ/kg per d	−0·8	−9·4	+0·4
%	−0·65	−7·4	+0·3
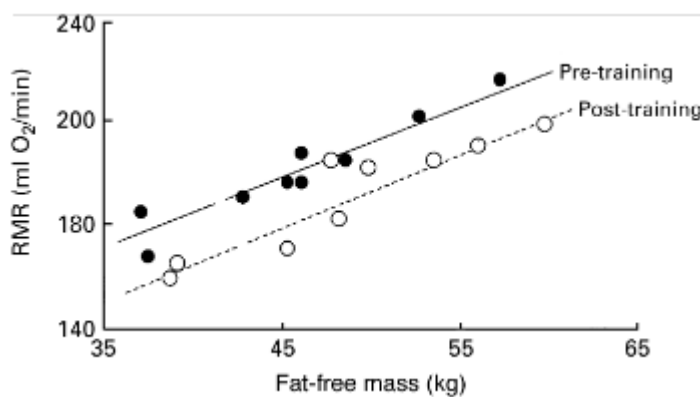
Figure 5.1 Relationship between fat free mass and resting metabolic rate (RMR) pre and post Interval training. Adapted from Bryne and Wilmore (2001).

The null hypothesis 'High intensity interval training does not significantly improve resting metabolic rate when compared with high intensity sprint training or aerobic training' can therefore be accepted.

5.2 Resting cardiac output and resting stroke volume

All groups including control began the research study with similar cardiac output (Q) and stroke volume levels and neither group significantly reduced or increased Q at any point during the programme. This is understandable given Q is affected by considerable intra-individual variation at rest. Q is effected by the emotional responses of an individual which accounts for changes in cortical outflow to cardiovascular accelerator nerves as well as to that act on the resistance and capacitance sympathetic fibres (Willmore et al., 2001). The

neuro-transmitter Acetylcholine is up regulated as a result of aerobic training translating to an increased influence on the sinus node of the heart causing bradycardia at rest, which also influences a reduced activity of parasympathetic fibres that innervate the sinoatrial node. These variables take an extended period of time to show any significant exercise induced changes. Willmore (1994) hypothesised that Q would increase with a sedentary individual by 100ml and decrease by 100ml after a six month aerobic exercise intervention. No noteworthy changes occurred in the current research study possibly due to the exercise intervention being twelve weeks in duration meaning Q had not had significant time to produce improvements.

Similarly as stroke volume is an integral part of cardiac output, without a rise in one it is unlikely to find any significant increases in the other, especially considering that recreationally trained individuals appear to facilitate increased cardiac output during exercise with an increased heart rate rather than increasing stroke volume (Scheuer & Tipton, 1977) This was the case in the current study with no significant improvements in stroke volume occurring. Traditionally stroke volume increases based on four factors. Increased internal left ventricular volume and mass, reduced cardiac and arterial stiffness, increased diastolic fill time and potentially improved intrinsic cardiac contractile function. It is interesting to note that all training groups significantly improved VO_{2max} without significantly improving stroke volume. Potential reasons for this link back to sedentary or recreationally trained individuals facilitating increased cardiac output by increasing heart rate to compensate for low stroke volume. Conversely as stroke volume was not measured during exercise it is entirely possible that resting stroke volume remained unchanged yet maximal stroke volume significantly improved however Scheuer and Tipton (1977) found that improvements in maximal stroke volume also increased stroke volume at rest so it is unlikely that maximal stroke volume was effected during the current research study.

The null hypotheses 'High intensity interval training does not significantly improve resting cardiac output when compared with high intensity sprint training or aerobic training' can therefore be accepted. The null hypothesis 'High intensity interval training does not significantly improve resting stroke volume when compared with high intensity sprint training or aerobic training' can also be accepted.

5.3 Total cholesterol and High density lipoprotein cholesterol

All groups including control began the research study with similar total cholesterol levels and neither group reduced or increased total cholesterol levels at any point during the programme. All groups including control began the research study with similar HDL cholesterol levels and neither group reduced or increased HDL cholesterol levels at any

point during the programme. This is consistent with research conducted by Leon et al. (2002) whereby significant increases in HDL were only prevalent after twenty weeks of aerobic exercise based on 55-75% VO_{2max} three times per week for twenty weeks. Interestingly Leon et al. (2002) found that HDL actually fell by 9.3% after five weeks of such training and only significantly increased from baseline results after fifteen weeks of aerobic training with an increase of 6.5% and continuing to increase a further 11.5% after 20 weeks.

Leon et al. (2000) and Kodama (2007) found a 3.6% increase in plasma HDL cholesterol as a result of a comparable aerobic training programme which was consistent throughout a mixed cohort of males and females ranging from ages 18-47. Examining numerous studies involving an exercise intervention and subsequent HDL measuring, it is apparent that there is no conclusive evidence to the influence of exercise on HDL cholesterol, revealing around 50% of research on males and even less so with females exhibiting a significant change in HDL cholesterol. A meta analysis of 2000 subjects indicated a 5% average increase in HDL cholesterol following an exercise intervention although the nature of the training be it endurance or HI training was not specified (Leon et al., 2002).

Perhaps the most reasonable explanation for no significant improvements in the current study comes from Zmuda et al. (1998) who found that men with low initial HDL cholesterol levels were less likely to increase HDL cholesterol levels through endurance exercise training when compared to men with normal HDL levels. Furthermore it was suggested that men with high base HDL levels will significantly improve HDL levels over those with normal base HDL levels. Considering 42% of subjects in the current research study would be categorized as having low HDL cholesterol levels (<1.0 mmol/l) it is entirely likely that Zmudas research provides the explanation for no change in HDL. Zmuda suggests that subjects with baseline HDL values lower than average are less able to improve HDL cholesterol ratios as a result of endurance training due to a reduced influence on triglyceride metabolism.

Another possible basis for HDL to have remained unchanged is from research by Kodama (2007) suggests that HDL is influenced primarily by the exercise duration, while exercise intensity most favourably modifies blood pressure and waist girth in relation to HDL. Potential mechanisms for favourable exercise related lipoprotein changes could result from enhanced triacyl-glycerol clearance from plasma in response to exercise.

It is important to note that HDL and total cholesterol are influenced by a great number of factors besides exercise. Even trained endurance athletes express substantial variability in HDL levels, with some runners values, around the average value, for the population as a whole. No single factor, be it training status, body composition or nutritional intake separates

runners that exhibit high HDL values to those that have lower values. This suggests a genetic aspect exerts a strong correlation on blood lipid profiles, in particular, endothelial lipase, a specific enzyme that is key in the production of HDL. An up regulation of this gene catalyses endothelial lipase with potential causes including a reducing HDL production and therefore an increase in cardiovascular risk.

The null hypothesis 'High intensity interval training does not significantly improve total cholesterol when compared with high intensity sprint training or aerobic training' can therefore be accepted. The null hypothesis 'High intensity interval training does not significantly improve high density lipoprotein when compared with high intensity sprint training or aerobic training' can also be accepted.

5.4 Lactate threshold

All groups including control began the research study with similar wattage at lactate threshold and neither group reduced or increased lactate threshold at any point during the programme.

This finding is both consistent and conflicting with other studies evaluating the effect of varying intensities on lactate threshold. Some studies (Maffuilli, Capasso & Lancia, 1991) demonstrate a significant improvement in running speed at lactate threshold in high intensity groups, but show no significant improvements in low-moderate aerobic training after 6 weeks and 5 months of training respectively. Other studies have shown no significant differences between training intensities (Londeree, 1997). However these studies showed significant improvements in lactate threshold irrespective of comparisons to other training modalities.

Maffuilli et al. (1991) found that HI training 4 times per week at 80-100% VO_{2max} for 4 weeks improved the wattage produced at lactate threshold by 7% as opposed to endurance training which elicited no effect. This finding is concurrent with more recent research by Esfarjani and Laursen (2007) that also found a significant improvement in Lactate threshold from HI interval training similar to the interval training of the current study. Favier, Constable, Chen and Holloszy (1986) suggests training at or above lactate threshold is necessary to improve lactate threshold, which would explain why submaximal endurance training fails to change lactate threshold. Mechanisms behind this include endurance training recruiting more slow twitch type I muscle fibres resulting in more use of oxygen in the muscles during exercise contributing to a lower production of blood lactate which therefore results in no training effect

in clearing lactate (Donovan & Brooks, 1983) and a reduced utilization of anaerobic ATP-production.

If the oxygen supply to the working muscle is adequate enough, as previously mentioned the lactate will be oxidised and therefore will not build up to near lactate threshold. Conversely the oxidation of the lactate may not be distributed evenly across a muscle, meaning one part of a muscle may receive enough oxygen to properly undertake aerobic metabolism efficiently whereas another part may in fact require lactate to complete the aerobic metabolism cycle to prevent the build up of lactic acid (McCullagh, Poole, Halestrap, O'Brien & Bonen, 1996). HI training may benefit from this mechanism as lactate is transported when lactic acid begins to build and therefore a maintained concentration of monocarboxylate could prove important for improved performance.

The null hypothesis 'High intensity interval training does not significantly improve lactate threshold when compared with high intensity sprint training or aerobic training' can therefore be accepted

5.5 Maximal oxygen uptake

All groups began the research study with statistically insignificant differences between relative VO_{2max} scores, meaning all groups had a similar VO_{2max} beginning testing. None of the groups significantly improved VO_{2max}.

Jeukendrup and Martin (2001) suggest that untrained cyclists elicit 20-40% increase in VO_{2max} after 9 weeks of aerobic endurance training at 60% VO_{2max} 4-5 times per week. Norris and Petersen (1998) compared the VO_{2max} of trained endurance cyclists (VO_{2max} 57 ml.kg.min^{-1}) pre and post an 8 week aerobic training intervention consisting of 4 to 5 sessions per week between 40 and 50 minutes per session. After 4 weeks significant increases in VO_{2max} were observed and by the end of the 8 weeks of training VO_{2max} increased by 5%. The same author also compared the aerobic training programme to a HI training programme involving 30 second to 1 minute sprints at max effort for 12 weeks resulting in a significant ($p<0.05$) 14.22% increase in relative VO_{2max} over that of the aerobic group.

Tabata et al. (1996) showed that HI intermittent training 5 days a week for six weeks can elicit VO_{2max} improvements in just 3 weeks. After 3 week of training, VO_{2max} increased significantly ($P<0.01$) by 5ml.kg.min^{-1} as seen in figure 5.2. Post training, at week 6, VO_{2max} significantly increased by 7ml.kg.min^{-1} compared to baseline. The potential mechanisms behind the relatively fast improvements in VO_{2max} due to HI training, could be a result of

training at a higher percentage of VO_{2max} compared to endurance training, which therefore provides a training stimulus. A factor which determines VO_{2max} is the ability of muscles to receive and utilise oxygen. As previously discussed the ability of HI training to facilitate an up regulation of PGC-1α could then directly influence VO_{2max} (Gibala et al. 2012).
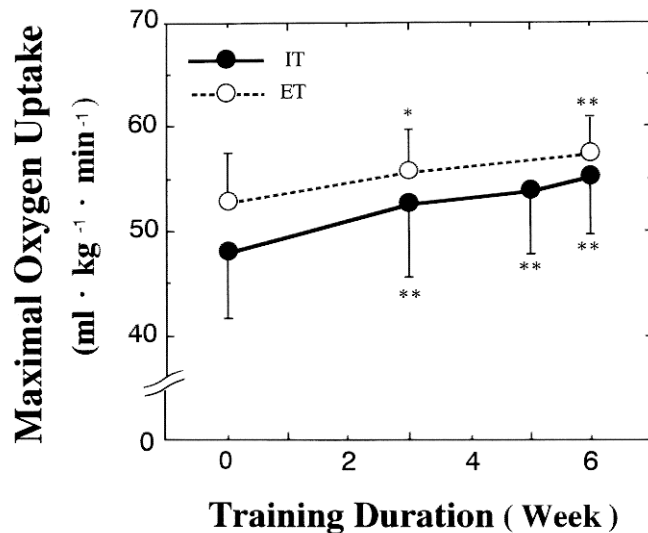
Figure 5.2 Comparisons between endurance training (ET) and HI intermittent training (IT) in VO_{2max} after six weeks of training. Adapted from Tabata et al. (1996).

The null hypothesis 'High intensity interval training does not significantly improve VO_{2max} when compared with high intensity sprint training or aerobic training' can therefore be accepted.

5.6 60 km time trial performance

Firstly there was no significant difference between any training group for the time taken to complete the time trial performance at baseline, meaning all subjects started at the same ability regardless of group. Firstly there was no significant difference between any training group for the time taken to complete the time trial performance at baseline, meaning all subjects started at the same ability regardless of group. All training groups did not significantly change the time taken to complete the time trial at any point during the study however at week 12 the HI training group displayed a significantly different 60km time trial completion time compared to both the aerobic group (2.9% P=0.002) and the interval training group (2.01% P=0.027).

These training adaptations are comparable to other research studies, including a study by Norris and Peterson (1998) involving subjects of a similar baseline VO_{2max} as the subjects in

the current study (56.8 ± 6.6 ml.kg.min^{-1}) reported increases in VO$_{2max}$ (an increase of 7%, also consistent with the current study) and 40km time trial performance increased by 8% after 8 weeks of HI training similar to the HI training in the current study ($P<0.05$). Other researchers have examined the influence of a variety of HI training programmes. Stepto (2001) investigated the influence of five different HI training programmes, performed twice per week for three consecutive weeks, on the rate of performance improvements in 20 moderately trained cyclists.

Stepto, Martin, Fallon and Hawley (2001) compared 2 different HI training modalities and the influence each had upon 40 km time trial performance. The first involving a traditional approach of 8, 4 minute sessions and 85% peak power output with rest periods up to 90 seconds. This form of HI training has improved aerobic performance in a number of different subject groups ranging from novice cyclists (Stepto et al., 2001) to highly trained cyclists (Stepto et al., 2001). The second HI training programme involved 12, 30 second sprints at maximum exertion with 3-5 minutes rest between sets, both forms of high intensity training showed similar improvements in 40 km time trial performance. Laursen and Jenkins (2002) noted that this form of training (the second HI training programme) had not previously been linked with improvements in endurance training which could have implications for improved performance.

The current research supports this claim that HI sprint training could be more important to an endurance athlete, especially if improving time trial performance is a priority as the HI sprint training group in the current study improved 0.6% more than the aerobic training group.

The null hypothesis 'High intensity interval training does not significantly improve 60km time trial performance when compared with high intensity sprint training or aerobic training' can therefore be accepted.

5.7 Limitations and future research

An issue and potentially a reason for the results of the 60 km time trials being completed within very similar times, week after week, especially in the control group who were prescribed no training, could be that although feedback from the Metalyser was hidden from the subject, data from the 3D visualisation in front of the subject was visible during exercise. Of this data the subjects could read the average Kph they were travelling and also distance travelled, which could have influenced how hard they were working knowing how fast they were cycling and therefore how long it would have taken to complete. Feedback to the subject has been shown to influence performance. Nikolopoulos, Arkinstall and Hawley (2001) demonstrated this effect when athletes were told they were completing three 40 km

time trials when in fact the time trials were 34 km, 40 km and 46 km respectively. When subjects were aware of the 46 km trial, subjects on average worked 13 watts less than the other distances, suggesting that subjects work less when the distance is known. Although all subjects in the current study were aware they were undertaking a 60 km time trial it could be argued that knowing were in the 60 km they currently are could have a similar effect to knowing the overall distance.

In an effort to ensure the training programmes were comparable, the weekly training volumes were proportional to the training. The aerobic programme accounted for 2250 kj per week whereas the HI training group accounted for 10% of volume at 225 kj per week. The weekly training volume for the interval training programme was not calculated but every effort was made in its design to incorporate both aerobic and HI elements and was estimated to have a weekly training volume at 50% of the aerobic training programme. Proper investigation in to the calorific load of the interval training programme would have served to better compare the three programmes.

An issue discussed previously was the initially low levels of HDL throughout all groups of the current research study. As no parameters were set in the inclusion criteria for the current study regarding a minimum requirement of HDL levels, subjects with varying HDL levels were recruited. As a result 42% of the subject base would've been classified as having low HDL levels (<1 mmol/l). The implications of this could have produced skewed results in relation to any possible improvements in HDL as a result of training, as it has been suggested that individuals with initially low HDL levels will not respond as readily to exercise when compared to individuals with normal or high HDL levels.

Another potential limitation of the current research relates to the RMR measurement. As previously stated training produces an EPOC effect up to forty eight hours after a training bout. Due to the nature of the training in the current study, subjects were rarely in a situation where the previous training session was more than forty eight hours ago. This, coupled with the logistics of organising a testing session in regards to subject availability and laboratory accessibility meant most of the RMR readings would've been recorded whilst the subject was under the effect of EPOC. Although the implications of this have been reported to create a inflated RMR response, the current study showed no significant differences in RMR. However this could imply that the current study could possibly have reduced RMR if tested greater than forty eight hours after the last training bout.

Finally, the implications of analysing 8 variables on the current study has confused the reader to the purpose of the research, meaning the reader struggles to grasp the homogeneity of the variables studied. In light of this, were the research to be carried out

again, 2 separate studies would have been investigated. 1 would focus on the influence of HI training on performance, with a subject population whose goal is to improve performance, including variables of time trial performance, VO_{2max} and lactate threshold, and a second would focus solely on the potential health related benefits to HI training, utilising a subject cohort that would have no benefit or interest in increasing performance, but would benefit to a health related improvement from exercise for example individuals with diseases and sedentary populations, which are currently inconclusive and therefore crucial to understand.

Potential considerations for future research include exploration into how calorically demanding a HI training programme is, meaning how few training sessions per week are needed to elicit significant improvements in markers of aerobic fitness, namely VO_{2max} or time trial performance.

Chapter 6. Conclusion

In conclusion, of the three training modalities, none elicited any significant change in any of the variables tested over the period of 4 weeks of training. This both contradicts and in some cases supports previous literature, exposing the opportunity for further research into optimising training techniques and exploring the influence of training on novel variables of fitness and health.

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8.0 Appendix

RMR guidelines

Several factors may alter apparent resting metabolic rate (RMR) during measurement with indirect calorimetry. Likewise, numerous indirect calorimetry measurement protocols have been developed over the years, and the methodology employed could influence test results. As part of a larger project to determine the role of indirect calorimetry in clinical practice, a systematic review of the literature was undertaken to determine the ideal subject condition and test methodology for obtaining reliable measurement of RMR with indirect calorimetry by Compher (2006). Food, ethanol, caffeine, and nicotine affect RMR for a variable number of hours after consumption; therefore, intake of these items must be controlled before measurement. Activities of daily living increase metabolic rate, but a short rest (≤20 minutes) before testing is sufficient for the effect to dissipate. Moderate or vigorous physical activity has a longer carryover effect and therefore must be controlled in the hours before a measurement of RMR is attempted.

Limited data were found regarding ideal ambient conditions for RMR testing. Measurement duration of 10 minutes with the first 5 minutes deleted and the remaining 5 minutes having a coefficient of variation <10% gave accurate readings of RMR. Individuals preparing for RMR measurement via indirect calorimetry should refrain from eating, consuming ethanol and nicotine, smoking, and engaging in physical activity for varying times before measurement. The test site should be physically comfortable and the individual should have 10 to 20 minutes to rest before measurement commences. A 10-minute test duration with the first 5 minutes discarded and the remaining 5 minutes having a coefficient of variation of <10% will give an accurate measure of RMR (Compher, 2006).

Raw Data

60k time trial

	Baseline	week 8	week 12
1	118.13	117.02	116.1
1	123.07	119.58	117.48
1	117.37	116.11	113.2
1	121.15	119.52	116.59
1	119.05	117.08	115.54
1	119.55	118.33	116.58
2	120.04	119.49	113.2
2	120.57	118.45	114.52
2	118.09	116.55	112.5
2	114.58	112.57	111.5
2	114.2	112.56	110.56
2	116.59	115.56	113.01
3	118.55	116.01	113.59
3	118.21	115.28	113.59
3	117.08	115.01	114.41
3	117.25	116.21	115.57
3	119.01	118.46	116.54
3	117	116.05	115.21
4	120.04	119.54	119.58
4	119.11	119.05	118.51
4	116.21	116.25	117
4	117.26	117.28	117.24
4	119.54	119.49	119.51
4	118.21	118.24	118.22

Lactate threshold

	Baseline	week 8	week 12
1	164	167	187
1	183	203	202
1	168	165	185
1	137	157	157
1	177	184	176
1	157	164	161
2	141	147	158
2	158	155	162
2	183	184	178
2	151	154	164
2	137	148	151
2	145	144	138
3	124	131	138
3	141	148	154
3	157	161	167
3	167	169	178
3	182	189	201
3	188	186	197
4	150	153	151
4	162	164	159
4	139	135	138
4	141	143	142
4	133	133	134
4	142	140	146

VO$_{2max}$

	Baseline	week 8	week 12
1	38	43	46
1	44	46	52
1	48	52	56
1	48	51	56
1	53	57	60
1	49	53	58
2	51	54	55
2	49	53	55
2	51	55	58
2	47	51	54
2	55	58	62
2	56	59	63
3	49	54	56
3	48	53	56
3	52	56	58
3	51	55	59
3	56	58	61
3	53	56	58
4	59	60	58
4	54	53	53
4	51	51	52
4	47	46	46
4	49	48	47
4	39	38	39

Total Cholesterol

	Baseline	week 8	week 12
1	3.39	3.68	3.55
1	4.21	4.58	4.22
1	3.79	3.24	3.89
1	2.99	3.54	3.48
1	4.51	4.66	4.61
1	3.95	3.91	3.94
2	2.87	2.68	2.99
2	3.55	3.45	3.54
2	4.21	4.25	4.26
2	4.55	4.68	4.58
2	5.01	4.78	4.79
2	3.54	3.48	3.56
3	4.27	4.24	4.58
3	3.99	3.89	3.84
3	2.57	2.68	2.64
3	3.59	3.58	3.44
3	4.44	4.15	4.11
3	5.11	5.01	4.89
4	4.46	4.47	4.41
4	3.38	3.34	3.79
4	4	3.91	3.95
4	3.44	3.48	3.39
4	3.79	3.8	3.74
4	4.11	4.16	4.2

HDL Cholesterol

	Baseline	week 8	week 12
1	0.58	0.67	0.59
1	1.3	1.43	1.21
1	0.46	0.47	0.58
1	1.36	1.34	1.35
1	0.97	1.06	1
1	1.05	0.95	1.07
2	1.83	1.65	1.72
2	0.73	0.81	0.76
2	0.79	0.81	0.85
2	0.73	0.89	0.82
2	0.37	0.37	0.5
2	1.54	1.44	1.49
3	1.64	1.74	1.62
3	1.44	1.47	1.52
3	0.98	0.91	0.94
3	0.88	1	0.94
3	1.89	1.88	1.76
3	1.49	1.67	1.68
4	0.83	0.65	0.73
4	1.05	1.09	1.15
4	1.18	1.24	1.28
4	0.98	1.05	0.99
4	1.99	1.88	1.89
4	1.22	1.19	1.14

Stroke Volume

	Baseline	week 8	week 12
1	76	79	77
1	79	83	78
1	89	84	85
1	94	91	94
1	63	68	67
1	59	70	65
2	78	73	76
2	84	84	83
2	91	84	83
2	59	65	68
2	66	69	68
2	78	75	74
3	72	78	75
3	70	66	69
3	83	81	85
3	85	74	86
3	79	71	75
3	69	76	72
4	67	78	76
4	58	66	66
4	93	81	87
4	75	74	72
4	64	71	72
4	62	76	63

RMR

	Baseline	week 8	week 12
1	2285	2300	2258
1	2300	2254	2358
1	2148	2168	2247
1	1999	2057	2047
1	2465	2300	2311
1	2100	2014	2275
2	2301	2157	2299
2	2005	2045	2100
2	2009	2001	2087
2	2105	2201	2222
2	2165	2144	2178
2	2458	2354	2501
3	2009	2101	2209
3	2017	2132	2101
3	2103	2004	2203
3	2101	2200	2208
3	2611	2588	2534
3	2100	2168	2145
4	2311	2345	2301
4	2456	2510	2405
4	2004	2100	1999
4	1997	2006	2087
4	2000	2078	2153
4	2056	2106	2047

Q

	Baseline	week 8	week 12
1	5.4	5.3	5.7
1	5.1	5.4	5.1
1	5.7	5.2	5.6
1	4.9	5.1	5.2
1	5.2	5.2	5
1	3.9	4.2	4.3
2	5	4.9	5.4
2	5	5.3	5.1
2	3.8	3.9	4.1
2	4.8	4.7	5
2	5.6	5.8	5.2
2	6.1	5.7	5.7
3	4.9	5.4	5.3
3	5.3	5.4	5.1
3	5.2	5	4.9
3	5.4	5.1	4.9
3	5.2	4.9	5
3	4.8	4.9	5
4	4.7	5.6	5.3
4	5.2	4.9	5.4
4	5.8	5.5	6
4	6.4	6.6	5.5
4	6	5.8	5.7
4	3.8	3.7	3.6