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The effects of an ultra-endurance event on heart rate variability and cognitive performance during induced stress in Ironman Triathletes

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University of Cape Town

Declaration

I, Ilse Joubert, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Signed by candidate

10 October 2009

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List of Abbreviations

HRV	Heart rate variability
HRR	Heart rate recovery
HR	Heart rate
RF	Respiratory frequency
RSA	Respiratory sinus arrhythmia
RT	Reaction Time
WM	Working Memory
LF	Low Frequency
HF	High Frequency
VLF	Very low Frequency
ULF	Ultra low Frequency
NN50	Successive R-R differences exceeding 50ms
pNN50	NN50 as a percentage of R-R intervals measured
RMSSD	Root Mean Square successive difference of the R-R intervals
SDNN	Standard Deviation of the average R-R intervals
SDANN	Standard Deviation of the 5-min means of R-R intervals
PSD	Power spectral density
FFT	Fast Fourier Transforms
STFT	Short Wave Fourier Transform
AR	Autoregressive Modelling
ANS	Autonomic Nervous System
RAS	Reticular Activating System
CVC	Cardiovascular Control Centre
SWS	Short Wave Sleep
ECG	Electrocardiography
CTG	Cardiotocography
SA	Sinoatrial
RV	Right Ventricle
LV	Left Ventricle
BMI	Body Mass Index

BP	Blood Pressure
bpm	Beats per minute
HT	Hypertension
CAD	Coronary Artery Disease
CVD	Cardiovascular Disease
MI	Myocardial Infarction
VF	Ventricular Fibrillation
SCD	Sudden Cardiac Death
BRS	Baroreflex Sensitivity
VO ₂ Max	Oxygen Consumption Maximum
VT	Ventilatory threshold
RPE	Rate of Perceived Exertion
FFA	Free Fatty Acids

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Abstract

Background: The effects of long-term participation in ultra-endurance exercise on the cardiovascular system have recently been the subject of much interest. It is well known that HRV, a marker of autonomic activity, is enhanced with long-term aerobic exercise training. However, after acute exercise, HRV is reduced, but recovers over time depending on the intensity of the prior bout of exercise. A limitation of previous research is that exercise bouts of only up to 120 minutes have been studied. A modified Stroop Task is a laboratory stressor to assess executive cognitive function by means of reaction time and accuracy. The resting HRV is directly related to these prefrontal neural functions, but the effect of an altered HRV on cognitive function has never been investigated.

Objective: We determined the effects of an ultra duration (10 – 15 hours) exercise event on parameters of HRV and cognitive function during a Modified Stroop Task, 60 – 200 minutes after the 2007 South African Ironman Triathlon event (3,6km swim; 180 Km cycle; 42,2 Km run).

Methods: 1 Female and 13 male competing triathletes (IRON; ages 33.7 ± 7.9) and 7 control subjects (CON; 2 female and 5 males aged 42 ± 4.5) completed a Modified Stroop Task before and after the event. The individual HRV parameters, heart rate (HR), respiratory frequency (RF), reaction time (RT) and % of mistakes made were recorded via the Biopac MP150WSW System (Goletta, California, USA). Data was transformed by auto regressive analyses (Biomedical signal analysis group, University of Kuopio, Finland) into LF (0.04 - 0.15 Hz) and HF (0.15 - 0.5 Hz) components. Additional calculations included %LF and %HF as well as the central or peak frequencies in both the LF and HF bands.

Results: The IRON group showed significant decrements in all HRV parameters post-event compared to their pre-event values. These IRON

parameters included the pNN50 ($20.6 \pm 20.0\%$ pre vs. $1.3 \pm 1.9\%$ post, $p < 0.001$), the RMSSD (43.8 ± 24.4 ms pre vs. 14.2 ± 7.3 ms post, $p < 0.001$), SDNN (43 ± 16 ms pre vs. 20 ± 11 ms post, $p < 0.001$), VLF (276 ± 224 ms² pre vs. 26 ± 28 ms² post, $p < 0.001$), HF (270 ± 424 ms² pre vs. 21 ± 24 ms² post, $p < 0.001$). Post-event total power recovered only a third of pre-event values after 60-200 minutes, with ~ 70% represented by the LF around 0.1 Hz (%LF $36 \pm 16\%$ pre vs. $69 \pm 22\%$ post, $p < 0.005$). This accounted for the 6-fold increase in the LF/HF ratio in the IRON group (2 ± 1 pre vs. 12 ± 9 post, $p < 0.001$) vs. only a 0.5 fold increase in the CON group (4 ± 5 pre to 6 ± 7 post, $p < 0.05$). During post-event testing the IRON %HF recovery correlated well with their respective RF decline.

There were no significant changes in the cognitive performance parameters in any of the groups despite possible physical and mental exhaustion.

Conclusions: The findings of this study suggest that i) HRV parameters are significantly altered for an undetermined period following an Ironman Ultra Triathlon. These changes may in part explain the changes in the markers of cardiac dysfunction observed in previous studies after Ironman events; ii) Cognitive function, as measured by RT and % missed/incorrect responses during the Stroop Task, was unaffected by participation in this event.

Keywords: heart rate variability; vagal reactivation, triathletes; ultra-endurance; cognitive performance; reaction times; accuracy, fatigue, Stroop

Chapter 1

Introduction and scope of the thesis

Due to the substantial health benefits of moderate physical activity, there has been a trend towards increased participation in sport and exercise globally¹⁻⁴. This has led to increasing interest on the part of athletes, in ultra-endurance sport including the Ironman Triathlon. Yearly, the number of athletes who participate in this discipline increases, and this has indeed been the case in South Africa where entries have reached 1600 in the 2007 event. The Ironman event places considerable physical and mental stress on the participants both on race day and during training and this inevitably leads to the potential of medical complications.

The physical demands and the profile of medical complications associated with participation in the Ironman Triathlon have been documented elsewhere⁵⁻⁷. Recently, however, the focus of attention has shifted to the effects of this type of sports participation on the cardiovascular system⁸⁻¹⁰. Indeed, recent reports have documented potentially negative effects following ultra-duration events on the heart with respect to contractile function and other markers of cardiac damage¹¹. In fact, it has been suggested that increased occurrence of sudden cardiac death following exercise might be related to changes in autonomic function^{12;13}. Yet, very few studies have investigated the physiological changes that follow ultra-endurance exercise, particularly with respect to autonomic activity associated with cardiovascular function.

HRV is a quantitative marker of autonomic activity¹⁴ and has become a popular measure of cardiac health the last 10 years¹⁵⁻¹⁹. It is a non invasive tool to evaluate sympathovagal balance, which is an indicator of cardiovascular and autonomic function in both healthy and disease states. Thus in clinical medicine HRV proved to be a useful prognostic tool to assist in risk stratification for sudden cardiac death, mortality and morbidity from heart failure²⁰.

The effects of exercise training on HRV have been studied over the past 10 years. Indeed, the improvement of HRV parameters with long-term moderate exercise training has led to the vigorous implementation of various forms of exercise in cardiac rehabilitation programmes²¹⁻²⁵.

In the athletic population, recent studies have concentrated on the immediate effects of exercise on HRV and found that mainly exercise intensity determined the delay in recovery if depression of HRV occurred (usually around 55-65 % VO_2 max). Better trained athletes tend to have a higher threshold for these changes, and recover more rapidly with respect to all parameters of HRV^{26;27}. However, the effects of exercise bouts of long duration (more than two hours) on HRV have not been investigated²⁶.

Cognitive performance (central processing and motor execution) during mental stress (sympathetic stimulation) has been shown to simultaneously activate vagal parasympathetic effects on the heart, leading to a decreased heart rate (HR) and raised HRV to counteract the global sympathetic effects on the heart²⁸. This is more prominent with complex cognitive tasks involving executive function (Cognitive Stroop Task)²⁹.

Chapter 2 is a review of the literature on HRV as it pertains to cognitive function and exercise. The effects of long duration exercise (> two hours) like the Ironman on HRV, particularly during stress (competition related) and during a cognitive function challenge test, have never been investigated. We wanted to determine whether HRV responses are altered with exercise of this duration and if the exercise and HRV changes influences cognitive function. This study is the first to monitor cognitive performance after exercise of this duration.

Thus in Chapter 3 the details of an original investigation designed to identify changes in HRV parameters and cognitive function will be presented. The main findings and practical clinical applications of these findings will be summarised in Chapter 4 of this dissertation.

Chapter 2

Literature Review

Heart rate variability and endurance exercise: Implications for health and disease

1. Historical overview of HRV measurement

Since the 19th century and the development of the galvanometer, researchers have expanded on the knowledge and methods applied to ECG recordings to demonstrate heart rate variability (HRV). Hon and Lee (1956) were the first to notice alterations in the heart beat intervals prior to fetal stress³⁰ and Sayers followed by documenting the physiological rhythms embedded in the HRV signal³¹. Studies by Wolf and co-workers in 1977 noted the positive relationship between decreased HRV and post infarct mortality risk³². From the 1980s, Akselrod et al. (1981) expanded on the existing knowledge by introducing power spectral methods to accurately evaluate HRV³³.

Over the years the application of HRV research in the clinical setting were conducted by two major disciplines, cardiology and obstetrics³⁴. Furthermore, physiologists studied HRV in relation to cognition²⁹ and metabolism³⁵, while psychologists studied HRV as an index of 'attention, mental effort, or mental load'^{14;28;29;31;36}.

2. HRV measures and recording

HRV is used as a reliable non-invasive method to investigate vagal activity at the heart and thus can be seen as a quantitative marker of autonomic activity. It is derived by measuring successive R-R intervals from the raw ECG tachogram, or alternatively from arterial pressure recordings. HRV can be analysed in both time- and frequency-domains. Measures were recently standardised by the Task Force of the European Society of Cardiology (see

their report 1996)³⁷. Non-linear measures, like the Poincare plot, fit data points (the value of a given heart period against the subsequent heartperiod)³⁸ into an ellipse; with the length and breadth of the radii of the 2 axes giving a picture of HRV at a glance³⁹.

2.1 Time-domain

Table 1.1: Time-domain parameters

SDNN	standard deviation of R-R intervals
SDANN	the standard deviation of the average R-R intervals calculated over short 5 min periods over time
RMSSD	square root of the mean squared differences of successive R-R intervals
NN50	number of intervals of successive R-R intervals greater than 50 ms
pNN50	the percentage derived by dividing NN50 by the total number of R-R intervals

HRV can be measured as the variance between successive R-R intervals over a period of time. Normal-to-normal (NN) intervals (i.e. all normal intervals between adjacent QRS complexes), are calculated from continuous ECG recordings. Variables derived from direct measurements of the R-R intervals and those derived from the differences between successive R-R intervals can then be calculated. Most commonly used are the NN50 (number of intervals of successive R-R intervals greater than 50 ms) and the pNN50 (the percentage derived by dividing NN50 by the total number of R-R intervals). These parameters represent predominantly parasympathetic activity^{37;38}. The following four measures are usually also recommended: SDNN (the standard deviation of R-R intervals) is an estimate of overall HRV as a square root of the R-R interval variance and represents the general ANS balance. HRV triangular index is a good measure of overall HRV, but permits only casual pre- processing of the ECG signal. SDANN (the standard deviation of the average R-R intervals calculated over short 5min periods over time) is an estimate of the changes in heart rate for cycles longer than 5 minutes, i.e. the

long-term components of HRV. Conversely, RMSSD (the square root of the mean squared differences of successive R-R intervals) estimates the short-term components of HRV. The RMSSD method has better statistical properties than pNN50 and NN50, and is therefore preferred. Only recordings of the same duration can be directly compared in this way³⁷.

2.2 Frequency-domain

Table 1.2: Frequency-domain parameters

HF	0.15 - 0.4 Hz	Parasympathetic activity
LF	0.04 - 0.15 Hz	para- and sympathetic activity
VLF	0.003 - 0.04 Hz	under humeral and thermal regulation
ULF	0 – 0.0033 Hz	day/night variation
LF/HF ratio		marker of sympathovagal balance
HF/(LF + HF)		marker of parasympathetic modulation
LF/(LF + HF)		marker of sympathetic modulation

Abbreviations: HF, high frequency; LF, low frequency; VLF, very low frequency; ULF, ultra low frequency

Power spectral density (PSD) curves of R-R interval traces measures the relative activity in the different frequency bands. The high frequency (HF) band, which is respiratory-dependant and appears to be under parasympathetic influence, lies between 0.15 - 0.4 Hz. The low frequency (LF) band - influenced by both para- and sympathetic activity, appears to reflect mostly oscillations in the baroreflex loop - lies between 0.04 - 0.15 Hz. The LF/HF ratio is seen as a reliable marker of sympathovagal balance⁴⁰.

Other frequencies less defined and not commonly used in shorter duration (1 - 5 minutes) HRV analyses are the very low frequencies (VLF: 0.0033 - 0.04 Hz), possibly under humeral and thermal regulation and the ultra-low frequencies (ULF: 0 – 0.0033 Hz), influenced by circadian cycles. Slow rhythms, especially ULF and VLF measured over longer durations (Holter recordings) have been found to be good predictors of cardiac mortality after MI^{41;42}. Malliani et al. (1994) found that the normalised LF/ (LF + HF) and HF/

(LF + HF) ratios are reliable markers of resting sympathetic and parasympathetic modulation, respectively, even on an individual basis⁴³.

2.3 Recording of HRV

Frequency-domain analyses are preferred over time-domain analyses for short recordings. The recording duration must be at least 10 times the wave length of the lower frequency band, thus at least one minute for HF, and two minutes for LF, are required to assess these two components. For clinical studies, a standard recording period of five minutes has been recommended³⁷. In this regard, it has been found that five-minute recordings track 24-hour recordings adequately for clinical trials in HF and LF bands, while PSD (power spectral density) calculations for VLF band measurements over short duration (<30 min) should be avoided.

HRV in general is subject to circadian rhythms, thus factors like posture, state of arousal, changes of activity and breathing influence HRV⁴⁴. Recordings of HRV have been done supine, standing, during exercise and during 24-h Holter recordings. It is speculated that SWS (short wave sleep), the stage of sleep when most recovery takes place⁴⁵, is under vagal predominance⁴⁶. Combined with very regular breathing and very stationary ECG, SWS provides optimal conditions for HRV recordings^{44;47}.

Spectral analysis can be calculated non-parametrically (Fast Fourier Transforms-FFT), which is simplistic and fast; or parametrically, which is smoother with easy post-processing and identification on smaller samples^{48;49}. FFT assumes that the time series contains only deterministic components. On the other hand, autoregressive (AR) modelling, where data are treated as a composite of deterministic and stochastic components, provides a more statistical approach⁴⁰.

Additional measures of HRV, like the quantification of RSA, can be evaluated by cross-spectral analysis as introduced by Porges et al. (1980)⁵⁰. Finally,

wavelet transform analyses divide the heart rate fluctuations into the various frequency components of the signal⁵¹.

Artefacts contaminating the raw ECG tachogram can be resolved by correcting the original ECG trace.

3. HRV regulation

3.1 Autonomic control

The heart is regulated by stimulating sympathetic nerves and inhibitory parasympathetic nerves of the autonomic nervous system (ANS) and influences heart rate and contractility. The vascular circulation is neurally controlled by the central circulatory demand centre in the subthalamic region, the cardiovascular control centre (CVC) in the pons and medulla as well as peripheral afferents (hypothalamus, baro- and chemo receptors and muscle afferents). HRV mainly depends on parasympathetic input from the vagal nerve. The presence or withdrawal of parasympathetic input is controlled cerebrally by the central circulatory demand centre, by integrating cognitive processes, brainstem nuclei and peripheral afferent input⁵².

3.2 Non-autonomic control (Hormonal)

Hormonal fluctuation (catecholamine, cortisol, growth hormone, renin-angiotensin) influences HRV frequencies below 0.3 Hz, while circadian rhythms determine hormonal levels. It is believed that these indirect effects of circadian cycles are represented by LF variations⁵³. Lastly, respiratory-related mechanical stretch of the sinoatrial node also influences the heart rate⁵⁴.

3.3 HRV balance

A healthy HRV is determined by the balance between parasympathetic tone and sympathetic input via the autonomic nervous system (ANS). The combined effect also depends on the condition of the heart, i.e. the autonomic reactivity of the heart or its sensitivity/ability to respond to the impulses that

determine its rhythm^{55;56}. Thus a decrease in HRV may be indicative of a lowered vagal discharge and/or resistance of cardiac muscarinic parasympathetic receptors⁵⁷.

Sympathetic activation occurs after mental- (cognition or emotions)^{58;59}, physical-(exercise)⁶⁰ or chemical- (adrenergic agonists, atropine antagonists)^{57;61;62} stress, which releases metabolic and hormonal factors⁶³ into the circulation like adrenaline, noradrenaline, cortisol and growth hormones. These hormones react on the β 1 cells of the myocardium to induce increased heart rate and contractility. It is noteworthy that HRV responses differ depending on the mode of β adrenergic stimulation⁶².

The vagus nerve mainly regulates autonomic control of the heart during rest, by slowing heart rate at the SA node, and dampening contractility power of the rest of the myocardium. The activity of parasympathetic vagal input is determined by factors like age⁶⁴, exercise⁶⁵, training⁶⁶, disease and body fat⁶⁷. BMI is for example inversely related to HRV⁶⁸.

The total sympathetic effect is dependant on the amount of vagal withdrawal as seen in animal studies where vagal dominance prevented ventricular fibrillation (VF), but poor vagal activity (vagal block by atropine) rendered them susceptible to ischemia-induced VF⁶⁹. During cognitive challenging tasks, a strong vagal drive will override excessive sympathetic effects - thus increasing stroke volume (lengthened diastole which will allow increased filling of the ventricle)^{70;71} - and thereby improve concentration⁶⁴ and reduce natural tremor to enhance accuracy (rifle shooting)⁷².

3.4 Determinants of HRV

Spontaneous HRV is dependent on 3 physiological factors; namely, respiration, BP regulation, and thermal regulation. These 3 components affect the HRV power spectrum as follows:

- Respiration generally results in RSA activity in the HF band. However, RSA frequency is heavily influenced by breathing frequency^{73;74}, and the RSA amplitude is strongly related to RMSSD.
- Maintaining peripheral resistance via adrenergic vasomotor activity results in LF oscillations around 0.1 Hz at the heart⁷⁵, while thermal vasomotor regulation peaks at VLF < 0.04 Hz⁷⁶.

Modifiable factors of HRV include exercise training^{65;66} or higher activity levels⁷⁷, lower BMI and increased lean body mass⁶⁷, better lipid profiles, improved glucose tolerance and insulin sensitivity, lowered blood pressures⁶⁸⁶⁵ ⁷⁸, as well as gene expression⁷⁹.

Non-modifiable factors, like age and gender^{65;80}, genetics⁸¹, ion channel activity⁸² and autonomic receptor expression⁸³, might be responsible for the large variations in baseline HRV.

In subjects over 70-years-old, more than 50% of total spectral power lies in the VLF range which peaks at 0.03 Hz⁷⁶, indicative of the higher sympathetic drive seen with age. In the aged and in CHF patients, the constantly elevated sympathetic tone leads to down-regulation of adrenergic receptors. This augmented sympathetic activity occurring with age⁸⁴, shifts the whole spectrum to the lower frequencies. In a healthy population VLF rhythms are potentially determined by intravascular acidosis/alkalosis, high altitudes, asphyxia, and vasoactive agents⁸⁵. They are influenced by thermoregulatory processes⁸⁶, by the renin-angiotensin system,³³ and hemodynamic feedback delays, as well as by the mechanical and central effects of breathing⁸⁷.

HRV is also subject to circadian rhythms; thus factors like posture, state of arousal, changes of activity, and circulating hormonal levels exert an influence on HRV⁴⁴. Perini et al. (2003) summarised the effects of body position as a shift from vagal to sympathetic dominance⁷⁶. The sitting position results in a decrease in HF compared to a supine position via vagal withdrawal induced by orthostatic pressure changes. Thus the sitting position results in

sympathetic dominance, thereby masking effects of training on vagal activity in both HF and LF bands. Long-distance runners and cyclists tend to have the highest HF power in the supine position only. This increased HF power is even more pronounced in the LF/HF ratio – a good estimate of sympathovagal balance⁴⁰. Surprisingly, it does not seem as if the lowered LF/HF ratios in trained subjects are dependent on body position⁷⁶. LF power peaks around 0.1 Hz, especially during head tilt or mental stress, due to sympathetic activity⁷⁶.

Respiration may also affect the HRV via the mechanical stretch of the SA node, and furthermore, respiratory modulation of autonomic nerves is proportional to levels of activity in the baroreceptor reflexes. Changes in the intrinsic mechanisms of the SA node, responsible for training-induced bradycardia, may also influence HRV^{65;88;89}.

Psychosocial factors like emotions (anxiety and depression)⁹⁰ and mental activity (complex tasks) have been shown to reduce HRV, while relaxation improves HRV⁹¹.

4. Clinical application of HRV

The clinical relevance of HRV is substantial. For years obstetricians have used HRV monitoring via CTG (cardiotocography) recordings to monitor fetal health and in anaesthesiology it serves as an index of the depth of anaesthesia⁹². Psychosocially HRV biofeedback training is used to improve emotional and psychiatric disorders. Lately, reduced HRV has been found to be associated with diabetes^{93;94}, hypertension⁹⁵ and overtraining in athletes⁹⁶. A study completed during and after a training camp suggested that HRV can be used as a monitoring tool for the training state in athletes to determine and prevent the overtraining syndrome⁹⁷.

Furthermore, in clinical cardiology, HRV as a measure of sympathovagal balance at the heart¹⁴ is a well-researched subject. This is especially so in known heart diseased patients¹⁵ and over the past 20 years has proven to be an extremely useful tool in determining cardiac health. In fact, it is one of the

single most important independent determinants of not only the health or status of the cardiovascular system, particularly the heart, but also an accurate indicator of the heart's recovery after a cardiovascular incident^{16-19;98}

Indeed, prospective studies have shown HRV to be a reliable indicator of survival after myocardial infarction; of incidences of ischemia; and of the risk of sudden death due to reperfusion-induced arrhythmias⁹⁹⁻¹⁰¹. Thus it serves as a good prognostic tool in risk stratification for sudden cardiac death¹⁰² and mortality and morbidity from heart failure^{20;41;99;103}. A reduction in HRV has also been shown to be directly correlated to angiographic severity of CVD^{104;105}. R-R intervals of less than 50 ms increases CVD mortality risk 5.3 times compared to HRV > 100 ms¹⁰⁶.

Sudden Cardiac Death (SCD) in healthy young athletes is a great cause of concern among coaches and medical staff, as well as athletes and their families¹⁰⁷⁻¹¹¹. The causes of SCD and the anatomically pathological conditions leading to it, are well described and are well-known in cardiology, and protocols are in place to screen athletes clinically for underlying heart conditions before competing¹¹²⁻¹¹⁵. Important factors associated with SCD are previous myocardial ischemic injury and its size¹¹⁶; the presence of coronary artery thrombi¹¹⁷; and alterations in cardiac autonomic regulation¹¹⁸. Furthermore, the risk of SCD increases significantly (20-fold) during and immediately after exercise, possibly related to the acute exercise-induced changes in autonomic tone^{12;13}, which include parasympathetic withdrawal and increased sympathetic activity¹¹⁹. Markers of impaired parasympathetic activity, such as low HRV, a small HR response to an increased BP i.e. low baroreflex sensitivity (BRS)^{120;121}, slow HRR after exercise^{101;122-126} and an increased QT interval⁶¹, accurately predicts increased risk for SCD and arrhythmias, in both CHD patients¹²⁷⁻¹²⁹ and the general population¹³⁰⁻¹³².

Therefore HRV is accepted as a sensitive cardiac health marker to assess the relative risk of cardiac conditions or to enhance risk stratification for sudden cardiac death in young presumably healthy athletes¹³³. Voss et al.(1998) established that the sensitivity of predicting arrhythmia risk improves more

with a multi-parametric approach (using time- and frequency-domain parameters combined with HRR and BRS), than by standard measurement of global HRV alone¹³⁴. VF during a first ischemic attack in healthy individuals can be better predicted together with HRV and BRS¹³⁵. Thus combined with HRR indices, HRV more accurately predicts risk in asymptomatic CAD, independent of the severity of CAD and the left ventricular ejection fraction^{124;124}.

5. HRV and Cognitive Function

The four factors that play a role in the outcome of cognitive functionality after exercise are the fitness of subjects, exercise intensity and duration, the specific psychological task used and the time until testing¹³⁶.

5.1 Exercise as stressor

Acute aerobic exercise positively influences psychological and cognitive brain functions^{137;138}, while a 15-week aerobic exercise training regime enhanced executive function in overweight children compared to sedentary controls¹³⁹. More specifically, aerobically trained subjects¹⁴⁰ improved their reaction times and accuracy during complex central processing. This correlates with the faster reaction times found in active adults during formal central information processing tests^{141;142}. However, detrained subjects still maintained their reaction times in non-executive simple cognitive tasks¹⁴³. On the other hand, short term aerobic exercise training (16 weeks) did not alter sympathetic nervous system activity or behavioural responses during the completion of a Stroop Task in middle-aged men¹⁴⁴.

Apart from physical fitness, HRV also seems to be an important factor for competent functioning in a complex environment¹⁴⁵; and correlates with better performance on tasks involving executive function¹⁴³, which rely on an intact and active prefrontal cortex¹⁴⁶.

Task performance is often an inverted U-shape function of arousal¹⁴⁷. Exercise induced arousal is dependent on sympathetic mediation from the reticular activating system (RAS)^{137;148}. Arousal levels are optimal after moderate exercise, but reduced after high-intensity exercise. Immediate cognitive function thus improves with exercise lasting less than 1 hour¹⁴⁹, due to raised metabolic rate, blood-flow and brain neurotransmitters (catecholamines, endorphines)^{150;151}.

Grego et al. (2004) suggested that, apart from arousal, central fatigue resulting from exercise of more than two hours and hallmarked by heat, dehydration and hypoglycemia¹⁵⁰, can negatively impact cognitive function¹⁵². Grego and co-workers found that P300 latency was slower during the 2nd hour of exercise, and it occurred concomitantly with metabolic changes, such as increases in cortisol, epinephrine and FFA and decreases in blood glucose. Polich and Kok (1995) suggested that delayed P300 latency is associated with an alteration in the speed of cognitive processing¹⁵³.

Excessive sympathetically mediated arousal can impair cognitive function when performing under pressure. Strong vagal drive to dampen HR and increase HRV is necessary to maintain or improve executive cognitive function under these conditions. This was first noted by Bea and John Lacey in 1958²⁸. They found that vagal activation occurred paradoxically during raised alertness, counteracting global sympathetic input on the heart. Heart rate decreased despite sympathetic responses like raised skin conduction velocity and sweating^{36;36}. This was seemingly more prominent with tasks that needed central processing prior to peripheral motor activation. In other words, response inhibition was required during active anticipation. Termination of this process also terminated the slowing of the heart. The more complex the task or the more mental processing was needed prior to response inhibition, the more central inhibition of processing occurred and the longer the interval between two heart beats became.

5.2 The Stroop task as stressor

The Stroop task and the reliability of correct responses have been shown by several studies to be a valid laboratory induced stressor^{154;155}. Standard Stroop tasks include variants of congruent (colour matching words) and incongruent (colour different from word) colour-word and neutral-word or picture-word tasks on a computer screen. The subject must inhibit responses/executive function to these tasks, until complex central cognitive processing took place. This Stroop task interference is defined as the added stress associated with naming the colour of the word rather than the more automatic reading of the word^{156;157}. This interference effect is very robust and reliable, especially if emphasis is placed on speed¹⁵⁸. Outcomes can then be measured according to reaction times and accuracy, namely the faster and more accurate, the higher the cognitive executive function

Age seems to slow down performance in all forms of Stroop tasks^{157;159}, although performance is unaffected by hand dominance and gender¹⁶⁰. The effects of anxiety and tension provoked by the Stroop task may improve reaction times, probably from increased concentration and muscular tension¹⁶¹.

Thus the Stroop task provides an adequate complex task under performance stress conditions²⁸, and should be especially challenging to the fatigued Ironman group of participants.

6. HRV in sports medicine

6.1 Acute effects of exercise on HRV

Exercise is characterised by parasympathetic withdrawal, sympathetic stimulation, and increased circulating catecholamines¹⁶². During recovery, these processes are reversed, firstly, by the prompt restoration of vagal tone/parasympathetic reactivation^{124;163;164}, followed by the slow withdrawal of sympathetic activity and circulating humoral factors¹⁶⁵. During exercise, vagal withdrawal should be accompanied by a decrease in HRV values. However,

studies in HRV during exercise consistently delivered conflicting results, with HF, LF, and LF/HF ratios not reflecting the decreased vagal activity. Parasympathetic effects seem to persist even during high intensity exercise^{119;166}. Perini et al. (2003) concluded that HRV seems to be a reliable indicator of autonomic regulation during rest and recovery periods, but not during exercise⁷⁶.

6.2 Long-term effects of exercise on HRV

Aerobic fitness depends on training intensity and endurance. The current best known determinants of aerobic fitness are the vagal-related indices, namely HR, HRR, and HRV, especially the HF and LF components and the LF/HF ratio¹⁶⁷. Markers of intensity and endurance are VO_{2max} and VT (ventilatory threshold) respectively. The VT¹ (1st ventilatory threshold) is the exercise intensity where a decrease in arterial oxygen levels occurs and is expressed as a percentage of VO_{2max} (usually around 55-65% VO_{2max}) or can be expressed in ml O₂/min/kg. The VT² (second ventilatory threshold) represents the exercise intensity where PaO² and PaCO² levels decrease simultaneously around 85% VO_{2max} . Aerobic endurance can be determined by constant intensity performance, by the duration of maintained performance, by blood lactate concentration, by the ventilation during graded exercise, by the O₂ uptake, and by the VT¹⁶⁸.

6.2.1 HR and HRR

For years sports coaches have used resting heart rate and post-exercise heart rate recovery (HRR)¹²² as indicators of the aerobic training response, or to guide against overtraining or fatigue¹⁶⁹, even though many studies have failed to confirm this¹⁷⁰. It has therefore been suggested that HRV should also be used as an indicator of athlete fatigue¹⁷¹; however, Bosquet et al. (2008) warned that for the correct interpretation of HR and HRV, it must be compared with other signs of overreaching before conclusions can be drawn¹⁷².

Furthermore, a study by Buchheit et al. (2006) showed that HRV and HRR can be dissociated. While HRV is more specifically related to cardiorespiratory fitness, HRR is better associated with training load as determined by the RPE/ Baecke Sport Scores¹⁷³. Thus effects of fitness on HRR are training-load induced and represent acute changes in cardiac autonomic function. On the other hand, the effect of increased cardiorespiratory fitness on HRV is more related to the genetically determined VO_{2max} and gives insight into chronic cardiovascular changes¹⁷³. Bosquet et al. (2007) and Javorka (Javorka 2002) also found no correlation between HRV and HRR^{167;174}.

Though high VO_{2max} values have persistently been associated with better autonomic control (increased HRV and HRR), Bosquet found no correlation between aerobic endurance (as measured by VO_{2max} , and VT) and HRV or HRR¹⁶⁷.

6.2.2 HRV

It is well documented that physically trained subjects have increased parasympathetic activity and lowered heart rates at rest¹⁷⁵. Aerobic exercise training has been shown to both lower cardiac sympathetic activation and increase parasympathetic tone at rest and during exercise¹⁷⁶. Moderate- to high-intensity training seems to be more effective than low intensities of the same energy expenditure¹⁷⁷. The Whitehall II study of civil servants in the UK (1997-1999), also confirmed the association of moderate to vigorous activity on HRV¹⁷⁸. Recently it was confirmed that training improves BRS¹⁷⁹. Meanwhile, HR and HRV responses to ischemia or submaximal exercise are reduced with training¹⁰⁶.

Exercise duration, combined with intensities above the VT1 provides the necessary stress response and metabolic demand to activate the sympathetic autonomic branch, which leads to acute and long-term ANS changes and adaptation due to the shift in autonomic balance²⁷.

Recent studies by Iwasaki (2003) and Buchheit et al. (2004) described the relationship between exercise load and HRV graphically as bell-shaped and confirmed that only a moderate training load is necessary to increase vagal-related HRV indices^{44;180}. In highly trained athletes - despite lower HR - HRV returned to pretrained values or equalled that of sedentary controls, if training loads were excessive; even in the absence of overload, competition and fatigue.

Goldberger et al. (1996) also showed that HRV only increased up to a critical point. Further increases in parasympathetic activity resulted in a decrease in HRV¹⁸¹. HRV as an index for cardiovascular vagal activity may be unreliable in subjects with originally high vagal activity¹⁶⁷ and extreme training loads (i.e. athletes preparing for competition) and may lead to persistently elevated sympathetic tone¹⁸².

Furthermore, although increased HRV in moderately trained athletes seem to be reproducible regardless of changes in the amount of weekly activity¹⁸³, HRV did not increase in a dose-dependant manner with increasing activity. This agrees with Buchheit et al. above, that physical activity level does not seem to be an accurate correlate of HRV.

6.3 HRV, exercise and disease states

The linear correlation between improvement in HRV (an increase in HRV parameter values) and moderate exercise, or simply increased daily physical activity, is a remarkable finding, and has led to the vigorous implementation of exercise in cardiac rehabilitation programmes to reduce mortality and morbidity²⁵.

Billman et al. (1991) showed that exercise has anti-arrhythmic intervention properties¹⁸⁴. These authors found that endurance exercise training can improve vagal function such that vagal regulation is maintained even when the heart is stressed by exercise or ischemia. A possible mechanism is that healthy parasympathetic activity counteracts the sympathetically induced QT

prolongation during the post-exercise recovery period, thus serving as a natural anti-arrhythmic⁶¹

The cardio-protective effect of increased HRV (improved vagal tone) appears to be mediated through enhanced cardiac electrical stability¹⁷⁶ of the cardiac cell-membrane⁴⁴. In the diseased heart, β_1 receptor sensitivity decreases. Consequently, the inotropic support relies on the remaining β_2 receptors. β_2 receptor activation results in an elevation in intracellular calcium, provoking oscillations in membrane potential, which reduces cardiac stability¹⁸⁴. Exercise seems to alter this β receptor imbalance by reducing β_2 receptor density or altering signalling, thus restoring electrical stability¹⁰⁶. Therefore a robust cardiac system that can adapt rapidly to a change in environmental demands via negative feedback has good prognostic properties, while a monotonic HR response may indicate electrical instability.

It is known that acute reductions in HRV resembles stress placed on the cardiovascular system, either physiological or due to pathological disease. We also know this happens in both healthy and diseased subjects¹⁸⁵, but to a lesser extent in trained individuals⁶⁹.

Past HRV studies concentrated mostly on known cardiac disease or patients at high risk for cardiac disease, with the goal of determining the long-term manipulative possibilities of exercise to amend risk factors.

7. Ultra duration exercise and cardiac health

Concern over whether exercise of ultra duration would cause temporary or permanent cardiac damage has led to numerous publications on Ironman triathletes with sometimes contradictory results.

In 2004, La Gerche et al. found no markers of sustained damage 4.7 days after an Ironman event¹⁸⁶. Recent studies did, however, report cardiac dysfunction after an ultra-endurance event, although the duration needed for these changes are not clear. Full- and half-Ironman races both seem to

transiently lower left ventricular contractility and alter relaxation. This is shown in the impaired diastolic and systolic left ventricular function on echocardiography, up to 2 days post-event¹⁰. Diastolic function changes do not seem to be influenced by training status or exercise duration⁸. Furthermore, the cumulative effect of repeated long duration exercise bouts does impede LV systolic function¹⁸⁷ which may influence performance in a multistage event. Right ventricular (RV) function impairment up to 1 week post-event seems to be of even greater clinical relevance if due to pulmonary hypertension¹⁸⁸. Rifai and co-workers (1999) assessed highly sensitive and specific markers of cardiac damage, cardiac troponin T and troponin I, and confirmed that these reversible abnormalities that caused cardiac dysfunction after an Ironman event, were due to cardiac damage¹¹. In this regard, Tulloh et al. (2006) observed post exercise ECG changes in all participants, and found linear correlations between ejection fraction decreases and peak troponin T levels¹⁸⁹. Several mechanisms have been postulated: transient coronary spasms due to exercise with myocardial stunning due to reflow^{190;191}; oxidant stress or disturbed calcium homeostasis¹⁹²; LV pressure gradients¹⁹³; altered preload and afterload¹⁹³; cardiac autonomic modulation; cardiac cellular metabolism¹⁹⁴ and the desensitising of β_1 -receptors due to prolonged exposure to increased catecholamine levels¹⁹⁵.

Cardiac damage may even become fatal. It has been hypothesised that some individuals may be more prone to developing fibrotic plaques, or scar tissue, due to minor myocardial injury¹⁰. Lesions near the atrioventricular bundle and bundle branches may lead to fatal arrhythmia in veteran athletes independent of the size or density of the lesion¹⁹⁶.

8. Effects of acute exercise on HRV

8.1 Exercise type/Frequency

The resultant fatigue from heavier training loads has been shown to reduce HRV¹⁹⁷. Previous studies found that aerobic exercise reduced HRV in both the HF and LF frequency bands^{76;198}, with recovery periods of more^{199;200}, or less than one hour^{174;201}.

Prolongation of recovery periods were even more pronounced after anaerobic exercise⁷³. Thus resistance exercise may promote prolonged disturbance of autonomic activity, which may increase risk of a cardiovascular event during this period in older or sedentary susceptible individuals.

HRV is also decreased more in continuous than in interval exercise and the degree of lowering correlates with the intensity of exercise²⁰².

8.2 Exercise intensity

Perini et al. (2003) found that HRV fluctuations seem to correlate with exercise intensity and postulated that this may specifically reflect muscular work⁷⁶. A study by Kaikkonen et al. (2007) found slower recovery and lower levels of HF and total HRV after moderate- to high-intensity exercise, but found that doubling the running distance had no added effects to that of the intensity²⁶.

After submaximal exercise ($VO_{2max} < 55\%$) autonomic control of HR normalised within five minutes²⁰³, except if aged >70 years, when 7-8 minutes were required for full recovery of HRV²⁰⁴.

Seiler et al. (2007) further established that only exercise intensities done above VT_1 (around 55-65% VO_{2max} depending on the training condition of the athlete), clearly marked the threshold for delayed ANS recovery²⁷. This may be more pronounced above VT_2 in recreational athletes.

Below VT_1 minimal changes occur in blood hormone levels (cortisol, growth hormone, adrenaline and noradrenalin) and immune function, with no increases in blood lactate²⁰⁵. This is indicative of minimal metabolic changes. On the other hand, exercise above VT_1 ($VO_2 > 65\%$), increases blood lactate concentrations and oxygen consumption. Combined with the increased metabolic demand, increases in HR and respiratory frequencies seem to correlate with changes in the recovery of autonomic function post exercise,

which influences HF especially. Thus HRV may be a good indication of whether exercise was performed above or below the ventilatory threshold²⁰⁶.

8.3 Exercise duration

Prolonged distance exercise does not seem to have the same effect on metabolism as does moderate to high intensity exercise²⁶. It is unsure what duration must be performed to induce autonomic changes, or cause significant delays in recovery, especially if performed below VT_1 ²⁷.

8.4 The recovery period

Recent studies have investigated the effects of exercise of different intensities and durations on HRV during the immediate recovery period. Recovery of HRV to resting levels after mild to moderate exercise has been shown to occur within 5 minutes. However, higher intensity exercise delays the recovery period for up to 1 hour, independent of the duration of the exercise bout²⁷.

It is speculated that the initial fast recovery of HR and HRV post exercise, is due to prompt restoration of vagal tone (possibly incomplete)⁷³. Immediate changes in cardiac function (e.g. pre-load, after-load and contractility) combined with the loss of central command and baroreflex activation, leads to fast vagal reactivation^{198;201}. This produces a decrease in HR of 30 - 35 beats/min within the 1st minute of recovery⁷⁶. The withdrawal of sympathetic nerve activity and hormonal factors, starting after the 1st minute, contribute to a further decrease in HR after medium-high intensity exercise^{163;198;207}.

The prolonged recovery of HRV is predominantly down to the role of the parasympathetic system⁷³. This has also been shown by Raczak et al. (2006), who found that the change in autonomic profile that occurred due to long-term intensive training, promoted parasympathetic dominance¹⁸².

In this regard, a characteristic of highly trained athletes is faster post-exercise ANS recovery, even after exercise above VT_2 , reflecting parasympathetic dominance²⁷.

The effect of intensity and duration of exercise on HRV in the 1st minute post exercise was explored by Kaikkonen et al. (2007)²⁶ and Martinmaki et al. (2008)²⁰⁸ who both used Short-Time Fourier transform (STFT) methods to calculate HRV²⁰⁹. HRV recovery was complete in the HF band as well as in the other frequency bands about 1-2 min after moderate- to low-intensity exercise^{26;208}. This correlates with the blockade study of Goldberger et al. (2006) who found increases in all time-domain parameters, which included the RMSSD and RMS, within five minutes. These authors also found RMSSD and RMS to be reliable markers of vagal activity²¹⁰ as noted by the Task Force (1996)³⁷.

Exercise intensities exceeding 50% VO_{2max} leading to very low HRV during exercise, resulted in significant slower recovery of only HF power, not detected in the other frequencies²⁶. This indicates that only HF power represents vagal modulation changes and seems to reflect intensity more accurately²⁶. Furthermore, using conventional spectral analysis methods, HF and LF expressed in absolute values (ms^2) seem to provide more consistent results than normalised values²⁰⁸.

Seiler et al. (2007) found that HRV recovery, independently of duration (60 or 120 minutes), took place within 5-10 minutes if done below VT_1 , but was delayed to 30 minutes if done above VT_1 . They found a higher VT_1 threshold in the highly trained athletes with more rapid recovery of all parameters, compared to controls²⁷. Thus the latest studies (Kaikkonen & Seiler et al. 2007) established ANS recovery to be related to the intensity of exercise, regardless of the duration of exercise. However, this research only examined exercise durations up to 120 minutes; the effects of longer duration exercise have yet to be investigated²⁶.

As far as we are aware, no studies have investigated presumably healthy athletes and the extent of their HRV perturbations during an ultra-endurance event like the Ironman triathlon (duration of up to 15 hours), where the role of hormonal and metabolic factors are likely to present the athletes with extreme cardiovascular challenges.

We studied 14 highly trained triathletes and compared their resting and post Ironman data to that of control subjects, who worked similar if not longer hours, at the Ironman under severe stress. We investigated the effects of below VT_1 exercise over durations >10 hours on cognition and HRV and its rate of recovery. We measured HRV before and after the Ironman during a five minute cognitive test, to standardise the mental state of all participants.

University of Cape Town

Chapter 3

The effects of an ultra-endurance event on heart rate variability and cognitive performance during induced stress in Ironman Triathletes

Introduction

Due to the substantial health benefits of moderate physical activity, there has been a trend towards increased participation in sport and exercise globally¹⁻⁴. This has led to increasing interest on the part of athletes, in ultra-endurance sport including Ironman Triathlon. Annually, the number of athletes who participate in this discipline increases, and this has indeed been the case in South Africa where entries reached 1600 in the 2007 event. The Ironman event places considerable physical and mental stress on the participants both on race day and during training and this inevitably leads to the potential of medical complications.

The physical demands and the profile of medical complications associated with participation in the Ironman Triathlon have been documented elsewhere⁵⁻⁷. Recently however, the focus of attention has shifted to the effects of this type of sports participation on the cardiovascular system⁸⁻¹⁰. Indeed, recent reports have documented potentially negative effects following ultra-duration events on the heart with respect to contractile function and other markers of cardiac damage¹¹. In fact it has been suggested that increased occurrence of sudden cardiac death following exercise might be related to changes in autonomic function^{12;13}. Yet, very few studies have investigated the physiological changes that follow ultra-endurance exercise, particularly with respect to autonomic activity associated with cardiovascular function.

HRV is a quantitative marker of autonomic activity¹⁴ and has become a popular measure of cardiac health the last 10 years¹⁵⁻¹⁹. It is a non-invasive

tool to evaluate sympathovagal balance, which is an indicator of cardiovascular and autonomic function in both health and disease states. Thus, in clinical medicine it has proven to be a useful prognostic tool to assist in risk stratification for sudden cardiac death, and mortality and morbidity from heart failure²⁰.

The effects of exercise training on HRV have been studied over the past 10 years. Indeed, the improvement of HRV parameters with long-term moderate exercise training has led to the vigorous implementation of various forms of exercise in cardiac rehabilitation programmes²¹⁻²⁵.

In the athletic population, recent studies have concentrated on the immediate effects of exercise on HRV and found that mainly intensity determined the delay in recovery if depression of HRV occurred (usually around 55-65 % VO₂max). Better trained athletes tend to have a higher threshold for these changes, and recover more rapidly with respect to all parameters of HRV^{26;27}. However, the effects of exercise bouts of long duration (more than two hours) on HRV have not been investigated²⁶.

Cognitive performance during mental stress has been shown to simultaneously activate vagal parasympathetic effects on the heart²⁸ in the presence of sympathetic stimulation. This is more prominent with complex cognitive tasks involving executive function (e.g Stroop Task).

The effects of long duration exercise (> two hours) like the Ironman on HRV, particularly during stress (competition related) and during a cognitive function challenge test, have never been investigated.

Aim of the study

This study sought to determine the effects of fatiguing exercise following the participation in the Ironman Triathlon on indices of HRV and cognitive performance during induced mental stress. The changes in variables within the group of athletes were compared to those of a control group of

recreational and non-athletes who did not participate in the event but remained awake for at least the same duration as the event.

Methodology

Study design

This was a prospective cohort study.

Subjects

21 Triathletes, competing in the 2007 Ironman Triathlon in Port Elizabeth, were recruited as subjects for this study at registration. The event comprised a 3.8km surf swim (2 laps of 1.9km each), 180km cycle (3 laps of 60km each), and finally a 42.2km run (3 laps of 14km each) which all have to be completed within the cut-off time of 17 hours. 7 Control subjects were made up of staff of the UCT/MRC Research Unit for Exercise Science and Sports Medicine who were not competing, but attended to the medical tent and research activities during the event.

All participants were fully informed as to the nature of the study, the investigations involved (Appendix B) and the questionnaires (Appendix C) to be completed. Those agreeing to proceed with the study completed and signed an informed consent form. (Appendix E). The experimental protocol was approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town in accordance with the Declaration of Helsinki.

Participants (subjects and controls) had to be non-smokers and relatively active with no history of cardiac symptoms, disease or previous collapse. The complexity of the Stroop test required a good and intact cognitive function and a fast working memory (WM). Exclusion criteria thus included cardiac disease, colour blindness, previous head injuries, psychiatric disorders or any current use of drugs or medication. In particular, participants had to be free from

especially heart rate altering medications, CNS drugs like anti-epileptics, stimulants (caffeine) and recreational drugs

Testing Procedure

The 7 Control subjects were tested prior to leaving Cape Town in a soundproof dark room under rested conditions. Each test took about 30 minutes to complete. In all, 21 Ironman subjects were tested during the Ironman registration in a darkened, separate and relatively quiet room. They completed a questionnaire regarding personal detail and training hours, experience and injuries (Appendix C). Subjects then underwent a familiarisation Modified Stroop Task to allow them to become used to the environment and computer test. After the familiarisation test, electrodes and transducers were applied to the subjects and connected to a Biopac MP150WSW System (Goletta, California, USA) to record ECG and respiratory frequency (RF).

Subjects were instructed to relax with their eyes closed for 1 min during which time measurements of baseline ECG and breathing rates were recorded. Immediately after the baseline period and without moving, subjects were instructed to open their eyes and complete the Stroop task (5 min 24 s in duration). After the Stroop task, subjects completed a further 1 minute rest period with their eyes closed. ECG and RF were recorded throughout testing.

Post-event 14 of the 21 athletes (IRON) completed the testing procedure again. This test was done as soon as the athlete reported to our temporary laboratory, which consisted of a minibus parked in a relatively secluded area behind the finishing tent. Following completion of the testing of the 14 IRON subjects after the midnight cut-off time, we proceeded to test the 7 CON subjects between 00h30 and 03h00 in the morning.

The Modified Stroop Task

The modified Stroop task entailed the individual presentation of cues (2 cm in height) appearing every 3 seconds and displayed for 400 ms followed by a 2600 ms black screen (which constituted the response period), in the centre of a computer monitor on a black background¹⁶¹. This cue word was the name of one of 4 colours (red, blue, green or yellow), which was presented either in: (i) grey colour with the word reading either red, blue, green or yellow; or (ii) red, blue, green or yellow colour with the colour being different to the word, but not in grey. The subject was required to respond as quickly and correctly as possible by pressing one of four buttons to indicate either the colour of the word on the screen (if the colour of word was red, blue, green or yellow), or of the word itself (if colour of word was grey). Subjects were required to use only their two index fingers to press the relevant response button (red and blue buttons with left index finger and green and yellow buttons with right index finger).

In total 96 cues were randomly presented, 25% neutral (grey) and 75% incongruent colour words, to increase the cognitive demand on the subjects. The use of the grey words ensured that subjects had to read and recognise the colour words rather than just noticing the colours, thereby invoking the Stroop effect¹⁵⁸. Subjects were requested to focus on the centre of the screen during each trial.

Respiratory rate

The respiratory rate per minute was measured via a force transducer fixed to a belt placed around the chest wall. Subjects were asked to expel all the air from their lungs when the transducer belt was first fitted and then instructed to breathe normally. The chest transducer was connected to an amplifier with a low-pass 10Hz filter. The respiratory frequency (RF), i.e. breaths per second, was calculated from the respiratory rate.

ECG Measurement

ECG activity was recorded from 3 electrodes placed in positions representing Eindhoven's triangle namely, subclavicular bilaterally and over the anterior superior iliac crest. The skin surface was cleaned and gently abraded with an alcohol swab before electrodes were attached. The 3 electrodes were connected to the Biopac ECG amplifier set to band-pass filter between 0.5 and 35Hz. ECG and recordings were analysed with AcqKnowledge for Mackintosh OS X (version 3.9.0). This software uses a modified Pan and Tompkins algorithm to detect QRS complexes²¹¹. All ECG traces were then again off line band-pass filtered between 0.5 and 35Hz to reduce interference noise. The filtered ECG recording tachograms were then visually inspected to determine the correct recognition of QRS complexes and T waves. Noise and missed or ectopic beats were corrected by either enhancing R peaks to distinguish them from T peaks or by spacing beats. Undefined QRS complexes were corrected by replacing it with a previous adequately spaced QRS complex. Without this spacing of beats, which was only necessary in one subject where we corrected 3 beats, the ECG tracing are not identifiable for the analysing software^{37;38}.

Data analysis

Only after each tachogram showed no spurious beats were the data transformed by autoregressive analysis, using software from the Biomedical signal analysis Group (Department of Applied Physics, University of Kuopio, Finland) into LF (0.04 - 0.15 Hz) and HF (0.15 - 0.5 Hz) components. It is generally accepted that the HF component is a marker of parasympathetic activity, while the LF component, which includes both sympathetic and parasympathetic activity, is a marker for vasomotor sympathetic modulation. The LF/HF ratio is generally accepted to be indicative of sympathovagal balance. We analysed HF power up to 0.5 Hz due to the high RF post-event which resulted in activity in frequencies up to 0.5 Hz. Additional calculations included %LF and %HF as well as the central or peak frequencies in both the LF and HF bands.

Statistical analysis

All data are presented as means and standard deviations. Comparisons between the subject characteristics of the CON and IRON groups were made using independent-sample *t*-tests. A *p*-value of <0.05 was considered statistically significant. Data were evaluated to determine if they were parametrically or non-parametrically distributed by applying Levene's Test. Parametric data was then analysed for interaction, time and group effects by using a two way ANOVA. Non-parametric data was analysed using the Mann-Whitney for group effects and the Wilcoxon test to determine time effects.

RESULTS

Subject characteristics

Descriptive characteristics of the CON and IRON groups are shown in Table 1.3. In total, 13 male and 1 one female Ironman triathletes aged 33.7 ± 7.9 years, with a mean BMI of 22.6 ± 0.6 , and a mean of 15.7 ± 4.4 hours training per week, completed the study. The CON group consisted of 5 males and 2 females aged 42.0 ± 4.5 years, with an average BMI of 25.8 ± 6.5 , and mean training hours of 4.0 ± 2.4 per week. Age and training hours per week differed significantly between the 2 groups ($p < 0.00005$), but height, weight and BMI matched.

Table 3.1: Subject characteristics of the CON and IRON groups upon entry to the study

	<i>Age(yrs)</i>	<i>Weight(kg)</i>	<i>Height(cm)</i>	<i>BMI</i>	<i>Training(hrs/week)</i>
CON	42 ± 4.5	78 ± 12.9	1.75 ± 0.1	25.8 ± 6.5	4 ± 2.4
IRON	$33.7 \pm 7.9^*$	72.7 ± 8.0	1.79 ± 0.1	22.6 ± 0.6	$15.7 \pm 4.4^{**}$

Abbreviations: **yrs**, years; **kg**, kilogram; **cm**, centimetres; **BMI**, body mass index; **hrs/week**, hours per week

IRON vs. CON (t-test), * - $p < 0.05$, ** - $p < 0.00005$

Race and recovery times

The IRON subjects had an average racing time of 757 ± 88 min (12h37min), with a range 621 – 948 min (10h21 – 15h48). Comparatively, the winner of the event finished in 8h33 min and the last competitor in 16h56 min. Furthermore, the IRON subjects recovered for an average period of 106 ± 36 min (1h46min), with a range of 59 – 200 min, before being tested.

Heart rate (HR) and respiratory frequency (RF)

The HR, RF and their % change in the IRON and CON groups from pre- to post-event are displayed in Table 3.2.

Table 3.2: Heart rate, Respiratory Frequency and HRV Time-domain parameters in the CON and IRON groups during the 5 minute Stroop Task both pre and post Ironman Event.

	<i>CON</i>		<i>IRON</i>	
	Pre Event	Post Event	Pre Event	Post Event
<i>HR (bpm)</i>	71 ± 13	$73 \pm 17^*$	70 ± 10	$84 \pm 8^{***}$
<i>% increase in HR</i>		5 ± 27		23 ± 22
<i>RF (Hz)</i>	0.28 ± 0.03	0.33 ± 0.05^T	0.33 ± 0.06	0.39 ± 0.05^{GT}
<i>% increase in RF</i>		16 ± 13		23 ± 18
<i>SDNN (ms)</i>	38 ± 10	39 ± 15	43 ± 16	20 ± 11^{AA}
<i>RMSSD (ms)</i>	30.6 ± 14.9	$33 \pm 21^* \#$	43.8 ± 24.4	$14.2 \pm 7.3^{***}$
<i>pNN50 (%)</i>	11.7 ± 15.8	$16.6 \pm 21.4^* \#$	20.6 ± 20.9	$1.3 \pm 1.9^{***}$

Abbreviations: **HR**, heart rate; **bpm**, beats per minute; **RF**, respiratory frequency; **Hz**, Hertz; **SDNN**, standard deviation of the R-R intervals; **ms**, milliseconds; **RMSSD**, root mean square of successive difference of the R-R intervals; **pNN50**, percentage of R-R intervals > 50 ms; **%**, percentage

Interaction effect (ANOVA): ^A - $p < 0.05$; ^{AA} - $p < 0.01$;
 Group effect (ANOVA): ^G - $p < 0.05$
 Time effect (ANOVA): ^T - $p < 0.00005$
 Pre vs. post (Wilcoxon) * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$
 CON vs. IRON (M-Whitney) # - $p < 0.05$

The CON group showed a slight increase in HR ($p < 0.05$) from pre to post-event, but the IRON group had a significant increase from 70 ± 10 to 84 ± 8 bpm ($p < 0.001$).

Upon closer analysis of the data it was noted that 4 CON subjects had lower HR post-event, while one subject showed a 52% increase in HR. This accounts for the high SD in the CON % increase in HR value. Only 1 IRON subject had a lower HR post-event. Surprisingly this athlete was among the ones that we tested the earliest (75 minutes) after completion. The % increase in HR in the IRON group also had a high SD value, which resulted in there being no interaction effect between the CON and IRON groups.

Furthermore there was no interaction effect in the RF between groups. But there was a significant time effect ($p < 0.00005$). CON RF increased by $16 \pm 13\%$ and IRON RF by $23 \pm 18\%$. There was also a group effect, with IRON RF significantly ($p < 0.05$) greater than CON RF throughout (Table 3.2).

HRV

Time-domain Parameters

The time-domain values are displayed with HR and RF in Table 3.2. There was a significant interaction effect with respect to SDNN ($p < 0.01$), with CON SDNN remaining around 40 ms pre and post-event, while IRON SDNN were halved from 43 ± 16 to 20 ± 11 ms post-event. The time-domain parameters pNN50 and RMSSD were both non-parametric and therefore no interaction effect was observed. There was however a slight increase in the CON RMSSD from pre to post-event (30.6 ± 14.9 to 33 ± 21 ms) $p < 0.05$, while the IRON RMSSD decreased significantly from 43.8 ± 24.4 to 14.2 ± 7.3 ms ($p < 0.001$). The same phenomenon was seen with respect to the pNN50 which increased in CON from 11.7 ± 15.8 to $16.6 \pm 21.4\%$ ($p < 0.05$), but decreased in IRON from 20.6 ± 20.0 to $1.3 \pm 1.9\%$ ($p < 0.001$). The post-event RMSSD and pNN50 values were also different between groups ($p < 0.05$).

Frequency-domain Parameters

Frequency-domain data for both CON and IRON groups pre and post-event is summarised in Table 3.3. There were no interaction effects in HRV absolute power in any of the frequency bands in IRON vs. CON groups. However, VLF and HF as well as total power values were significantly lower post compared to pre-event in the IRON group ($p < 0.001$). The IRON VLF ($p < 0.01$) and HF ($p < 0.05$) as well as total ($p < 0.01$) powers were also significantly lower than those of the CON subjects post-event.

Table 3.3: HRV Frequency-domain parameters in the CON and IRON groups during the 5 minute Stroop Task pre and post the Ironman Event.

	<i>Controls</i>		<i>Ironman</i>	
	Pre Event	Post Event	Pre Event	Post Event
VLF (ms^2)	268 ± 182	231 ± 212 [#]	276 ± 224	26 ± 28 ^{***}
LF(ms^2)	206 ± 100	282 ± 218	243 ± 165	170 ± 189
HF(ms^2)	132 ± 96	199 ± 350 [#]	270 ± 424	21 ± 24 ^{***}
VLF + LF + HF (ms^2)	605 ± 293	711 ± 602 [#]	789 ± 649	216 ± 198 ^{***}
%LF	38 ± 20	47 ± 25 ^l	36 ± 16	69 ± 22 ^{G-trend, l}
%HF	21 ± 14	17 ± 18 ^{l-trend}	25 ± 14	12 ± 12 ^{l-trend}
LF/HF	4 ± 5	6 ± 7 [*]	2 ± 1	12 ± 9 ^{***}

Abbreviations: VLF, very low frequency; LF, low frequency; HF, high frequency; LF/HF, ratio; ms^2 , milliseconds; % , percentage

Group effect (ANOVA): ^{G-trend} - $p = 0.055$

Time effect (ANOVA): ^{T-trend} - $p = 0.074$; ^T - $p < 0.005$

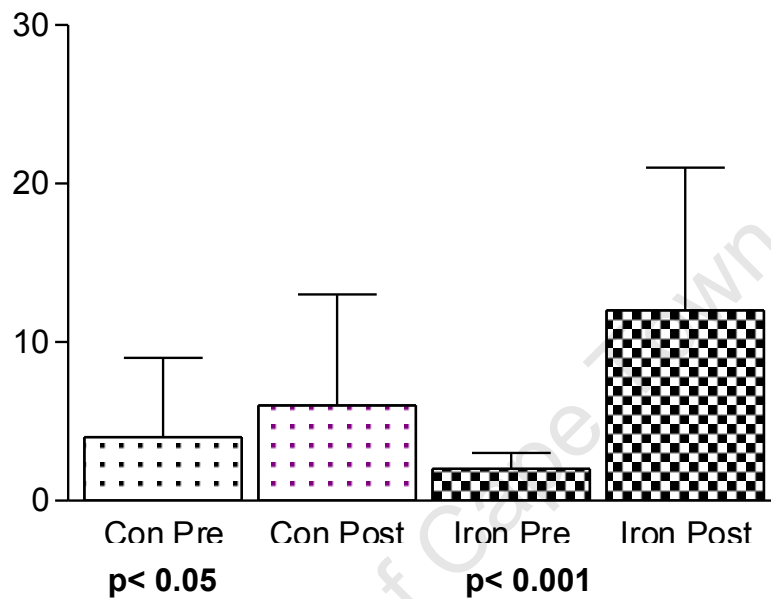
Pre vs. post (Wilcoxon time): * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$

IRON vs. CON (Mann-W group):[#] - $p < 0.05$; ^{##} - $p < 0.01$

The %LF parameter, which indicates the percentage of the total HRV spectrum lying in the LF range, showed no interaction effect. There was however a significant time ($p < 0.005$) effect, with post-event %LF being significantly greater than pre-event values. There was also a strong trend for the IRON group to have a greater %LF than the CON group ($p = 0.055$). Though the %HF in the IRON group seemed lower post-event than both their pre values and the CON post values, there were no statistical differences between groups. There was only a trend towards a time effect ($p = 0.074$).

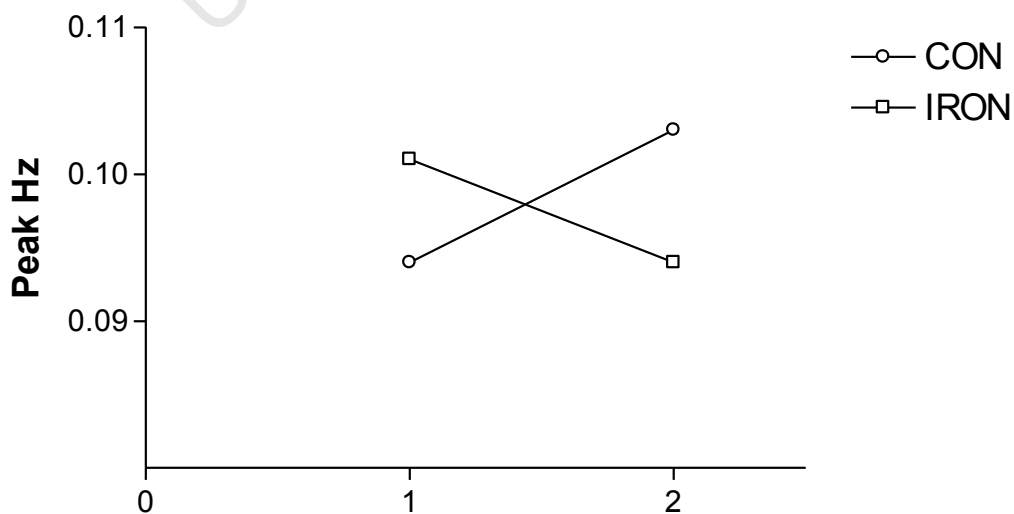
The LF/HF ratio in the IRON group was increased by a factor of 6 from pre to post-event ($p < 0.001$), while the CON ratio increased only fractionally ($p < 0.05$, figure 3.1).

Figure 3.1: LF/HF ratios in the CON and IRON groups Pre and Post Ironman Event



Pre vs. post (Wilcoxon) - CON $p < 0.05$;
 - POST $p < 0.01$; *** - $p < 0.001$

Figure 3.2: LF peak Hz in the CON and IRON groups Pre and Post the Event



Interaction effect (ANOVA): - $p < 0.015$

There was a significant interaction effect with respect to the LF peak Hz. While CON LF peak frequencies increased, IRON frequencies decreased over the course of the event ($p < 0.015$, Figure 3.2).

Cognitive Performance Parameters

Table 3.4 summarises the cognitive performance parameters of the IRON and CON groups during the Stroop task, pre- and post the event.

Table 3.4: Cognitive Performance in the CON and IRON groups pre and post the Ironman Event

	<i>CON</i>		<i>IRON</i>	
	Pre Event	Post event	Pre Event	Post Event
RT (ms)	1123 ± 246	1080 ± 311	1256 ± 255	1241 ± 413
%IR	4 ± 3	5 ± 4	6 ± 8	4 ± 5
%MR	0	4 ± 10	3 ± 4	2 ± 4
%CR	96 ± 3	91 ± 9	92 ± 10	94 ± 6

Abbreviations: **RT**, Reaction Time; **ms**, milliseconds; **IR**, Incorrect Responses; **MR**, Missed Responses; **CR**, Correct Responses;

Post-event RT in both groups tended to be fractionally faster. There were no group or interaction effects. The CON group showed less accuracy after the event, due to the missed responses in a subject who fell asleep briefly during the test. The IRON group showed no decrements in RT post-event and a slight improvement in accuracy, though not significant, from $92 \pm 10\%$ pre to $94 \pm 6\%$ post.

DISCUSSION

To the best knowledge of the author, this study was the first of its kind to investigate the effects of a particularly gruelling endurance event on HRV and cognitive function during induced cognitive stress. Importantly, the IRON group formed a good representative sample of the Ironman population tested, the first subject finishing 55th, while the last subject finished in position 1256

out of the ~1600 finishers. The IRON subjects were tested between 1 hour and 3h20min after they finished the event, giving a good spread of recovery times. We found no clear correlation between the %HF power recovered vs. recovery time²⁶.

The first major finding of this study was that IRON subjects showed steep decrements in all the post-event time-domain HRV parameters measured, with the pNN50 being the most affected, only recovering to 11% of its pre-event value. In fact not even one of the 14 athletes was able to recover to pre-event pNN50 values. Only one subject (7% of sample) had the similar RMSSD values pre vs. post-event (11.2 vs. 13.5 ms) and another subject (7%) similar SDNN values pre and post-event (26 vs. 27 ms). The CON subjects, on the other hand showed a more mixed response, 4 subjects (57%) having greater RMSSD, SDNN and pNN50 values post-event.

The severe decrement in HRV in the IRON group was also observed in the frequency-domain. The recovery of total power (VLF + LF + HF) was incomplete in all IRON subjects even up to three hours after the event, the average being only a third of pre-event total power. The decrement in total power in the IRON subjects is interesting and in keeping with the findings of Whyte (2000) and Rifai (1999). They respectively reported cardiac biochemical marker and echocardiographic changes in Ironman athletes for 24 hours after a half Ironman event²¹² and up to four days after a full Ironman event^{9;11}. It is uncertain if these markers and echo changes truly represent cardiac damage in these fit athletes or only indicate severe strain on a physiological level. If anatomical micro damage is caused, it is unsure whether normal restoration occurs over the 4 days of recovery, or if damaged tissues are actually replaced by fibrotic tissue, which can predispose these athletes later in life to arrhythmias as in the unfortunate case of the veteran athlete Sy Mah¹⁹⁶.

Whether the HRV changes are the result, the cause, or independent of any anatomical cardiac damage (as indicated by the elevated biochemical markers) or of physiological strain, remains to be determined. We can only confirm that autonomic changes accompany this left ventricle function

depression and may contribute to congestive heart failure incidents in the absence of cardiac disease after marathons²¹³.

Evaluation of the specific frequency bands in the triathletes revealed that the power in both the HF and VLF bands was significantly reduced, by over 90% in each band, even after a 1h45 min recovery period. In sharp contrast, LF power was only reduced by ~30%, which did not reach significance. This means that ~70% of the total HRV power was found in the LF band post-event, double the 35% power in the LF band pre-event. Equally important is the fact that only 12% of total power was found in the HF band post-event, as opposed to 25% pre-event. Interestingly, though we found no correlation between %HF power recovered vs. recovery time in the 14 IRON athletes as a group, we nevertheless did find a correlation (r^2 of 0.93) in a subgroup of 7 IRON athletes. In this regard, it is well known that the individual conditioning of athletes would play a great role in the speed of post-exercise %HF power recovery²⁷. It was noticeable that post-event RF was significantly lower (0.37 ± 0.03 vs. 0.42 ± 0.04 , $p < 0.03$ Hz) in those 7 IRON subjects who had a correlation, compared to the 7 IRON subjects who showed no correlation between HF power recovery vs. recovery time. Lower post-event RF may be indicative of better conditioning and could explain the correlation between the %HF powers recovered vs. recovery time in some, but not all, of the triathletes tested.

In comparison to the above findings, the CON subjects again showed a more varied response. In agreement with the time-domain parameters, total HRV power increased between 1.5 and 3 times in 4 CON subjects (57%) post vs. pre-event. However, only 47% of total power was found in the LF band compared to the ~70% in the IRON group post-event. This difference in %LF in the 2 groups almost reached a level of statistical significance ($p = 0.55$).

This finding is in concordance with the suggestion that the % power in the LF band is indicative of a predominance of sympathetic activation⁴⁰.

Furthermore, it seems as if the LF/HF ratio in our groups is a good marker for sympathovagal balance. Both groups had significantly higher LF/HF ratios post-event, however, while the IRON group ratios increased 6 fold, the CON

group only showed a 0.5 fold rise. This is in line with the much greater sympathetic arousal expected in the triathletes than in the sleep deprived CON subjects post-event. Acute sleep deprivation has been found to be associated with increased sympathetic and decreased parasympathetic activity²¹⁴. These authors found that LF and the LF/HF ratio increased as early as after only 12 hours of sleep deprivation, while HF starts to decrease at 24 hours accompanied by the lowering of BRS. The subjects described in the CON group underwent a 20 hour period without sleep. In accordance with the findings of Zhong et al.(2005), increased LF/HF ratios were found in the CON group after this period without decrements in HF power²¹⁴.

The increased %LF in the IRON group is mainly attributed to a peak around 0.1 Hz, known as the Mayer wave. LF rhythms have been related to fluctuations in arterial pressure baroreceptor mechanisms⁷⁵. After an ultra-endurance event the sustained dilated peripheral vascular system, pooling of blood in the lower extremities and compliant splanchnic system and the additional Barcroft/Edholm reflex due to a drop in right atrial pressures²¹⁵⁻²¹⁸, stimulate the carotid, aortic and cardiopulmonary baroreceptors³⁸. The baroreceptor neural loop relays information regarding this low pressure state to the heart to keep HR elevated in order to maintain blood pressure. Under simultaneous sympathetic activation, peripheral constriction causes a pressure increase in the cardiovascular system, reversing the effects on the heart. Each increase and decrease in heart rate represents one oscillation which occurs rhythmically about every 10 seconds (0.1 Hz). Thus the power in the LF band is not indicative of the inherent HRV, but represents the baroreceptor feedback delay due to total peripheral resistance changes.

An interesting finding was noted with respect to the LF peak Hz between the CON and IRON groups. Both groups had LF peaks close to 0.1 Hz, however while the CON group had a raised LF peak Hz after the event, the IRON group had a lower LF peak Hz resulting in a significant interaction effect (Fig 3.2). The lower peak Hz in the IRON group may be indicative of a slightly longer baroreflex feedback delay, which could be related to fatigue. Thus the differences in the CON and IRON groups' LF peak Hz during cognitive stress

(pre and post-event) is in keeping with the suggestions that activity in the LF band is indicative of sympathetic vasomotor drive^{75;219}.

A further important finding of this study was with respect to the effects on the indices of cognitive performance. Previous studies indicated improved cognitive performance in complex and simple tasks after exercise lasting > 20 minutes and less than one hour^{220;221}, provided that the exercise bout is accompanied by a raised blood catecholamine concentration¹⁴⁹. Further studies found improved decisional task performance after exercise lasting less than 2 hours due to the raised metabolic load which increases arousal^{148;149;221}. After exercise lasting > 2 hours, when signs of exhaustion becomes evident, cognitive function possibly can decrease¹⁵⁰. Grego et al. demonstrated that this decline in the presence of metabolic changes is indicative of exhaustion¹⁵².

In the present study cognitive performance in both groups showed no significant difference between tests irrespective of the ultra-endurance exercise bout or the 20 hour sleep deprivation period. Thus we were unable to document neural fatigue using the methodology described in this study, despite the subjects' participation in an ultra-endurance race or long working hours. Alternatively, physical and neural fatigue might truly not affect cognitive function during induced cognitive stress. Mood state and anxiety provoking conditions have been shown to improve reaction time either by increased attention, faster central processing or increased muscular tension¹⁶¹. The mental stress and attractive challenge¹³⁸ provided by the Stroop Task might be the reason both CON and IRON groups were able to perform cognitively well, despite exhaustion.

A number of factors that may influence the variables measured in this study are worthy of mention. Firstly the CON and IRON subjects were not matched for age or training hours. In this regard, the known decrements in HRV with age²²² did not affect our subject population as we found no significant differences in the pre-event HRV data between the CON and IRON subjects. This may have been due to our standardisation of the subjects' state of arousal by recording HRV during a cognitive stress test.

The subjects' various states of arousal were evident in their individual heart and respiratory frequencies. Two control subjects most familiar with the Stroop task (and therefore the most relaxed), had the lowest HR and RF of all subjects both pre and post-event. The one control subject is qualified in Mindfulness Training which might also influence his control over autonomic and cognitive functions, to perform under pressure. A lower HR and RF, which is indicative of a lower level of anxiety, can be attributed to familiarisation with the test, mental state or ability to cope or perform under stress.

As a group, the IRON subjects had significantly higher RF than the CON subjects throughout. This was expected due to pre-race anxiety and increased post-race sympathetic drive. The 23% increases in both HR and RF in the IRON subjects post-event, a clear indication of their extreme sympathetic drive after the 10-15 hour event^{73;223}, may have been slightly more elevated due to performing the Stroop task while fatigued. Niewiadomsky et.al (2007) suggested that elevated post exercise HR could be interpreted as a sign of augmented sympathetic activity⁷³. Interestingly, post-event RF in the CON group (0.33 ± 0.05 Hz) was exactly equal to those of the IRON group pre-event (0.33 ± 0.06 Hz). Together with the concomitant increases in the CON group HR, this is suggestive of a comparable sympathetic drive in CON group post-event vs. IRON group pre-event.

As mentioned above, the pre-event HRV in both time- and frequency-domains did not differ significantly between groups even though CON subjects were significantly older and less well trained than IRON subjects. In this regard, previous studies have shown that HRV is better correlated to cardio-respiratory fitness (namely how the heart will react in reaction to stress like exercise or ischemia), than to aerobic fitness¹⁷³.

However, there were indications of greater HRV in the IRON vs. CON group pre-event, but due to high inter-subject variability no significances were found in any single parameter. The most notable indications of greater HRV in the younger, more highly trained IRON group were found in the practically 50% higher RMSSD values and virtually doubled pNN50 and HF powers pre-event. Although, the much higher HF power in the IRON group pre-event was

skewed by one triathlete whose HF power was 6 times higher than the group mean. This also explains the very large SD in the IRON group HF power.

Certain limitations were evident in conducting this study. As mentioned previously, subjects were not matched for age, gender or training fitness. However, no differences have previously been documented in the 30-min recovery of HRV between the genders²⁶. A small sample size may also contribute to the large standard deviation in some parameters.

Previous studies established that paced as oppose to spontaneous breathing is a much better protocol for more accurate measurement of HRV^{224;225}, as tidal volume and RF can influence HF power at rest^{48;226}. Thus interference of respiration rate due to anxiety cannot be excluded in this study. However, RF had no effect on HF power during recovery in a study by Kaikonen et.al (2007)²⁶.

Familiarisation with the cognitive test could also play a role in accuracy and even speed of reaction during the test. Some controls had more experience with this test and thus had less anxiety. Therefore they may have an advantage to show better HRV indices and reaction times than the IRON group. A four day interval between tests is preferred to exclude a training effect during the Stroop Task¹⁴⁷. This was accomplished with the CON group, but unfortunately not with the IRON group. Therefore memory from the two days before could enhance performance or relaxation during retesting.

The pretest in the IRON group was done at registration 1-2 days prior to the event. Due to anxiety before the event, adrenergic stimulation might have interfered with HRV and HR readings, as well as cognitive performance and does not represent a rested state. Furthermore, we had no control over the time of day, food or stimulants including caffeine ingested prior to testing at registration, which would have influenced concentration and reaction times.

As body position influences HRV readings, the supine position would have been more stable to record especially LF values, by excluding the sympathetic activation present in the sitting position. However, HF/LF ratios should be unaffected and reliable⁷⁶.

Finally, multiple recovery observations until full recovery of HRV might have provided more insight regarding the full recovery period needed after such a significant event. This should be the focus of future research in this field.

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Chapter 4

Summary and conclusion

Our study was the first to investigate HRV recovery and cognitive performance, in the form of reaction times and accuracy during a complex mental task, after an ultra duration event. In this prospective cohort study undertaken at the 2007 South African Ironman Triathlon held in Port Elizabeth we (1) recorded and analysed the ECG, HR, RF, RT and accuracy on triathletes performing a Modified Stroop Task before and after the event (2) identified the changes in HRV and cognitive performance, compared to their individual recovery times (time till testing) and that of a control group. 14 Triathletes were recruited as subjects for the study, while staff from the UCT/MRC Sports Institute served as controls.

Previous studies were performed only on exercise of different intensities and durations of up to 120 minutes. The results showed immediate vagal reactivation after exercise and normalising of all HRV parameters to basal levels within 5 minutes. A delay in HRV recovery was seen only with exercise intensities above the VT_1 ($VO_{2max} > 55\%$). HF was mostly affected with delays of up to 90 minutes.

In contrast, in our study HRV indices of the athletes did not return to basal levels between the 60 - 200 minutes when athletes were tested. These HRV decrements were seen in both time (especially pNN50) and frequency (especially VLF and HF) domains. If a measure of HF power recovery was present, it correlated with reduced RF, indicative of lower sympathetic drive.

There was a big increase in %LF and a 6-fold increase in LF/HF ratio in the IRON group. This was probably due to baroreflex activity in the presence of sympathetic vasomotor drive to combat the peripheral vasodilatation after the event.

A further interesting finding in the present study was that cognitively no significant changes in reaction times or accuracy occurred in either group, despite mental (controls) or physical (athletes) exhaustion. This possibly implies that neural fatigue (if present), associated with the perception of physical or mental fatigue, is not directly related to cognitive function as measured by a modified Stroop task.

In summary, the clinical implication of this study is that the sports physician should be aware that triathletes who participated in an ultra-endurance event such as an Ironman Triathlon, showed significant changes in the autonomic function of the heart, which accompanies the possible increase in cardiac biochemical markers and echocardiographic changes in other studies.⁹⁻¹¹. These athletes might be theoretically more susceptible to cardiac death from arrhythmias, due to the sustained sympathetic activation and the absence of the counteracting effects of the normal parasympathetic reactivation occurring after exercise.

Future studies need to investigate the return of vagal activity at shorter time intervals and for a longer period afterwards, even over the next few days, to track full recovery of HRV indices.

In conclusion the long term impact of these ultra endurance events on the cardiovascular system is not certain. Our study only confirms the immediate change of autonomic activity as reflected in the depressed HRV for several hours after an ultra-endurance event.

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Appendix A

Subject information sheet

HEART RATE VARIABILITY AND COGNITIVE PERFORMANCE CHANGES DURING PHYSICAL EXHAUSTION

Dear Subject

Thank you for your interest in and for agreeing to participate in this important study on the changes of heart rate variability and cognitive function during an ultra – endurance event.

The data for this study will primarily consist of the recording of HRV, respiratory frequency and accuracy during a reaction time cognitive test (STROOP TEST), while an ECG monitor will read your heart rate and measure your heart rate variability through 3 ECG leads attached to both arms and one leg. The 1st test will be done at registration prior the Ironman Triathlon Event, as well as 60 – 200 minutes after completing the event. Thus 2 tests need to be taken in total. The tests one day prior and immediately after the race will be done in PE near the registration facilities.

Prerequisites to the data collection dates will be:

1. Arrive in a well rested relaxed state
2. 24 hours restriction of intake of any central nervous system stimulants like coffee, nicotine amphetamines, cold or flu remedies

Before the test you will be guided as to the protocol of the STROOP test which will test your reaction time to words and colours appearing on a screen in front of you.

It is important for you to know that the information obtained from the questionnaires and the investigations, will be used for research purposes only, and will be kept confidential. All costs incurred for the tests, will be covered by the researchers.

Once again, thank you for agreeing to be a participant in this study.

I wish you a good, injury free Ironman that will do justice to your months of preparation!

Dr Ilse Joubert

MBChB

Appendix B

UCT/MRC

RESEARCH UNIT FOR EXERCISE SCIENCE AND SPORTS

MEDICINE

QUESTIONNAIRE

SURNAME :

NAMES :

DATE OF BIRTH :

HEIGHT : WEIGHT :

OCCUPATION :

TEL NO – WORK :

HOME :

CELL :

FAX NO :

POSTAL ADDRESS :

E- MAIL :

FAMILY HISTORY

Is there anyone in your family with any of the following?

Description	Yes	No	Relationship
High Blood Pressure			
Cancer			
Epilepsy			
Psychiatric Abnormalities			
Asthma			
Tuberculosis			
Diabetes			
Kidney Disease			

PERSONAL HISTORY

Do you have any of the following?

Description	Yes	No	Description	Yes	No
Surgery			Urinary tract infection		
Head Injury with unconsciousness			Genetic Disorder		
Kidney Stones			Sugar in urine		
Ear, nose & throat disorder			Epilepsy		
Post Nasal Drip			Weakness / Paralysis		
Chronic Cough			Hernia		
Tuberculosis			Disease of joints		
Rheumatic fever			Lower back pain		
Heart murmur			Cancer		
High blood pressure			Skeletal pain / cramps		
Chest pain during exercise			Blood in the stool		

Chest pain at rest			Stomach or intestinal disorder		
Recurrent diarrhea			Jaundice or Hepatitis		

1. Do you smoke? :

2. Approximately how many times have you had headache in your life :

3. How many hours per night do you sleep on average? :

4. Where do you live? :

5. Have you had any extraordinary social /emotional stress recently? E.g. death / illness in family, marital disharmony, work related)? :

6. How much alcohol do you drink (tots, glasses of wine / beers)? :

University of Cape Town

Appendix C

MEDICAL EXAMINATION

RACE NUMBER:

GENERAL APPEARANCE : Head and facial expression

Pulse :

Temperature : °C

Blood Pressure : / mmHg

Description	Yes	No	Specify
Lymph nodes in neck			
Pharynx inflamed			
Nasal Mucosa inflamed			
Ear drums inflamed			
Left lung apex clear			
Left lung base clear			
Right lung apex clear			
Right lung base clear			
Heart sounds normal			
Heart murmurs			

ADDITIONAL COMMENTS:

EXAMINING PHYSICIAN: NAME:

Appendix D

Informed consent form

Title of the study: Effects of ultra endurance on heart rate variability and cognitive performance

The data for this study will primarily consist of the recording of HRV, respiratory frequency and accuracy during a reaction time cognitive test (STROOP TEST), while an ECG monitor will read your heart rate and measure your heart rate variability through 3 ECG leads attached to both arms and one leg.

This test needs to be done one day prior the Ironman Triathlon Event and 60 – 200 minutes after completing the event. The tests one day prior and immediately after the race will be done in PE near the registration facilities.

Participants will be instructed to refrain from consuming tea, coffee, alcohol or any other central nervous system stimulant for four hours prior to the commencement of their participation in the trial.

The collection of the various data will be as follows:

1. EEG testing and reaction time (45 minutes)
2. ECG recordings of heart rate and variability (simultaneously)

Possible risks of participating in this trial

None.

I confirm that the exact procedure and techniques, and possible complications of the above tests have been thoroughly explained to me.

Possible benefits of participating in this trial

The completion of the questionnaires and results of the investigations may help to demonstrate HRV changes occurring after ultra-endurance events and the effects on cognitive performance. Participants will gain by learning more about their HRV changes, reaction times and accuracy after the race, compared to earlier assessments in a rested state. They will also benefit indirectly from the increase in knowledge in this field in future events.

I am fully aware of the procedures involved in:

1. The enrolment process

2. The prerequisites regarding diet prior testing
3. Prompt attendance of both tests

I am aware of the following precautions that will be taken by the investigators during testing:

- 1 A familiarisation process to familiarise myself with procedures used on the testing days
- 2 That medical treatment will not be neglected in the unlikely event of an injury during the race

I am aware that

1. The costs involved in the collection and analysis of the various samples involved will not be for my account.
2. That all staff involved in the collection of specimens are professionals and currently carry medical insurance.
3. All data collected will be kept confidential, stored in a secure environment with only the principle and associated investigators having access, and may be presented anonymously in a medical journal article, or at a medical conference.
4. I am free to contact the principle investigator , Dr Ilse Joubert on 0820767948
5. I am free to contact the chair of the UCT Faculty of Health Sciences Ethics Committee, Prof T. Zabow on 021 406 9111

I am free to withdraw from the study at any time, should I choose to do so. I understand that I may ask questions at any time during the testing procedure. I know that the personal information required by the researchers and derived from the testing procedure will remain strictly confidential and will only be revealed as a number in statistical analysis.

I have carefully read this form and understand the nature, purpose and procedures of this study. I agree to participate in this research project of the MRC / UCT Research Unit for Exercise Science and Sports Medicine

Name of volunteer:

Signature of volunteer:

Name of investigator:

Signature of investigator:

Date:



The effects of an ultra-endurance event on heart rate variability and cognitive performance during induced stress in Ironman Triathletes.

M Phil (Sports Medicine) dissertation: Ilse Joubert (JBRILS001)

Examiner's report: Dr U Holtzhausen

20 April 2009

Introduction

Thank you for the invitation to examine the dissertation. The topic is extremely relevant to current developments in the field of sports medicine. The dissertation was interesting to read. The inclusion of two areas of investigation, that of heart rate variability and mental exhaustion into one study was noted with interest.

Relevant literature

The literature consulted was extensive, and well beyond the requirements for a Master's dissertation. Recent and older sources were referenced from relevant journals and other publications. Referencing was done adequately. Suggestions for additional referencing have been made elsewhere in the report. Literature was presented in a systematic way. Because of the complexity of the topic, the literature review was not easy to read, but presented a thorough review of the topic.

Research methods

The aim of the study includes two research questions. It is unclear why the HRV and cognitive function are investigated in the same study. Although not implied, it would have been difficult to find a correlation between causes for HRV changes and cognitive ability after ultra-endurance exercise.

Although addressed in the discussion, the sample size of both the IRON and CON groups are rather small to make meaningful deductions. This was evident in the large influence that single variables in one or two subjects had on the data and final outcomes of the study. The constraints are understood, but the sample size influenced the study negatively.

The methodology is presented clearly and comprehensively. It would have assisted the non-informed reader if the methodology, measurements and outcomes of the Stroop test was explained in the literature review or elsewhere.



The exclusion criteria are not well formulated. Rather avoid vague terms such as "preferably". Learning difficulties may have an important effect on the validity and outcome of the Stroop test and should rather have been defined more specifically.

Analysis of data was comprehensive, with application of appropriate statistical methods.

Interpretation of data

Data was interpreted appropriately in the discussion. A multitude of outcomes and measurements were presented. Although it was done properly according to relevant literature, it was difficult to find the main outcomes of the study until late in the chapter. The candidate does, however, show a thorough understanding of HRV in exercise, as well as of cognitive function testing.

Presentation

The dissertation was presented neatly and in an appropriate format. The appropriate use of tables and figures in results section assisted in the reading thereof.

A number of typographical and other errors have been noticed, that may be addressed before final publication of the dissertation:

- The list of abbreviations is incomplete
- Abstract: A short description of what is tested with a Stroop task will enhance the reader's understanding of the objective of the study.

Introduction and scope:

- Entries have reached 1600 in this year's event – rather specify the year. Also in the introduction of chapter 3.
- P11 par 2, p33: Insert "exercise" before intensity
- P11 par 3: Does "cognitive performance" indicate cognitive activities being executed? reference needed at end of sentence.

Literature review:



- 2. HRV measurement and recording: Non-linear measures/measurements?
Reference?
- 2.1. Time domain: Text lacks referencing
- 2.3. Recording of HRV: It is speculated that SWS is under vagal tone. – the meaning of the sentence is unclear. Is SWS influenced/controlled/ by vagal tone, or does vagal tone dominate during SWS?
- 3.1 lacks reference

Chapter 3:

- Methodology: References to Stroop test, ECG etc are needed, p 35-7.
- P44 par2: Grammatical error: "if anatomical" rather than "anatomically"
- P44 par2: Typing error: After out study...

Originality

The dissertation presents original work that makes an important contribution to the ongoing investigation of HRV and physiological effects of ultra endurance exercise, especially as far as methodology is concerned.

Conclusion

The candidate displays a good understanding of research methodology. She also displays a thorough understanding of the selected research topic. The study would have been enhanced if the research question was more focused, and a bigger sample could have been studied.

A mark of 68% is awarded.

Dr LJ Holtzhausen

MBChB; MPhil(Sports Medicine); FAFP(SA)

Friday, 08 May 2009

Lorraine McDonald
Faculty of Health Sciences, UCT
Anzio Road
Observatory
7925
South Africa

Dear Lorraine

RE: Examiners Report: Ilse Joubert - MPhil in Sports Medicine Thesis - The effects of an ultra-endurance event on heart rate variability and cognitive performance during induced stress in Ironman Triathletes

I have completed the review of this thesis, and returned to you with this covering letter is my examiners report and summary report of the thesis. It was an absolutely excellent piece of work, of which the candidate, her supervisors and the University of Cape Town can be really proud, as will be evident in the mark I gave it, 85%, and in the category I classed it, namely that the dissertation be passed with distinction. It is one of the best Masters theses I have ever read, and this is one of the highest marks I have ever given, but I feel this mark is justly deserved due to the high quality of this piece of work.

Thanks for the opportunity for reviewing this thesis, which was a pleasure to mark. Please note it is my wish that I waive my external examiner examination fee, and ask that it be donated to the research funds of her supervisors, Professor Wayne Derman and Dr Laurie Rauch.

If any further information is required regarding the thesis I am happy to provide further input.

Best wishes,

Professor Alan St Clair Gibson MBChB PhD MD

MPHIL EXAMINATION REPORT – ILSE JOUBERT - THE EFFECTS OF AN ULTRA-ENDURANCE EVENT ON HEART RATE VARIABILITY AND COGNITIVE PERFORMANCE DURING INDUCED STRESS IN IRONMAN TRIATHLETES

Examiner: Professor Alan St Clair Gibson MBChB, PhD, MD – Northumbria University

Overview

This thesis examined heart rate variability, respiratory and cognitive function changes in athletes after competing in an ultra-endurance ironman triathlon. It was performed as a 'field' trial, before and after the event in what must have been extremely difficult conditions, yet used an incredibly complex battery of state-of-the-art neurophysiological and cognitive tests, in a well-controlled and scientifically robust way. It produced some very interesting findings which I am sure will be well received by the scientific community when published, and will lead to I am sure further research in this novel research area.

I can state with complete honesty that I perceive this thesis to be an absolutely exceptional piece of research work. The introduction and review of why the problem was being examined was logically set out, the methods used in the study were described in a manner which was easy to understand, and the results section showed in excellent detail the findings of each component of the study, using both tables and figures. In particular, I would like to commend the literature review section of the thesis, which is perhaps one of the best I have read at either a Masters or PhD level, and which was based on an astonishing number of previously published journals, all of which it was obvious from the text had been read and absorbed by the candidate when producing the literature review. At the end of it I felt as if I had read a review published in a good international journal, and that I had learnt a number of new insights, in a field where I think I can say I have a fair amount of knowledge of. This literature review is a wonderful piece of work on its own, and I hope it will be published as a stand-alone review in the future by the candidate and her supervisors.

A special congratulatory comment must also be made regarding the discussion section. Every point or query I had made in my reading of the methods or results sections was considered in the discussion section, whether they were shortcomings of the study, or contradictory findings, or findings which were difficult to understand. At the end of it, I felt that every possible angle had been covered in a logical and flowing manner, and it rounded off what was in its entirety an excellent piece of work.

Congratulations to the candidate and to her supervisors, they have done themselves the University of Cape Town proud with this work, and I am sure the candidate has a great future ahead of her should she choose to continue her career in science. This thesis really is a 'tour de force' piece of research bringing together field work done in difficult conditions with state of the art research techniques and post-hoc neurophysiological data analysis, and I enjoyed reading every page of it.

Several suggestions and queries are described below, none of which are major issues, but which are put forward to assist in perhaps strengthening and rounding off what is a very good piece of work, to be performed at the discretion of the candidate and her supervisors.

Specific Comments

1. Abstract, pg 9, para 1. An abbreviation is used in the abstract (SDNN) which I don't think was described in full earlier in the text of the abstract or list of abbreviations. While these are explained later in the thesis text, as a reader it did leave me scratching my head to try and work out exactly what the abbreviation meant, and perhaps this can be clarified in the text or list of abbreviations.
2. Literature Review pg 13 para 2. Near the end of the paragraph it was stated that SDANN was calculated over 'short periods' but is an estimate of 'changes in heart rate for cycles longer than 5 minutes'. Does this mean that SDANN is actually measured over one long period, or two short periods at different time points which are then divided or subtracted from each other to give an understanding of a 'change'?
3. Literature Review pg 14, para 3. Perhaps elaborate how slow rhythms are good predictors of cardiac mortality after MI - is there a change associated with them, or is their mere presence such a predictor?
4. Literature Review pg 15, para 1. Can the word 'good' ever be classified or scientifically validated? Should this word rather be replaced by 'reliable', or 'repeatable', or 'valid'?
5. Literature Review pg 17, para 2. Are beta blockers causal of 'sympathetic activation which occurs after stress', or do they reduce this (not sure if it comes across correctly as written)?
6. Literature Review pg 26, para 2. Why is the linear correlation between 'improvement in HR variability (what does this mean - what does an 'improvement' entail - clarify this) and moderate exercise a 'remarkable' finding. The use of the word 'remarkable' comes across a bit odd written here, and is subjective, and should perhaps be removed or toned down.
7. Literature Review, pg 28, para 1. Why is desensitising of B1 receptors the 'most popular' mechanism. How do you define 'most popular'? This statement or word choice should perhaps be removed.
8. Literature Review, pg 29, para 5. The word 'exercise' should perhaps be added after 'Prolonged distance...does not...'
9. Literature Review, pg 30, para 3. Why have the readers been made aware that the authors of this finding are 'Polish'? This information is not relevant to what is being described and should perhaps be removed.
10. Study Introduction, pg 33, para 5. Why is this piece of work described as a 'pilot' study? It seems to be a standard type full study to me, and this word should in my opinion be removed.
11. Methods, pg 37, para 1. It would be good if a reference describing the validity and reliability of the 'modified Pan and Tompkins algorithm to detect QRS complexes' is described in the text.
12. Methods, pg 37, para 1. A bit more description of the methodology of, and why 'spacing of the beats, was required in one subject, and what effect this would have on

the veracity of the data in this subject.

13. Methods, pg 37, para 2. I think several previous studies have used a 'cut off' higher than 0.15 to differentiate between LF and HF components (often between 0.20 and 0.25). Perhaps a comment put be added or references given why this specific value was used.

14. Results, pg 39, para 2. Perhaps the words 'marked increase' should be changed to 'significant increase'.

15. Discussion, pg 44, para 2. There should perhaps be gaps between Whyte and (2000) and Rifai and (2000).

16. Discussion, pg 46, para 2. Spelling error - 'ossillation'?

17. Summary and Conclusion, pg 51, para 1. In my opinion the words '...or that neural fatigue associated with the perception of physical or mental fatigue is not directly related to cognitive function' could be added to the last sentence of this paragraph.

18. References, pg 54, ref 28. The references are in capital letters and format is different to the other references. The same for references 29, pg 55

It must be noted again, that these points raised are done so in order to strengthen what is already a very good piece of work, and most are indeed semantic or concern fine grammatical details rather than any serious perceived shortcomings. Congratulations again to the candidate and her supervisors on an excellent piece of work.

201 Patty Park
Firdale Rd
Tamboerskloof
8001
1 June 2009

To Professor Wayne Derman

Re: Corrections of the Mphil.Sportsmedicine Dissertation: The effects of an ultra-endurance event on heart rate variability and cognitive performance during induced stress in Ironman Triathletes

I have reviewed the comments of the examiners and respond to them below. Please note that the reviewer's comments are introduced at the beginning of the paragraph in bold. My response follows and the text from the thesis (amended) is shown in italics.

Response to reviewer no 1: Dr Holtzhausen

1.1 Research Methods : Why HRV and cognitive function were compared.

Recent research by Lill Hansen et.al (2004) indicated the distinct relationship between HRV and cognitive function. Detraining, leading to decreased physical fitness as measured by VO₂ Max, as well as a lowered resting HRV, concomitantly led to slower reaction times and less true positive responses during tests of executive function. This was not the case with non-executive tasks. This study sought to investigate if HRV, known to be decreased after strenuous exercise, would also be associated with poor cognitive executive responses in these triathletes.

1.2 Small sample size

The small sample size of the two groups is a limitation of this thesis which has been expanded upon in the discussion. Based on the study outcomes, further research is planned to extend this study with larger numbers.

1.3 Methodology, measurements and outcome of the Stroop test added to the literature review P24 par 1:

5.2 *The Stroop task as stressor*

The Stroop task and the reliability of correct responses have been shown by several studies to be a valid laboratory induced stressor^{154;155}. Standard Stroop tasks include variants of congruent (colour matching words) and incongruent (colour different from word) colour-word and neutral-word or picture-word tasks on a computer screen. The subject must inhibit responses/executive function to these tasks, until complex central cognitive processing took place. This Stroop task interference is defined as the added stress associated with naming the colour of the word rather than the more

automatic reading of the word ^{156;157}. This interference effect is very robust and reliable, especially if emphasis is placed on speed ¹⁵⁸. Outcomes can then be measured according to reaction times and accuracy, namely the faster and more accurate, the higher the cognitive executive function.

1.4 The exclusion criteria rewritten

Participants (subjects and controls) had to be non-smokers and relatively active with no history of cardiac symptoms, disease or previous collapse. The complexity of the Stroop test required a good and intact cognitive function and a fast working memory (WM). Exclusion criteria thus included cardiac disease, colour blindness, previous head injuries, psychiatric disorders or any current use of drugs or medication. In particular, participants had to be free from especially heart rate altering medications, CNS drugs like anti-epileptics, stimulants (caffeine) and recreational drugs.

PRESENTATION

1.5 The list of Abbreviations

This has now been completed.

HRV	Heart rate variability
HRR	Heart rate recovery
HR	Heart rate
RF	Respiratory frequency
RSA	Respiratory sinus arrhythmia
RT	Reaction Time
WM	Working Memory
LF	Low Frequency
HF	High Frequency
VLF	Very low Frequency
ULF	Ultra low Frequency
NN50	Successive R-R differences exceeding 50ms
pNN50	NN50 as a percentage of R-R intervals measured
RMSSD	Root Mean Square successive difference of the R-R intervals
SDNN	Standard Deviation of the average R-R intervals
SDANN	Standard Deviation of the 5-min means of R-R intervals
PSD	Power spectral density
FFT	Fast Fourier Transforms
STFT	Short Wave Fourier Transform
AR	Autoregressive Modelling
ANS	Autonomic Nervous System
RAS	Reticular Activating System
CVC	Cardiovascular Control Centre
SWS	Short Wave Sleep
ECG	Electrocardiography
CTG	Cardiotocography
SA	Sinoatrial
RV	Right Ventricle

LV	Left Ventricle
BMI	Body Mass Index
BP	Blood Pressure
bpm	Beats per minute
HT	Hypertension
CAD	Coronary Artery Disease
CVD	Cardiovascular Disease
MI	Myocardial Infarction
VF	Ventricular Fibrillation
SCD	Sudden Cardiac Death
BRS	Baroreflex Sensitivity
VO ₂ Max	Oxygen Consumption Maximum
VT	Ventilatory threshold
RPE	Rate of Perceived Exertion
FFA	Free Fatty Acids

1.6 Abstract: Description of what is tested with the Stroop test? A section has now been added in the text

Background: *The effects of long-term participation in ultra-endurance exercise on the cardiovascular system have recently been the subject of much interest. It is well known that HRV, a marker of autonomic activity, is enhanced with long-term aerobic exercise training. However, after acute exercise, HRV is reduced, but recovers over time depending on the intensity of the prior bout of exercise. A limitation of previous research is that exercise bouts of only up to 120 minutes have been studied. A modified Stroop Task is a laboratory stressor to assess executive cognitive function by means of reaction time and accuracy. The resting HRV is directly related to these prefrontal neural functions, but the effect of an altered HRV on cognitive function has never been investigated.*

1.7 Introduction Corrected:

Yearly, the number of athletes who participate in this discipline increases, and this has indeed been the case in South Africa where entries have reached 1600 in the 2007 event.

1.8 P12 Par 2 Corrected:

The effects of exercise training on HRV have been studied over the past 10 years. Indeed, the improvement of HRV parameters with long-term moderate exercise training has led to the vigorous implementation of various forms of exercise in cardiac rehabilitation programmes²¹⁻²⁵.

1.9 P12 Par 3 Added:

Cognitive performance (central processing and motor execution) during mental stress (sympathetic stimulation) has been shown to simultaneously activate vagal parasympathetic effects on the heart, leading to a decreased heart rate (HR) and raised HRV to counteract the global sympathetic effects

on the heart²⁸. This is more prominent with complex cognitive tasks involving executive function (Cognitive Stroop Task)²⁹.

Literature Review

2. Measures/Measurements? Corrected

2.HRV measures and recording

HRV is used as a reliable non-invasive method to investigate vagal activity at the heart and thus can be seen as a quantitative marker of autonomic activity. It is derived by measuring successive R-R intervals from the raw ECG tachogram, or alternatively from arterial pressure recordings. HRV can be analysed in both time- and frequency-domains. Measures were recently standardised by the Task Force of the European Society of Cardiology (see their report 1996)³⁷. Non-linear measures, like the Poincare plot, fit data points (the value of a given heart period against the subsequent heart period)³⁸ into an ellipse; with the length and breadth of the radii of the 2 axes giving a picture of HRV at a glance³⁹.

2.1 Time-domain : References added and 5 minute periods over long recording times explained

HRV can be measured as the variance between successive R-R intervals over a period of time. Normal-to-normal (NN) intervals (i.e. all normal intervals between adjacent QRS complexes), are calculated from continuous ECG recordings. Variables derived from direct measurements of the R-R intervals and those derived from the differences between successive R-R intervals can then be calculated. Most commonly used are the NN50 (number of intervals of successive R-R intervals greater than 50 ms) and the pNN50 (the percentage derived by dividing NN50 by the total number of R-R intervals). These parameters represent predominantly parasympathetic activity.^{37,38} The following four measures are usually also recommended: SDNN (the standard deviation of R-R intervals) is an estimate of overall HRV as a square root of the R-R interval variance and represents the general ANS balance. HRV triangular index is a good measure of overall HRV, but permits only casual pre- processing of the ECG signal. SDANN (the standard deviation of the average R-R intervals calculated over short 5 minute periods over time) is an estimate of the changes in heart rate for cycles longer than 5 minutes, i.e. the long-term components of HRV. Conversely, RMSSD (the square root of the mean squared differences of successive R-R intervals) estimates the short-term components of HRV. The RMSSD method has better statistical properties than pNN50 and NN50, and is therefore preferred. Only recordings of the same duration can be directly compared in this way³⁷.

2.3 Yes vagal tone dominates during SWS (p16)

HRV in general is subject to circadian rhythms, thus factors like posture, state of arousal, changes of activity and breathing influence HRV⁴⁴. Recordings of HRV have been done supine, standing, during exercise and during 24-h Holter recordings. It is speculated that SWS (short wave sleep), the stage of

sleep when most recovery takes place⁴⁵, is under vagal predominance⁴⁶. Combined with very regular breathing and very stationary ECG, SWS provides optimal conditions for HRV recordings^{44;47}.

3.1 Reference: This has now been corrected:

3.1 Autonomic control

The heart is regulated by stimulating sympathetic nerves and inhibitory parasympathetic nerves of the autonomic nervous system (ANS) and influences heart rate and contractility. The vascular circulation is neurally controlled by the central circulatory demand centre in the subthalamic region, the cardiovascular control centre (CVC) in the pons and medulla as well as peripheral afferents (hypothalamus, baro- and chemo receptors and muscle afferents). HRV mainly depends on parasympathetic input from the vagal nerve. The presence or withdrawal of parasympathetic input is controlled cerebrally in the central circulatory demand centre, by integrating cognitive processes, brainstem nuclei and peripheral afferent input⁵².

3.2 Non-autonomic control (Hormonal)

Chapter 3

3.2 Methodology references: Modified Stroop Task and ECG

The Modified Stroop Task

The modified Stroop task entailed the individual presentation of cues (2 cm in height) appearing every 3 seconds and displayed for 400 ms followed by a 2600 ms black screen (which constituted the response period), in the centre of a computer monitor on a black background¹⁶¹. This cue word was the name of one of 4 colours (red, blue, green or yellow), which was presented either in: (i) grey colour with the word reading either red, blue, green or yellow; or (ii) red, blue, green or yellow colour with the colour being different to the word, but not in grey. The subject was required to respond as quickly and correctly as possible by pressing one of four buttons to indicate either the colour of the word on the screen (if the colour of word was red, blue, green or yellow), or of the word itself (if colour of word was grey). Subjects were required to use only their two index fingers to press the relevant response button (red and blue buttons with left index finger and green and yellow buttons with right index finger).

In total 96 cues were randomly presented, 25% neutral (grey) and 75% incongruent colour words, to increase the cognitive demand on the subjects. The use of the grey words ensured that subjects had to read and recognise the colour words rather than just noticing the colours, thereby invoking the Stroop effect¹⁵⁸. Subjects were requested to focus on the centre of the screen during each trial.

ECG Measurement

ECG activity was recorded from 3 electrodes placed in positions representing Eindhoven's triangle namely, subclavicular bilaterally and over the anterior superior iliac crest. The skin surface was cleaned and gently abraded with an alcohol swab before electrodes were attached. The 3 electrodes were connected to the Biopac ECG amplifier set to band-pass filter between 0.5 and 35Hz. ECG and recordings were analysed with AcqKnowledge for Mackintosh OS X (version 3.9.0). This software uses a modified Pan and Tompkins algorithm to detect QRS complexes²¹¹. All ECG traces were then again off line band-pass filtered between 0.5 and 35Hz to reduce interference noise. The filtered ECG recording tachograms were then visually inspected to determine the correct recognition of QRS complexes and T waves. Noise and missed or ectopic beats were corrected by either enhancing R peaks to distinguish them from T peaks or by spacing beats. Undefined QRS complexes were corrected by replacing it with a previous adequately spaced QRS complex. Without this spacing of beats, which was only necessary in one subject where we corrected 3 beats, the ECG tracing are not identifiable for the analysing software^{37;38}.

P46 Par 3 Corrected:

If anatomical micro damage is caused, it is unsure whether normal restoration occurs over the 4 days of recovery

P46 Par 2 Corrected:

The first major finding of this study

Response to reviewer no 2: Prof Gibson

1. SDNN – see added to abbreviations (yellow highlighted)

2. SDANN- Measured over long or short periods?

“standard deviation of the 5-minute means of R-R intervals”. The SDANN is the mean R-R interval of multiple 5 minute R-R Interval recordings. The Taskforce paper only emphasized that for time domain measures, these recordings must be taken over a longer period of time (over 24 hours preferable) in order to include as many 5 minute intervals as possible.

3. Why slow rhythms are better predictors of mortality after MI? (p15)

“Slow rhythms, especially ULF and VLF measured over longer durations (Holter recordings) have been found to be good predictors of cardiac mortality after MI”

The reason for this is not clear currently and not well explained in literature. It was only noted through several studies to be more reliable. These slow wave recordings can only be measured if recorded over longer time periods. Thus in these studies continuous Holter recordings, which naturally include the stable SWS period at night, provide very reliable recording data.

4. Pg 16 Para 1: Corrected

Malliani et al. (1994) found that the normalised LF/ (LF + HF) and HF/ (LF + HF) ratios are reliable

5. Pg 18 Para 2: Adrenergic Antagonists Deleted

chemical- (adrenergic agonists, atropine antagonists).

I excluded betablockers, since they are used in the chemical treatment of stress symptoms and not used to elicit stress reactions in a lab setting.

6. Pg 27 Para 4: Remarkable Kept:

*The linear correlation between improvement in HRV (an increase in HRV parameter values) and moderate exercise, or simply increased daily physical activity, is a remarkable finding, and has led to the vigorous implementation of exercise in cardiac rehabilitation programmes to reduce mortality and morbidity. (Boulay P, Prud'homme D. Health-care consumption and recurrent myocardial infarction after 1 year of conventional treatment versus short- and long-term cardiac rehabilitation. *Prev.Med.* 2004;**38**:586-93.)*

This finding is nothing short of extraordinary, since it is one of our only scientifically measurable instruments to prove the efficacy of exercise. Thus I prefer this word in this context.

7. Pg 29 Para 1: Reference Added

*cardiac cellular metabolism*¹⁹⁴ (Liedtke AJ, Nellis S, Neely JR. Effects of excess free fatty acids on mechanical and metabolic function in normal and ischemic myocardium in swine. *Circ.Res.* 1978;**43**:652-61) and the *desensitising of β_1 -receptors* due to prolonged exposure to increased catecholamine levels¹⁹⁵.

8. Pg 31 Para 2: Corrected

Prolonged distance exercise does not seem to have the same effect on metabolism as does mode

9.Pg 31 Para 5 : Corrected

shown by Raczak et al. (2006), who found

I thus removed the detail 'polish'.

10.Pg 35 Para 6 : Corrected

This study sought

11.Pg 39 Para 1 : Reference added

*This software uses a modified Pan and Tompkins algorithm to detect QRS complexes²¹¹. (Meyer T, Gabriel HH, Kindermann W. Is determination of exercise intensities as percentages of VO₂max or HRmax adequate? *Med.Sci.Sports Exerc.* 1999;**31**:1342-5.)*

12. Pg 39 Para 1: Explained

Noise and missed or ectopic beats were corrected by either enhancing R peaks to distinguish them from T peaks or by spacing beats. Undefined QRS complexes were corrected by replacing it with a previous adequately spaced QRS complex. Without this spacing of beats, which was only necessary in one subject where we corrected 3 beats, the ECG tracing are not identifiable for the analysing software^{37,38}.

13.Pg39 Para 2 : High Frequency Values

According to most literature and especially with the standardization done by the Taskforce (2002), 0.15 – 0.18 Hz is the official cut off point between lower frequencies (LF) and higher frequencies (HF) is.

“The HRV spectrum contains two major components: the high frequency (0.18-0.4 Hz) component, which is synchronous with respiration and is identical to RSA. The second is a low frequency (0.04 to 0.15 Hz) component...” quoted from a summary prepared by Ichiro Kawachi in collaboration with the Allostatic Load Working Group. Last revised 1997. Also see: Sayers BM. Analysis of heart rate variability. *Ergonomics.* 1973;16:17-32.

14.Pg 42 Para 1 : Corrected

The CON group showed a slight increase in HR ($p < 0.05$) from pre to post-event, but the IRON group had a significant increase from 70 ± 10 to 84 ± 8 bpm ($p < 0.001$).

15.Pg 46 Para 3 : Corrected

and in keeping with the findings of Whyte (2000) and Rifai (1999).

16.Pg 48 Para 2 : Corrected

Each increase and decrease in heart rate represents one oscillation

17.Pg 53 Para 1 : Corrected

This possibly implies that neural fatigue (if present), associated with the perception of physical or mental fatigue, is not directly related to cognitive function as measured by a modified Stroop task.

18. Pg 54 Ref 28 Corrected.

29.Lacey JI. Somatic response patterning and stress: Some revisions of activation theory. In Appley MH, Trumbull R, eds. *Psychological Stress Issues in research.*, pp 14-37. New York: Applton-Century-Crofts, 1967.

30. Hon EH,.Lee ST. Electronic evaluation of the fetal heart rate. VIII. Patterns preceding fetal deat,further observations. *Am.J.Obstet.Gynecol.* 1963;**87**:814-26

I hope all is in order now

Yours truly,

Dr Ilse Joubert