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The heart of an endurance athlete: impact of and recovery after an ultra-endurance event.

A research report submitted to the Faculty of Health Sciences, University of the

Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree MSc Med in Biokinetics

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ABSTRACT

Background

Limited research currently exists on what the impact of an ultra-endurance mountain bike event has on the heart of an endurance athlete, and that exercise can potentially cause significant cardiac stress.

Aims

Our study set out to determine the stress and recovery of participation in a three-day ultra-endurance mountain biking event in the heart of athletes using HRV as an outcome measure.

Methods

Sixteen healthy participants (male and female) in a three-day ultra-endurance mountain biking event underwent a five-minute resting ECG recording in a supine position. Heart rate variability measurements were recorded two days before the race (baseline testing), after each race day (post-event testing) and at 24-hour recovery (recovery).

Results

Time-domain and frequency domain measures showed significant ($p \leq 0.05$) changes in HRV parameters after each race day. Our study found significant changes in HRV parameters all of which reflected an increase in sympathetic activity at rest after each day of the event. This data also indicates that the mean HR and RR variability variables did not returned to baseline value after 24-hours of recovery, showing ANS dysfunction and imbalances persisted up to at least 24-hours of post-event recovery.

The body was under continuous sympathetic dominance during rest as well as during each day of racing, meaning that each race day can be considered as physiological stress to the body. This may, in turn, cause a disturbance in homeostasis and an increase in ANS dysfunction.

Thus, recovery beyond 24-hours is needed before returning to further high intensity (greater than six metabolic equivalents) training after such an ultra-endurance event to prevent unwanted effects.

Conclusion

A three-day ultra-endurance mountain biking event causes ANS dysfunction which does not fully recovery 24-hours after the event. This has implications for further research and monitoring of athletes in advising a return to strenuous activity.

DECLARATION

I declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine in Biokinetics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

26 day of March 2019 at Windhoek

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ABBREVIATIONS

ANS	autonomic nervous system
A-V	atrioventricular
BP	blood pressure
BREC	biomedical research ethics committee
CNS	central nervous system
CR	cardiac remodelling
ECG	electrocardiogram
ET	endurance training
HF	high frequency
HIT	high intensity training
HPCNA	health professional council of Namibia
HPCSA	health professional council of South Africa
HREC-M	ministry of health and social services in Namibia and from the human research ethics committee – medical
HRV	heart rate variability
ISAK	international society for the advancement of kinanthropometry
LF	low frequency
LF/HF	low frequency to high frequency ratio
NN	normal-to-normal
NN50	normal-to-normal 50
NO	nitric oxide
O_2	oxygen
RMC	research management committee
RMSSD	square root of the mean of the sum of the squares of the differences between adjacent R-to-R peak intervals
RSA	respiratory sinus arrhythmia
S-A	sinoatrial
SDNN	standard deviation of the normal-to-normal RR-intervals

TP	total power
VLF	very low frequency
VO_{2max}	maximum oxygen uptake

DEFINITIONS LIST

Abrupt - characterized by or involving action or change without preparation or warning: sudden and unexpected

Acute - having a sudden onset, sharp rise, and short course

Allostasis - is the process of achieving stability, or homeostasis, through physiological or behavioural change.

Allostatic load - is "the wear and tear on the body" that accumulates as an individual is exposed to repeated or chronic stress. ... It represents the physiological consequences of chronic exposure to fluctuating or heightened neural or neuroendocrine response that results from repeated or prolonged chronic stress.

Anecdotally - based on or consisting of reports or observations of usually unscientific observers anecdotal evidence health benefits that may be more anecdotal than factual.

Baseline - a control measurement carried out before an experimental treatment.

Cascade - a sequence of successive activation reactions involving enzymes (enzyme cascade) or hormones (hormone cascade) characterized by a series of amplification of an initial stimulus.

Cessation - is an end to something, such as the stopping of a bad habit, as the cessation of smoking

Chronic - in medicine, lasting a long time.

Chronotropic - chronotropic effects (from chrono-, meaning time, and tropos, "a turn") are those that change the heart rate.

Clinical significance - in medicine and psychology, the clinical significance is the practical importance of a treatment effect—whether it has a real genuine, palpable, noticeable effect on daily life.

Constancy - a state of being constant or unchanging

Data - information [as measurements or statistics] used as a basis for reasoning, discussion, or calculation.

Deduced - to reach (a conclusion) by reasoning

Dependent Variable - a variable that varies due, at least in part, to the impact of the independent variable. In other words, its value "depends" on the value of the independent variable. For example, in the variable's "gender" and "academic major," academic major is the dependent variable, meaning that your major cannot determine whether you are male or female, but your gender might indirectly lead you to favour one major over another.

Gastrointestinal motility - is defined by the movements of the digestive system and the transit of the contents within it.

Haemodynamic - a branch of physiology that deals with the circulation of the blood.

Heart rate variability - is the physiological phenomenon of variation in the time interval between heartbeats.

Homeostasis - a property of cells, tissues, and organisms that allows the maintenance and regulation of the stability and constancy needed to function properly.

Independent Variable -the conditions of an experiment that are systematically manipulated by the researcher. A variable that is not impacted by the dependent variable, and that itself impacts the dependent variable. In the earlier example of "gender" and "academic major," (see Dependent Variable) gender is the independent variable.

Inotropic - affecting the force of muscle contraction

Instantaneous - done, occurring, or acting without any perceptible duration of time death was instantaneous

Methods - systematic approaches to the conduct of an operation or process. It includes steps of a procedure, application of techniques, systems of reasoning or analysis, and the modes of inquiry employed by a discipline.

Participant - individuals whose physiological and/or behavioural characteristics and responses are the objects of study in a research project.

Population - the target group under investigation. The population is the entire set under consideration. Samples are drawn from populations.

Reliability - the degree to which a measure yields consistent results. If the measuring instrument [e.g., survey] is reliable, then administering it to similar groups would yield

similar results. Reliability is a prerequisite for validity. An unreliable indicator cannot produce trustworthy results.

Stressor - a stimulus that causes stress psychological stressors

Sub-acute – a rather recent onset or somewhat rapid change.

Validity - the degree to which a study accurately reflects or assesses the specific concept that the researcher is attempting to measure. A method can be reliable, consistently measuring the same thing, but not valid.

CHAPTER 1

1. INTRODUCTION

There is every belief, substantiated by research, that exercise is good for one. What is unknown is whether there is a significant impact on the heart of an endurance athlete after completing an ultra-endurance mountain bike event.

The evidence is that the impact of exercise leads to healthy physiological adaptations and that most endurance athletes benefit from it (George, et al., 2012). The healthy heart of an endurance athlete can respond effectively to acute exercise and can delay fatigue during prolonged exercise (George, et al., 2012). George et al. (2012) who studied the acute and chronic adaptation on endurance athlete's hearts have shown that acute bouts of ultra-endurance exercise may cause an acute reduction in cardiac function, causing a cascade, which releases cardiac biomarkers. Thus, this evidence indicates that some endurance athletes may develop a pathophysiological cascade. Athletes and clinicians should be mindful of it and react adequately to this pathophysiological phenomenon (George, et al., 2012).

Heart rate variability (HRV) monitoring is a useful indicator in the diagnoses and prevention strategies of over-reaching in athletes (Meeusen, et al., 2012). Meeusen R. et al, (2012) reported over-reaching is an "accumulation of training and/or non-training stress resulting in a short-term decrement in performance capacity with or without related physical and psychological signs and symptoms of maladaptation in which restoration of performance capacity may take from several days to several weeks".

Often over-reaching is related to several warning signs, one of which includes autonomic nervous system (ANS) dysfunction and imbalances (Makivic, et al., 2013). It is known that the physiological system to be compromised due to an the increase in exercise stress either from increased intensity and/or from the duration of exertion (Aubert, et al., 2003).

1.1 Purpose of the Research

Considering this potential for exercise to cause significant cardiac stress, this study set out to determine the effect of participation in a three-day ultra-endurance mountain bike event in the heart of athletes using HRV as an outcome measure. The event consisted of a three-day mountain bike stage event covering a total distance of ~140km. This event, set in Namibia, is highly technical, and only advised for highly experienced riders. The event took place during high temperatures of 37- 40 degrees Celsius, with a total elevation gain of +-3100m across the three days (Figure 1.1). The average finishing time was nine hours. Results from this study would potentially advise endurance athletes and medical scientists on the impact of an ultra-endurance event on the heart, and the recovery needed before returning to high intensity (greater than six metabolic equivalents) training after such an ultra-endurance event.

Figure 1.1 Elevation gain and maximal elevation of day one, two and three.

The aim of the study was therefore to determine the effect of a three-day ultra-endurance mountain bike event on heart rate variability.

To achieve this, aim the following objectives were carried out:

- To determine the impact/stress of an ultra-endurance mountain biking event on the heart using HRV as an outcome measure;
- To assess the over-reaching in cyclists after an ultra-endurance mountain biking event using HRV analysis;
- To assess the recovery of the heart 24-hours after completing an ultra-endurance mountain biking event.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Allostasis, Allostatic load and Allostatic overload

The physiological response due to stress (either positive or negative) is well described through allostasis and allostatic load. Allostasis represents the adaptive process of maintaining homeostasis through complex physiological changes and therefore achieving a stable environment in the body (McEwen & Wingfield, 2003; Logan & Barksdale, 2008).

A deteriorated and cumulative allostatic process refers to allostatic load during which the physiological system is unable to adapt to (McEwen & Wingfield, 2003; Seeman, et al.,2004). Enduring challenges cause impairment of physiological regulating systems, which influences the sympathetic nervous system and the immune system (Schulkin, 2004).

Therefore, exercise over time has implications, which can be both either a good stress (eustress), and bad stress (distress) but the physiological change is determined by the individual's reaction to the stressor (McEwen & Wingfield, 2003).

2.2 Autonomic Regulation on Cardiovascular function

The autonomic nervous system regulates the body's internal functions where impulses are transmitted from the central nervous system (CNS) to peripheral organs, one which is the cardiovascular system (Freeman, et al., 2006). The ANS is responsible for the control of HR, the force of heart contraction, vasoconstriction, and vasodilatation of the blood vessels and contracting and relaxation of smooth muscles in various organs (Freeman, et al., 2006). The blood vessels will constrict due to the increase in sympathetic activity, which will decrease the gastrointestinal motility and constrict the sphincters, whereas the parasympathetic activity will induce the opposite response (Aubert, et al., 2003). The ANS is active in areas in the spinal cord, brain stem, and hypothalamus, which controls heart rate, blood pressure and stroke volume (Aubert, et al., 2003; Sarmiento, et al., 2013).

Two systems make up the autonomic nervous system, namely, the sympathetic and parasympathetic (vagal) system, which transmit automatic signals to the organs. The sympathetic system increases metabolic function to cope with challenges from outside the body, while the parasympathetic (vagal) system increases functions which is associated with growth and repair in the body (Aubert, et al., 2003; Makivic, et al., 2013; Sarmiento, et al., 2013; Dong, 2016; Gisselman, et al., 2016).

The cardiovascular function is control by the ANS, by both sympathetic and parasympathetic (vagal) nerves, which improves cardiac function (Aubert, et al., 2003; Makivic, et al., 2013). Cardiac inotropic and chronotropic function regulates between the relative sympathetic and parasympathetic stimuli. The natural pacemaker of the heart, the sinoatrial (S-A) node, controls the intrinsic heart rate (cardiac impulse). In general, increased parasympathetic (vagal) activity decreases the overall activity on the heart resulting in vagal predominance, which is distributed primarily by the S-A node to the atria and the atrioventricular (A-V) node to the ventricles (Aubert, et al., 2003; Makivic, et al., 2013).

Increased parasympathetic (vagal) activity increases the release of synaptic transmitter acetylcholine from the vagal nerve, which decreases the activity of the S-A node resulting in a decrease in heart rate (Aubert, et al., 2003) & (Makivic, et al., 2013). Increased sympathetic activity increases overall activity on the heart through the sympathetic nerves, which spreads to all areas of the heart, and mainly to the ventricles. The increased sympathetic activity increases the release of synaptic transmitter noradrenaline from the sympathetic ganglions, which increase the activity on the heart increasing cardiac contractility, blood pressure cardiac output and heart rate. (Aubert, et al., 2003; Makivic, et al., 2013; Sarmiento, et al., 2013; Dong, 2016; Gisselman, et al., 2016).

Furthermore, the information supplied by the sensory receptors, namely baroreceptors and chemoreceptors, determines the activity of the sympathetic and parasympathetic (vagal) activity. Baroreceptors (mechanoreceptors) are located within the heart and within the walls of the arteries (aortic arch and coronary arteries) (Aubert, et al., 2003; Makivic, et al., 2013).

Upon changes in pressure and due to the elastic elements of the arteries, the baroreceptors sense when the vessels are stretched due to increasing in blood pressure and therefore their function is vagally mediated (Aubert, et al., 2003; Makivic, et al., 2013). Two types of chemoreceptors exist, namely peripheral and central chemoreceptors. Peripheral chemoreceptors are located at the bifurcation of the carotid bodies and aortic bodies. Whereas central chemoreceptors are found within the medulla oblongata. The main function of these chemoreceptors is to control respiration and increases sympathetic activity on the heart and blood vessels (Aubert, et al., 2003; Makivic, et al., 2013; Sarmiento, et al., 2013; Dong, 2016; Gisselman, et al., 2016).

There is an increased risk of sudden cardiac death in athletes who present with a diminished vagal activity, which is indicated by a decrease in HRV and with the variability that becomes more constant (Dong, 2016). Currently, limited research exists on the effect of a single ultra-endurance event on HRV and its recovery.

Numerous studies (Table 2.1) have been done researching the effects of training load and intensity across a specific period. However, the acute (or subacute) effect of an ultra-endurance event on HRV and recovery is unknown. The importance of understanding the balance between physiological and potentially pathological cardiac stress needs to be understood.
Researchers suggested that providing valuable statistics on how the body recovers from exercise would have clinical significance, and still needs to be researched (Makivic, et al. 2013; Dong, 2016).

Cataldo et al. (2018) showed that baseline HRV measurements before a 10km race in master endurance athletes correlated well with the time of the 10km running event. It shows that with an increased HRV and increased parasympathetic activity before a race

can predict the performance and give insight on how the athlete will perform under a physical stress episode like a race.

2.3 Hear Rate Variability (HRV)

Heart rate variability is a measure using the time between the R-R intervals of the QRS complex on an electrocardiogram (ECG) recording and determining the variability between the consecutive R waves (Chen, et al., 2011; Makivic, et al., 2013; Vanderlei, et al., 2009). Heart rate variability is a physiological measurement used to evaluate the state of the autonomic nervous system (ANS) (Vanderlei, et al., 2009; Chen, et al., 2011; Makivic, et al., 2013).

The sympathetic and parasympathetic nervous system stimulates the heart and therefore influences heart rate (Makivic, *et al.*, 2013). When the constancy of the R-R intervals has maintained an equilibrium (homeostasis) can be maintained between the sympathetic and parasympathetic nervous system (Makivic, et al., 2013). Figure 2.1 illustrates the physiological phenomenon, namely the R-R interval otherwise known as HRV. Heart rate variability is measured in milliseconds from a series on instantaneous heartbeats, which can be recorded either with a short-term recordings of five-minutes or nominal 24-hour long-term recordings (Task Force of the European Society of Cardiology, 1996).

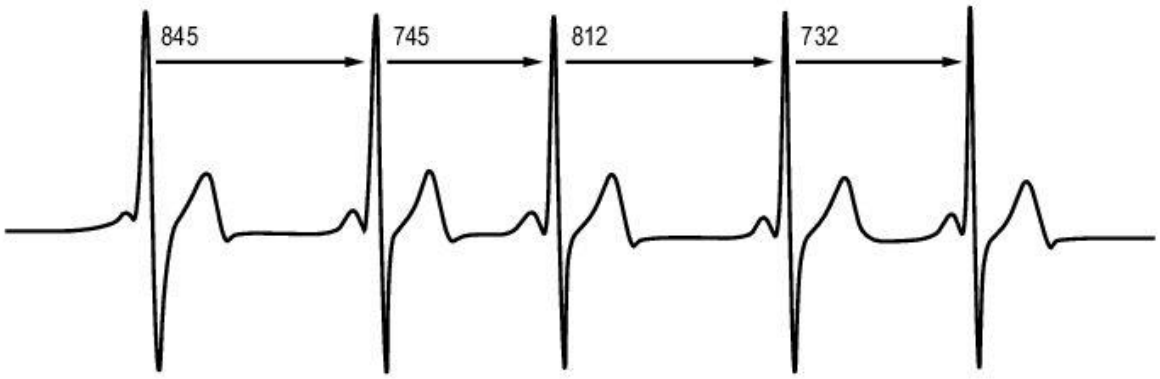

Figure 2.1 An example of an ECG signal – illustrating the physiological phenomenon of the R-R interval otherwise known as HRV (Makivic, et al., 2013)

Variations in HRV provide a profound and quick indicator of ANS impairments (Sehab, et al., 2009; Vanderlei, et al., 2009). A high HRV indicates an adaptation to exercises, which improves the function of the ANS. A lower HRV indicates a lack of adaptation or inadequate adaptation of exercise, which will depress the ANS.

Knowing that one can use HRV as a standard non-invasive assessment method for evaluating ANS function and that HRV is a powerful prognostic indicator of arrhythmic events following myocardial infarction (Sessa, et al., 2018). The sympathetic activity is related to the low frequency range (LF, 0.04–0.15 Hz) whereas the parasympathetic activity is related to the higher frequency range (HF, 0.15–0.4 Hz) of variation frequencies of the heart rate infarction (Sessa, et al., 2018). This frequency range difference represents an significant marker to detect the influence of sympathetic and parasympathetic systems. As described above, lower HRV is often associated to a autonomic dysfunction, and this parameter is widely researched between all arrhythmia risk markers infarction (Sessa, et al., 2018). This depression of the ANS can cause pathophysiology, including heart failure and myocardial ischemia (Sehab, et al., 2009; Vanderlei, et al., 2009).

Routledge et al. (2010) described that the mechanisms mediating the positive variation of HRV with exercise are unknown. However, angiotensin II and nitric oxide (NO) my potentially be contributors (Routledge, et al., 2010). In populations with low cardiovascular health exercise positively influences HRV via increasing vagal dominance and decreasing sympathetic tone (Routledge, et al., 2010).

An absence in the literature exists regarding the autonomic influence of cardio-deceleration after exercise, which is recognized as heart rate recovery (Javorka, et al., 2002; Kaikkonen, et al. 2007a). When an exercise stimulus from the central command stops the parasympathetic activity increases, which results in a decrease heart rate. Therefore, cardio-deceleration after exercise exists due to an increase in a parasympathetic drive (Javorka, et al., 2002).

On the contrary, when the exercise intensity progressively increases, the sympathetic nervous system will increase the sympathetic drive and decreases the vagal activity increases heart rate, stroke volume and myocardial contractility (Mertova, et al., 2017). These changes are dependent on the exercise intensity, duration and physiological capability of the athlete, and are associated with chemical and haemodynamic changes (Javorka, et. al., 2002; Makivic, et al., 2013; Sarmiento, et al., 2013). Kaikkonen et al. (2007a) believed that research on the exercise intensity and HRV have been studied to a large extent but that no studies exist on the effects of prolonged exercise duration on HRV and recovery.

2.4 Methods used to measure Heart Rate Variability

Time-domain methods

The simplest method to evaluate HRV is through the measurement of time-domain measures during which the intervals between successive normal complexes are determined. Time-domain method is considered the most validated and used HRV analysis method and is easily calculated through intervals between successive normal R-R complexes (Task Force of the European Society of Cardiology, 1996; Aubert, et al., 2003; Tarvainen, et al., 2013).

The most frequently used parameters include:

- Mean R-R interval, which measures the mean value of the R-R intervals across five minutes.
- The standard deviation of the normal-to-normal (SDNN) interval reflects the short- term and long-term variation during the recording. The SDNN involves both the sympathetic and parasympathetic nervous systems.
- Root mean square of successive differences (RMSSD) interval is used during short-term recordings to measure variability. The RMSSD is mediated by the parasympathetic nervous system and it is associated with the high-frequency power in the frequency-domain methods.
- The normal-to-normal 50 (NN50), which is the number of adjacent intervals, which differs with more than 50ms. It is seen as more reliable for short-term recordings compared to the SDNN. The NN50 is mediated by the parasympathetic nervous system.

Frequency-domain methods

Frequency-domain measures consists of various spectral methods to calculate and estimate the R-R intervals in series, which calculates the distribution of absolute and relative power into different frequency bands, thereby providing simple data of how power distributes as a function of frequency (Task Force of the European Society of Cardiology, 1996; Aubert, et al., 2003; Lampert, et al., 2008; Tarvainen, et al., 2013).

The most frequently used spectrum estimates are divided into:

- Very low frequency (VLF) (<=0.04Hz). Short-term measurements from the VLF band recording should be avoided and requires measurement of at least five minutes. Mechanism affecting the VLF might include temperature regulation, plasma renin fluctuations and endothelial processes. The sympathetic and parasympathetic nervous system mediates the VLF band.
  Low VLF power is said to be a good predictor for future adverse cardiac effects and is associated with increased inflammation.
- Low frequency (LF) (0.04-0.15Hz), requires measurements of at least two minutes. Disagreement exists regarding the ANS activity within the LF band. The sympathetic and high frequency mediates the LF band and resonance breathing affects the LF band.
- High frequency (HF) (0.15-0.40 Hz), requires measurements of at least one minute. As mentioned earlier it is associated with the RMSSD variability in the time-domain methods and affected by the respiratory sinus arrhythmia (RSA), which is mediated by the parasympathetic nervous system.
- Low frequency to high frequency (LF/HF) ratio measures the fractional distribution of power. It is seen as the ratio of sympathetic and parasympathetic activity. Thus, a low LF/HF ratio indicates parasympathetic nervous system dominance. Whereas a high LF/HF ratio indicates sympathetic nervous system dominance. A minimum measurement of five minutes is needed to determine the LF/HF.

- Total Power (TP) of overall RR-variability, reflects the overall power of the autonomic activity. Thus, a low TP indicates an increase in sympathetic nervous system dominance. Whereas a high TP indicates an increase in parasympathetic nervous system dominance.

Respiratory sinus arrhythmia known as the high-frequency component in HRV is considered the most noticeable periodic component in HRV measurements and ranges from 0.15Hz to 0.4Hz. The lower frequency (LF) component, which has a both sympathetic and parasympathetic origin, ranges from 0.04Hz to 0.15Hz and can be used to assess sympathetic efferent activity (Tarvainen, et al., 2014).

Some studies suggested that the LF component reflects sympathetic modulation and other studies to reflect both sympathetic and parasympathetic activity (Task Force of the European Society of Cardiology, 1996). Studies by Hottenrott et al. (2006) reported a significant decrease in HF domain measures as being indicative of a decreased vagal activity; and an increase in LF domain measures indicative of sympathetic activity during intensive boot camp training of six days.

Interestingly, Kaikkonen et al. (2007b) found that HF disappeared when exercise intensities exceed 50-60% of maximal oxygen uptake (VO_{2max}) and at high-intensity exercise (83% to 93% of VO_{2max}), the recovery of HF showed no differences. Furthermore, Kaikkonen et al. (2009) reported that exercise intensities, which exceed anaerobic threshold, might result in a decrease in HRV due to the metabolic load that increases. They report that during low to moderate exercise intensity there is minimal impact on the sympathetic nervous system, which agrees with the study of Perkins et al. (2015).

2.5 Endurance exercise and the heart

Athletes, especially endurance athletes, complete a high amount of training load and volume throughout their career and this training load leads to cardiac muscular adaptations due to the increased physiological and metabolic stimulus (George, et al., 2012). Three potential clinical areas in cardiac changes are posed by George et al, (2012), namely, "(1) how does heart structure, function or electrical activity adapt to endurance training? (2) are there any consequences for cardiac function and cardiomyocyte integrity that arise from undertaking acute bouts of (ultra) endurance exercise? (3) what, if any, clinically relevant cardiac changes can be observed in endurance athletes if lifelong endurance exercise can be documented?"

A study by Perkins et al. (2015) assessed both endurance training (ET) and high-interval training (HIT) and the impact thereof on HRV. The ET group completed a 45-minute continuous cycle ergometer session at a moderate exercise intensity of 62.5% of maximal heart rate.

The HIT group completed six sets of 30 seconds all-out supramaximal intensity on a cycle ergometer. They showed that in the HIT group the HF decreased significantly after the intervention at zero hours resulting in a decrease in vagal activity decreasing parasympathetic tone, subsequent increase in sympathetic tone and that after 24-hours there was a decrease in HF but not significant compared, to baseline values. With the ET group they showed that HF did decrease at zero hours after the intervention but was measured insignificant compared to baseline values and HF remained decreased up to 24-hours of recovery.

The following three studies showed that cardiac parasympathetic activity returned to baseline levels after 24-hours of recovery after an ultra-endurance event.

Firstly, a study by Gratze et al. (2005) studied triathletes who completed a full Ironman event (3.9km swim, 180.2 km cycle and 42.3km run). Heart rate variability was assessed one day before the event, one hour after the event and on one, three and seven days after the event. They reported that the sympathetic dominance and higher heart rate during the one-hour post-exercise resolved within three days during which heart rate and LF power decreased and HF power increased back to baseline values.

Secondly, a study done by Hautala et al. (2001) assessed HRV after a 75km cross-country skiing event. They found that heart rate (HR) remained the same one day after the event compared to baseline values but on the second day of recovery, they found that, HR was significantly lower compared to baseline values. The normalized high-frequency component and short-term R-R interval variability was significantly lower after day one but returned to baseline levels after day two. Furthermore, the normalized low-frequency component measured significantly higher after day one and returned to baseline values after day two.

Thirdly, a study by Bernardi et al. (1996) studied the effect of a 46 km rocky trail run at an average altitude of 2500m on blood pressure and HRV. It was found that 30 minutes after the race a relative increased sympathetic predominance and reduced parasympathetic baroreceptor activity was found and that 24-hours after the race, parasympathetic activation was increased.

The findings of the three above mentioned studies on ultra-endurance event suggest that cardiac parasympathetic activity returned to baseline levels after 24-hours of recovery after an ultra-endurance race. Findings from Stanley et al. (2013) shown that for complete cardiac autonomic recovery after a single exercise at least 24-hours of recovery is needed for low-intensity exercise, 24-48-hours of recovery for exercise at threshold intensity, and at least 48-hours of recovery after high-intensity exercise. Stanley et al. (2013) further proposed that the exercise duration is doubtfully to be the biggest factor affecting cardiac parasympathetic reactivation.

2.6 Other clinical Heart Rate Variability studies

Searched studies investigating changes in HRV during endurance exercise and training load is listed in Table 2.1. Exercise at various intensities and durations have variable effects on HRV.

Table 2.1 Summary of selected Studies investigating changes in HRV during endurance exercise and training load

Authors	Year	Subjects	Intervention	Method	Conclusion
Earnest, *et al.*	2003	8 professional male cyclist, age 27(1) years.	2001 Vuelta a España (Tour of Spain)	Examine HR and HRV. Baseline testing on day 0, testing on day 10, testing on day 17	Chronic exposure to heavy exertion showed the largest decrease in supine HRV
Mertová, *et al.*	2017	10 male sky-runners, age 37.2 ± 9.2 years	A sky-running trail marathon	Measuring HRV, Bassline testing 10, 2 and 1 day before the event, testing on race day, testing immediate post-race measures at 5 min intervals for 30 min and then at 5 hours and 30 hours post-race	The marathon caused a disturbance in the ANS. Which resulted in an increase in sympathetic activity up to five hours post-race and a vagal activity recovering at 30 hours
Baumert, *et al.*	2006	10 healthy athletes (track and field and triathletes) 5 men, aged 26.6 years and 5 women, age 24.8 years	2-week training camp. Stepwise increased cycling test followed by 40minutes of running and 80 minutes of cycling	Examining HRV, BPV, and BRS. Testing was done before, during and after the training camp	An abrupt increase in physical training resulted in altered autonomic cardiovascular activity. Resulting in parasympathetic inhibition and sympathetic activation
Vallverdú, *et al.*	2017	11 Male mountain trail runners, age 38.9 ± 2.6 years.	Gran Volt Cerdanya Ultrafons, a 35km mountain trail race	Analyse HRV response to high-intensity exercise. Measurements were taken during the race at 5min intervals	35km endurance mountain trail race showed to have multiple differential changes on HRV and the ANS which is related to the intensity
Plews, *et al.*	2014	9 Elite rowers, 4 female, 5 males	26 Week build-up training to the 2012 Olympic Games	Relationship between HRV and training intensity. All training sessions were recorded for analysis of training intensity. HRV was measured upon waking up on each day	Increased time spent at high-intensity training suppressed the parasympathetic activation whereas low-intensity training increased parasympathetic activation. Low-intensity training may be beneficial for optimal training programming

Perkins, *et al.*	2016	9 Untrained students, 6 female, 3 males	Two exercise protocols. Endurance protocol: 45min of moderate-intensity cycling. High-intensity interval protocol: 6 x 30sec of high-intensity cycling	HRV analysis measured over three days for two hours starting immediately after each session and each morning thereafter. Baseline measures were also obtained from a two-hour recording	High-intensity exercises showed a suppressed parasympathetic activity when compared to endurance training
Sarmiento, *et al.*	2013	11 elite cyclist, age 18.6 ± 3.0	Two protocols were performed one week apart, first an incremented load cycling test and then a six-minute constant load-test	HRV analysis during constant-load, high-intensity exercise. HRV was analysed at the start and end of the first phase ad minutes 2-3,3-4,4-5 and 5-6 after the first phase until completion of four one-minute intervals (second phase)	Wavelet transform allowed the researcher to observe how HRV is directly influenced by cycling cadence and power output at the end of the exercise
Ilellamo, *et al.*	2002	7 junior rowers, 18 years of age	Two daily training sessions, 7days a week, totalling to 26 to 30 hours of heavy exercise per week, which consisted mainly of rowing and field running	Baseline recording, 3 subsequent investigations done 3 months apart. 2nd and 3rd recordings done at a training load of 75% of maximum and final recording at 100% of maximum training load, 20 days before the rowing world championships	Highly intensive endurance training changes cardiovascular autonomic modulation. Changes are shown a predominance from parasympathetic towards sympathetic drive increase in cardiovascular performance at high training intensities
Coronoo, *et al.*	2005	Total of 24 men, 13 trained high-altitude group, age 27.8 ± 2.0 and 11 sedentary control high-altitude group age 27.4 ± 2.3	Running a marathon at 4220m	Orthostatic test and blood sampling for haematocrit were taken at rest before a maximal exercise test. Runners performed an orthostatic test before the race and after the marathon race 6-8 and 20-24 hours	Sympathetic predominance observed 6-8 hours after a high-altitude marathon recovered after 1 day of recovery. Living at high altitude does not impair cardiovascular autonomic response during training

HRV Heart rate variability; **ANS** Autonomic nervous system; **HR** Heart rate; **BPV** Blood pressure variability; **BRS** Baroreflex Sensitivity.

CHAPTER 3

3. METHODS

3.1 Study Design

The study used a prospective quantitative research design, which involved the collection and analysis of numerical data. The study was a repeated measure design. During the study, we assessed whether there is a significant impact on the heart of endurance athletes during and after completing a three-day mountain bike event and assessed the recovery status 24-hours after the event by using HRV as an outcome measure. The study consisted of the following measurement procedures; namely baseline testing, post-event testing, and a 24-hour post-event testing, which is discussed in detail under the data collection and analysis section.

3.2 Hypothesis

There are significant variations in HRV during and/or after the three days of competing in an ultra-endurance mountain biking event and HRV does not fully recover to baseline values within 24-hours.

3.3 Null-Hypothesis

There is no significant change in HRV during the three days of competing in an ultra-endurance mountain biking event.

3.4 Site of study

The collection of data was conducted in Namibia during the Standard Bank Klein-Aus Vista MTB Challenge, which is a three-day mountain bike stage event. The event took place in Aus, which is a small town in the South of Namibia in the desert. Anecdotally

the event is regarded as the toughest three-day stage event in Namibia. The event started on the 3rd of May 2018 and continued the 4th and 5th.

3.5 Study population

The study comprised of a sample of 17 participants (male and female) who participated in the three-day ultra-endurance mountain biking event and were selected through a sample of convenience. The race organizers of the Standard Bank Klein-Aus Vista MTB Challenge approved and provided written permission (Appendix 1) to conduct the study during their event.

Participants had the opportunity to volunteer to take part in the study after the race organizers sent an advert (Appendix 2) via email to all the participants. The researcher thereafter presented at a meeting (Appendix 2) to the participants to inform them in detail about the study. During the meeting, a consent form (Appendix 4) with all ethical standards were addressed. All participants had read, understood and signed their consent form before participation in the study. All participants received a copy of the participant information sheet (Appendix 5) to keep for their records. Participant information will remain confidential as discussed below in paragraph 4.1.

One participant failed to complete the event on the third day and therefore did not finish the event and was excluded from the study. Thus, 16 participants completed the full three-day stage event.

All participants were encouraged to avoid drinking alcoholic beverages and avoid smoking before and during the time of the experimental procedures. No participant reported taking any known medication or sympathomimetic drugs that could have affected the cardiovascular system. All participants were athletic, healthy and amateur mountain bike riders with mountain bike riding experience.

3.6 Inclusion criteria for the study were as follows:

- Male and female participants who entered the three-day ultra-marathon;
- 19 to 60 years of age.

3.7 Exclusion criteria were as follows:

- Any history or evidence of cardiovascular pathologies;
- Use of tobacco or alcohol (more than two drinks per day);
- Obesity with a body mass index ≥30 kg/m^2;
- Hypertension (systolic blood pressure (BP) ≥140 mm Hg, diastolic BP ≥90mm Hg);
- Use of cardiovascular medication;
- Any use of medication that may affect the ANS (sympathomimetic drugs).

3.8 Measuring tools/instruments

Welch Allyn PC based stress ECG system was used to obtain a resting ECG tracing. The HRV module in the Cardio Perfect software analysed short-term (five-minute recording) HRV in a series of heartbeat intervals up to a maximal resting ECG recording of five minutes and is validated software for RR-variability analysis (Cardio Perfect Software, Heart Rate Variability Module for Cardio Perfect Rest ECG (2015) Welch Allyn, Inc.). This module automatically detected the QRS complexes from which the R-R intervals are constructed. The R-R intervals were displayed along with the ECG for assessment of QRS classifications and localization of measurement artefacts and noise.

CHAPTER 4

4. DATA COLLECTION

4.1 Data collection

An overview of the study's data collection from baseline to 24-hour post-event measure is shown in Figure 4.1. The study consisted of conducting the following measurements: at baseline, post-event and at 24-hour post-event.

Baseline testing procedures

Two days before the event all participants' height (m) was measured using a Seca stadiometer (Seca, Hamburg, Germany), weight (kg) was measured using a Seca digital floor scale (Seca, Hamburg, Germany). Skinfolds (sum of seven skinfolds = triceps, subscapular, bicep, supraspinale, abdominal, frontal thigh and the medial calf was used) was measured using a Harpenden skinfold calliper (British Indicators, West Sussex, United Kingdom). Body composition of all participants was measured making using of the International Society for the Advancement of Kinanthropometry (ISAK) standards (Stewart, et al., 2011).

Participants underwent a pre-reading stabilization period of rest for five minutes to ensure that heart rate levelled before recording HRV. A resting ECG on each participant in a supine position for five-minutes provided baseline (pre-event) values. Electrode placement is done by following the standard 12 lead ECG electrode placement method, with moving the arm electrodes to the subclavian position and the lower extremities electrodes to the inferior rib-cage position (Jowett, et al., 2005).

As recommended, the interface between the electrode and skin was cleaned appropriately using 70% isopropyl alcohol swabs to decrease signal-to-noise ratio (Task Force of the European Society of Cardiology, 1996). During the five-minute recording, the athlete lay still to decrease interference and noise.

Baseline testing took place at Anton Swart Biokinetic Rehabilitation Practice in Windhoek two days before the event, which was performed by the principle researcher.

Post-event procedures

All participants were asked to move to a designated area once crossing the finishing line after each stage for the post-event measurement procedures. Participants underwent a pre-reading stabilization period within two-minutes after crossing the finishing line for five-minute rest to ensure that heart rate levelled before recording HRV. After the participants had completed each stage/day, a resting ECG was performed. Post-event measures were taken after each stage/day.

24-hour Post-event testing procedures

A 24-hour recovery measurement was recorded at Klein-Aus Vista the day after the participants had completed the third and final stage of the event. Participants underwent a pre-reading stabilization period of five-minute rest to ensure that heart rate levelled before recording HRV. A resting ECG in a supine position on each participant provided 24-hour recovery measurement.

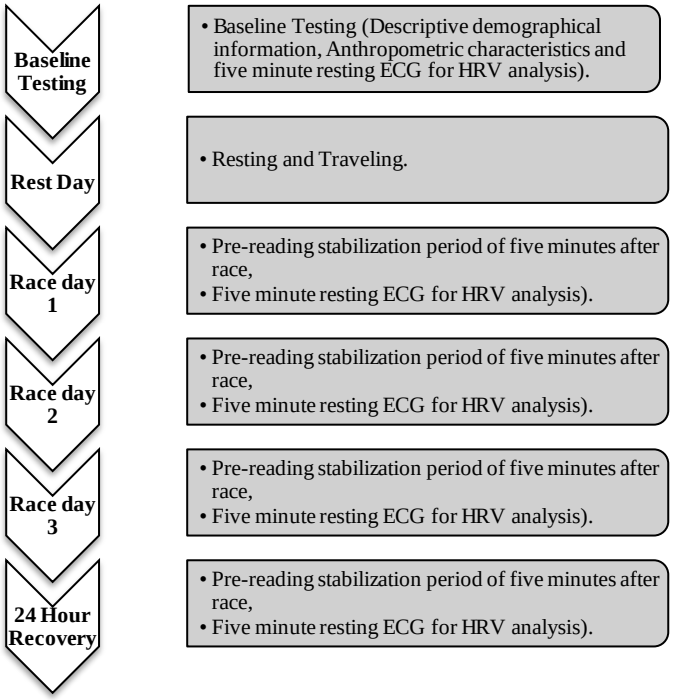

Figure 4.1. Overview of the study's data collection.

For the duration of retention of records, each participant's health records and measurements are held by following the guidelines of the Health Professional Council of South Africa (HPCSA) and Health Professional Council of Namibia (HPCNA). All hard copy records are stored in a safe place with electronic copies uploaded to online cloud storage, safeguarded by a password. All records and measurements will be stored for a period of not less than six years and only the principle researcher has access to these records. Indirect identifiers (numbers) replaced all obvious identifiers (example name and surname) of participants in the main data set to retain confidentiality. A separate data set known as the "Key" holds the indirect identifiers with the obvious identifiers.

4.2 Dependant variables

The summary of the dependant variables, which includes time-domain and frequency-domain methods, can be seen in Table 4.1.

Time-domain methods

The simplest method to evaluate HRV is through the measurement of time-domain measures during which the intervals between successive normal complexes are determined. It is considered the most valid and used HRV analysis method and is easily calculated. (Task Force of the European Society of Cardiology, 1996; Aubert, et al., 2003; Tarvainen, et al., 2013).

Frequency-domain methods

Frequency-domain consists of various spectral methods to calculate and estimate the R-R intervals in series, which calculates the distribution of absolute and relative power into different frequency bands. This provides simple data of how power distributes as a function of frequency (Task Force of the European Society of Cardiology, 1996; Aubert, et al., 2003; Lampert, et al., 2008; Tarvainen, et al., 2013).

Table 4.1 Dependent HRV variables of the study

Time0domain measures:	**Frequency domain measures:**
Mean Heart Rate	
Mean RR-variability	Total Power (TP) of overall RR-variability
Standard deviation of the sequence of NN intervals (SDNN)	High-Frequency Power (HF) (0.15-0.40Hz)
Number of pairs of successive RR interval which differ more than 50 ms (NN50)	Low-Frequency Power (LF) (0.04-0.15Hz)
	Very Low-Frequency Power (VLF) (<=0.04Hz)

Square root of the mean squared differences between successive RR intervals (RMSSD)	Low-Frequency power (LF)/High-Frequency Power (HF), the ratio between LF and HF powers

4.3 Independent variables

The independent variable measured the variations of HRV from baseline after each stage during the three-day stage event along with 24-hour recovery measurements after day three to assess the recovery status. Baseline HRV was established for each participant by measurement of HRV two days before the start of the race.

4.4 Validity and reliability

Welch Allyn PC Based stress ECG system

Accordingly, to the Task Force of the European Society of Cardiology, the technical requirements and recommendations for analysis should have a proper sampling rate. The optimal range is between 250 to 500Hz, and perhaps even higher (Task Force of the European Society of Cardiology, 1996). The Welch Allyn PC based stress ECG system has a sampling rate of 300, 600 and 1200Hz (Welch Allyn, CardioPerfect Workstation Resting ECG Module- User manual, 2015). The sampling was done at a sampling rate of 600Hz. The Cardio Perfect heart rate variability module analysis includes a wide variety of frequency-domain, time-domain and nonlinear analysis (Cardio Perfect Software, Heart Rate Variability Module for Cardio Perfect Rest ECG (2015) Welch Allyn, Inc.).

4.5 Statistical analysis

The descriptive data are expressed and summarized as means ± SD and were analysed using the statistical package STATISTICA (version 13.2). The study was a quantitative research design that used non-normal distribution. The normality of HRV

distribution was checked with the Shapiro-Wilk normality test, which confirmed non-normality.

The study used non-para-metric tests, which included Friedman two-way analysis of variance and Wilcoxon signed-rank test. Results are expressed as median and interquartile. The probability for the statistically significant difference was set at $p \leq 0.05$.

CHAPTER 5

5. ETHICS

Before the commencement of the study ethical clearance had been obtained from the Biomedical Research Ethics Committee (BREC) and Research Management Committee (RMC) (Appendix 6), Ministry of Health and Social Services in Namibia. Also from the University of the Witwatersrand Human Research Ethics Committee – Medical (HREC-M) (Appendix 7) (Certificate number M171037), Faculty of Health Science, University of the Witwatersrand, Johannesburg. The consent form addressed ethical standards (Appendix 4). All participants had read, understood and signed this consent form before participation in the study. All participants received a copy of the participant information sheet (Appendix 5) to keep for their records. Participant information remained confidential as discussed above in paragraph 4.1.

CHAPTER 6

6. RESULTS

Before data processing all ECG, tracing measurements were automatically corrected for artefact removal through the Welch Allyn Cardio Perfect software for ectopic and missed beats, hence the normal-to-normal interval (NN) obtained. A probability value

of ≤ 0.05 was considered significant. The descriptive demographical information and anthropometric characteristics of the 16 participants are presented in Table 6.1.

Table 6.1 Descriptive demographical information and anthropometric characteristics

Variable	Mean ± SD (range)
Age (years)	48.75 ± 7.41 (39-60)
Body Weight (kg)	78.48 ± 12.18 (63-100)
Height (m)	174.31 ± 6.90 (166-187)
Body mass index (m/kg2)	25.71 ± 2.64 (22-30)
Body Fat %	12.94 ± 3.28 (7-18)
Waist: hip ratio	0.86 ± 0.06 (0.74-0.92)

6.1 Time domain measures

Compared with baseline testing, mean HR increased and R-R variability decreased significantly ($p<0.05$, Table 6.2) and continued to increase and decrease until after day two and three of the event respectively, but did not recover back to baseline measurements after 24-hours after completing the three-day stage event. During the 24-hour recovery mean HR and R-R- variability measured significantly ($p<0.05$, Table 6.2) higher and lower respectively compared to baseline measurements.

The interval measures of both SDNN and NN50 decreased significantly ($p<0.05$, Table 6.2) from day one compared to baseline and continued to decrease until after day two. After day three, there was a slight increase compared to day two but significantly ($p<0.05$, Table 6.2) lower compared to baseline values. The 24-hour recovery revealed no significant difference between baseline and 24-hour recovery in SDNN and NN50.

The RMSSD decreased significantly ($p<0.05$, Table 6.2) as from day two compared to baseline and continued to decrease until after day three. After day three, there was a

slight increase in power compared to day two but significantly ($p<0.05$, Table 6.2) lower compared to baseline values.

The 24-hour recovery revealed no significant difference between baseline and 24-hour recovery in RMSSD.

6.2 Frequency domain measures

Changes in TP, HF, VLF and LF/HF indicative of cardiovascular modulation were associated with a significant ($p<0.05$, Table 6.2) change in HRV from day one compared to baseline and continued to change significantly until after day three. The 24-hour recovery revealed no statistically significant difference between baseline and 24-hour recovery in TP, HF, VLF and LF/HF.

The LF decreased significantly ($p<0.05$, Table 6.2) from baseline to after day one. No significant change was measured after day two, three and at 24-hours of recovery.

Box and Whisker Plot of HRV indicative of minimum, quartile 1, median, quartile 3 and maximum is shown in Figure 6.1 to Figure 6.10 with all the time domain measures and frequency domain measure respectively.

Table 6.2 Means, standard deviations and p-value (p<0.05) changes in Time domain and Frequency domain measures from baseline to 24-hour recovery

Time domain measures										
	HR		**RR-Variability**		**SDNN**		**NN50**		**RMSSD**	
Measurement	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value
Baseline	59.50 ± 8.45	-	1031.81 ± 150.00	-	47.63 ± 21.57	-	47.25 ± 50.01	-	43.69 ± 30.16	-
Day 1	82.00 ± 8.66	0.00***	738.90 ± 71.83	0.00***	23.81 ± 10.18	0.00***	5.56 ± 13.79	0.01**	38.06 ± 103.32	0.84
Day 2	89.00 ± 9.00	0.00***	681.75 ± 69.04	0.00***	20.44 ± 11.12	0.00***	2.56 ± 3.58	0.00***	11.56 ± 5.53	0.00***
Day 3	88.06 ± 8.65	0.00***	688.88 ± 70.23	0.00***	22.06 ± 17.33	0.00***	7.69 ± 19.31	0.01*	16.50 ± 17.49	0.01**
24 Hour Recovery	65.38 ± 8.40	0.03*	931.75 ± 125.36	0.03*	43.44 ± 21.69	0.57	27.60 ± 41.07	0.10	34.44 ± 25.18	0.23
Frequency domain measures										
	Total Power (TP)		**High Frequency Power (HF)**		**Low Frequency Power (LF)**		**Very Low Frequency Power (VLF)**		**LF/HF**	
Measurement	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value
Baseline	2847.06 ± 3127.77	-	930.56 ± 1133.74	-	1012.44 ± 1271.96	-	904.13 ± 1230.69	-	1.43 ± 0.88	-
Day 1	512.56 ± 499.47	0.00***	60.25 ± 67.39	0.01**	215.69 ± 180.74	0.01**	236.56 ± 268.88	0.02*	6.23 ± 6.73	0.02*
Day 2	465.06 ± 624.08	0.01**	35.19 ± 38.23	0.01**	299.31 ± 534.66	0.07	130.69 ± 135.55	0.03*	8.24 ± 6.93	0.00***
Day 3	562.94 ± 945.72	0.01**	137.94 ± 347.65	0.02*	296.13 ± 522.87	0.07	129.00 ± 133.87	0.02*	5.14 ± 4.37	0.00***
24 Hour Recovery	1807.38± 1770.36	0.23	523.88 ± 705.22	0.19	739.75 ± 1175.16	0.50	543.75 ± 445.89	0.30	2.09 ± 1.46	0.11
Level of significant differences from *p≤0.05, ** p≤0.01, ***p≤0.001										

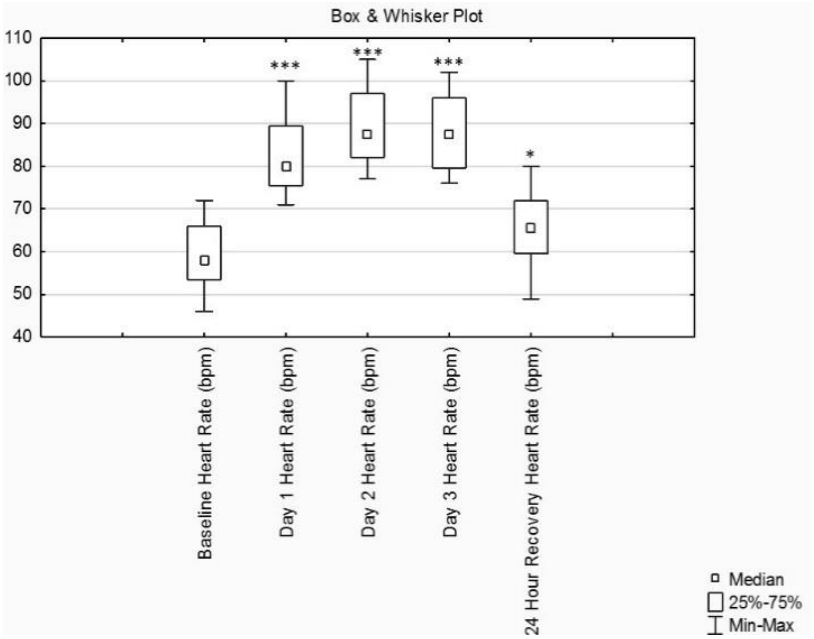

Figure 6.1 Box & Whisker Plot of the Heart Rate. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

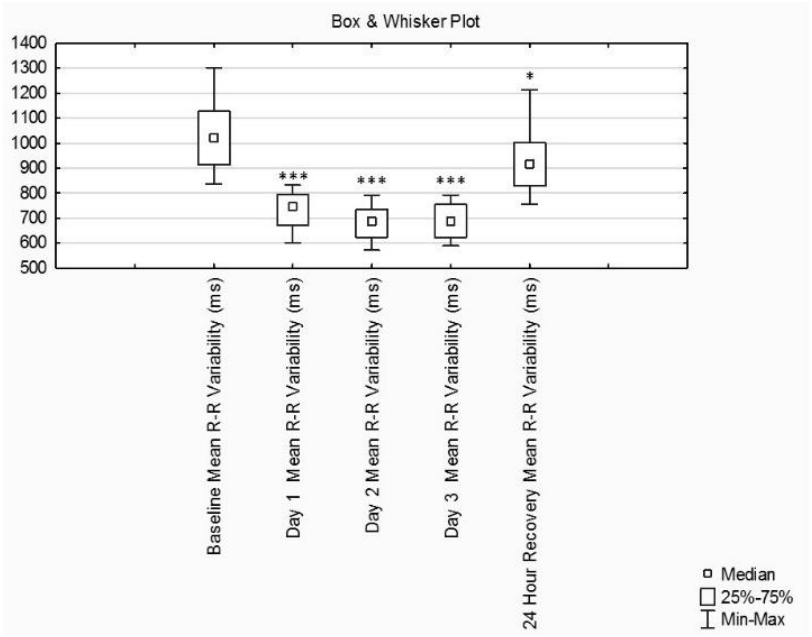

Figure 6.2 Box & Whisker Plot of the Mean R-R Variability of HRV. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

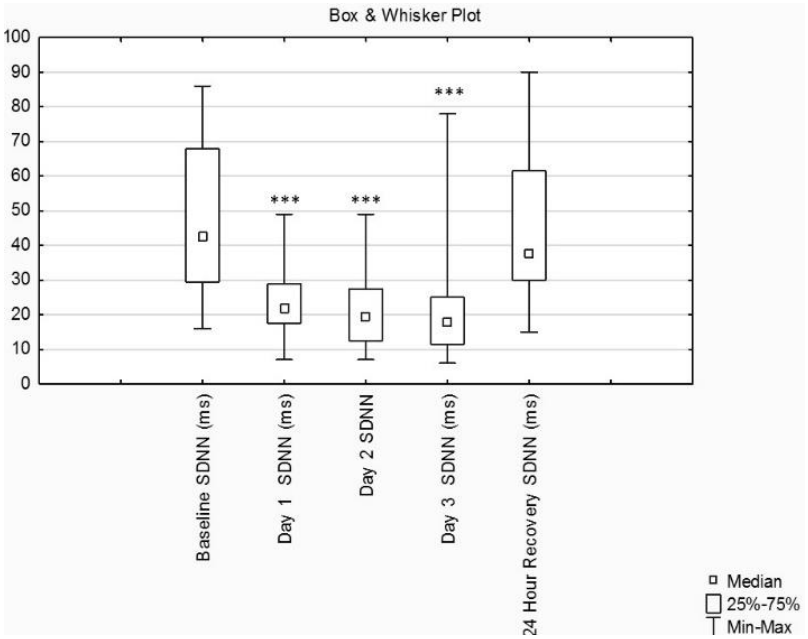

Figure 6.3 Box & Whisker Plot of the Standard deviation of the sequence of NN intervals (SDNN) of HRV. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

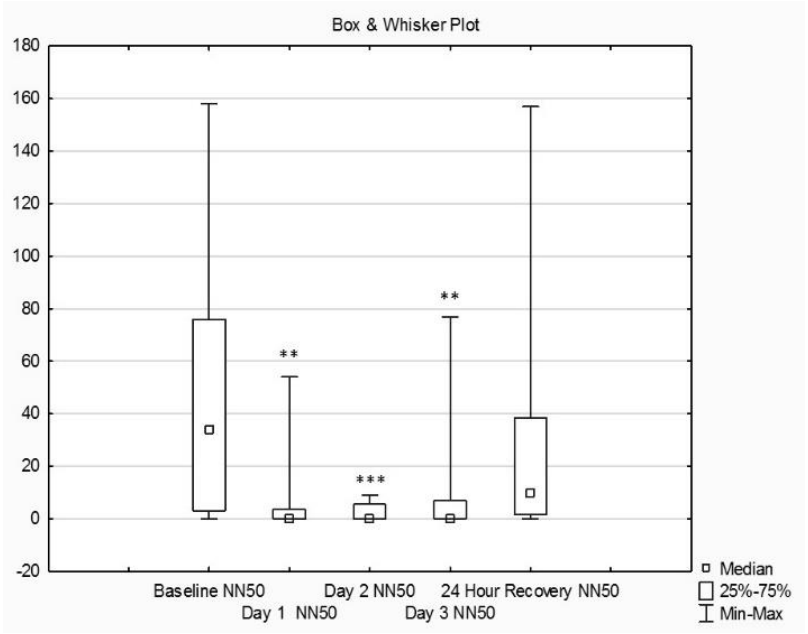

Figure 6.4 Box & Whisker Plot of the Number of pairs of successive RR interval which differ more than 50 ms (NN50) of HRV. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

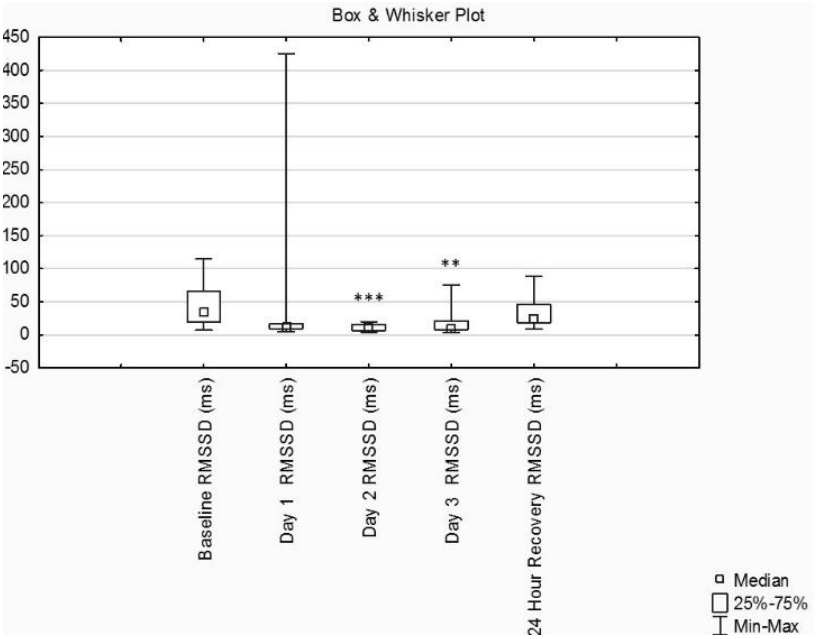

Figure 6.5 Box & Whisker Plot of the Square root of the mean squared differences between successive RR intervals (RMSSD) of HRV. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

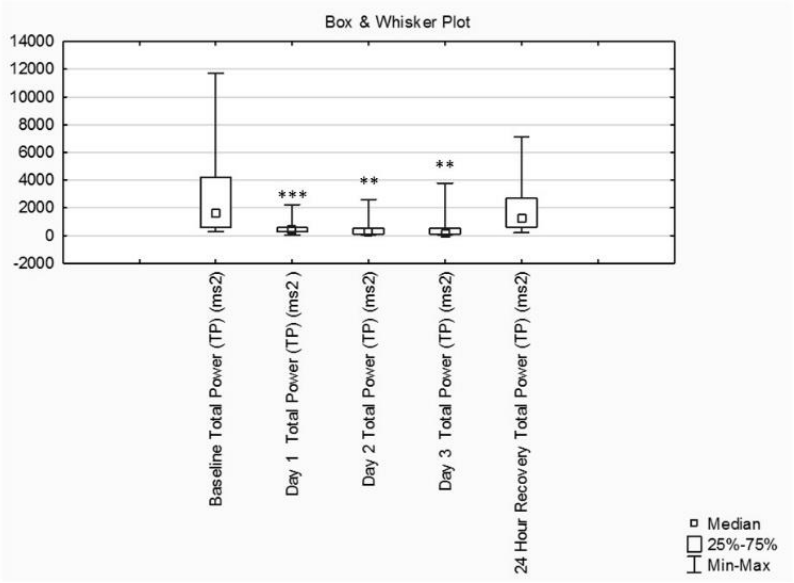

Figure 6.6 Box & Whisker Plot of the Total Power (TP) of HRV. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

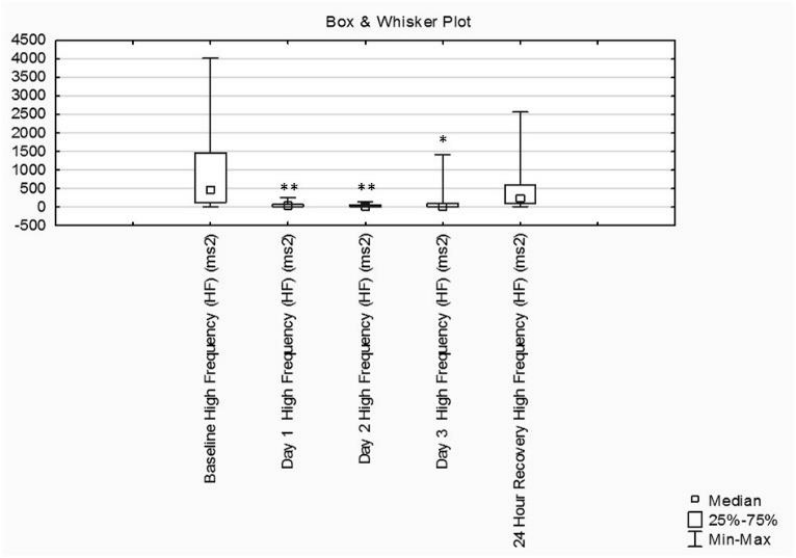

Figure 6.7 Box & Whisker Plot of the High Frequency (HF) of HRV. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

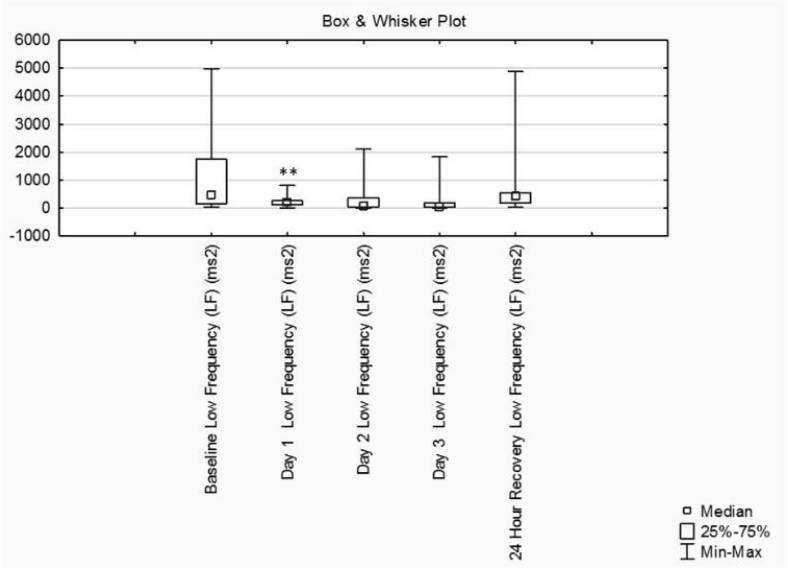

Figure 6.8 Box & Whisker Plot of the Low Frequency (LF) of HRV. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

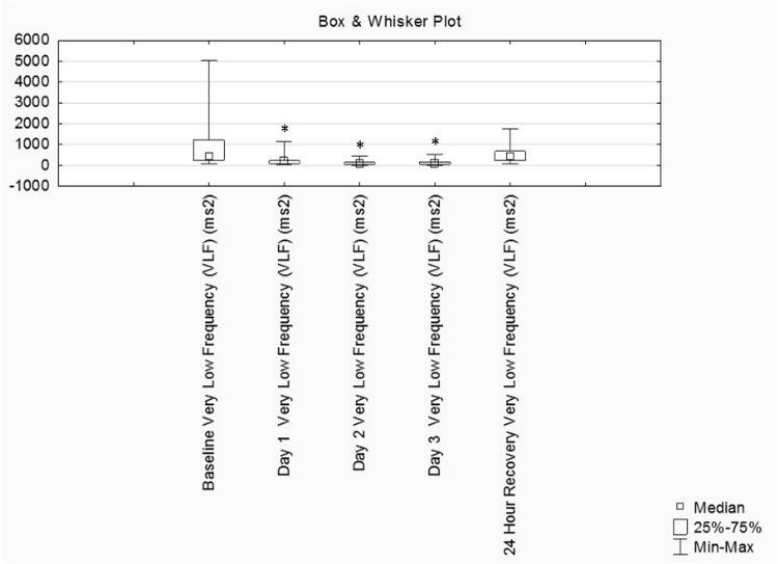

Figure 6.9 Box & Whisker Plot of the Very Low Frequency (VLF) of HRV. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

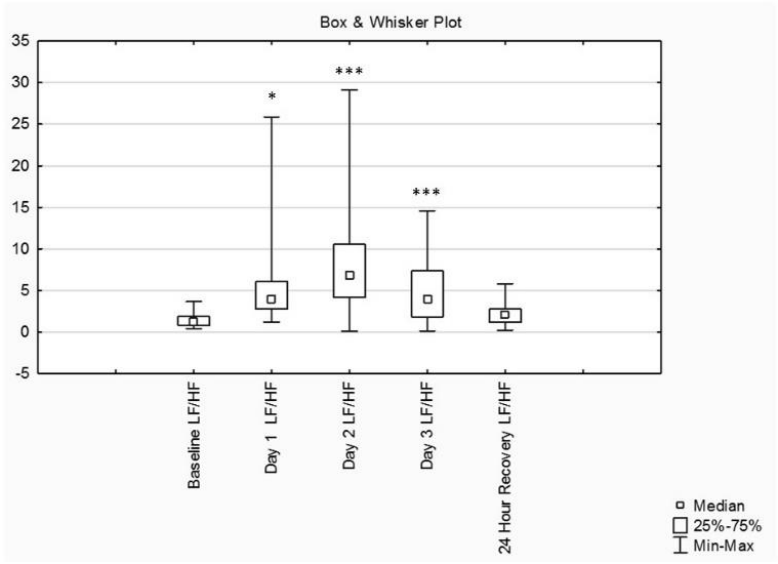

Figure 6.10 Box & Whisker Plot of the Low Frequency/High Frequency ratio (LF/HF) of HRV. Significant differences *p≤0.05, ** p≤0.01, ***p≤0.001.

CHAPTER 7

7. DISCUSSION

The main aim of this study was to investigate the effect of a single ultra-endurance event in the heart of athletes using HRV as an outcome measure and indicative of autonomic nervous system state. Little research exists in this field of the recovery dynamics of HRV after endurance exercise (Kaikkonen, et al. (2007a). In recent studies by Kaikkonen et al. (2007a) and Perkins et al. (2016), the key factors determining post-exercise HRV appeared to be the exercise intensity and exercise duration.

Limited research exists where data collection on HRV has been done during prolonged endurance events in uncontrolled environments (Earnest, et al., 2003; Mertova, et al., 2017). Most papers in the field are laboratory-based testing during controlled exercise sessions where intensity and exercise duration is pre-determined.

Therefore, the intensity and duration of the exercise play a significant role in the dynamics of HRV and environmental factors will contribute to the changes. No research exists currently regarding the effect of environmental factors within vivo studies versus laboratory-based testing during endurance exercises on HRV and needs to be researched.

7.1 Effects on Heart Rate response after an ultra-endurance event

It is known that after finishing an exercise HR decreases which reflects sympathetic withdrawal and in turn activating parasympathetic activation. (Imai, et al., 1994). In our study, it showed that heart rate did decrease once the activity come to an end.

However, the HR at post-event resting remained higher compared to that of the baseline values. Due to the work rate and accumulation of work across the three days of cycling, the post-event resting HR increased over the three days of the event. This increased post-event resting HR compared to baseline values may be due to the withdrawal of acetylcholine by the vagus nerve due to the withdrawal of the exercise stimulus, therefore leads to increased activity of the S-A node resulting in an increased heart rate at rest (Task Force of the European Society of Cardiology, 1996).

Furthermore, this increased sympathetic activity increases overall activity on the heart through the sympathetic nerves. Therefore, the sympathetic activity increases the release of synaptic transmitter noradrenaline from the sympathetic ganglions, which increase the activity on the heart increasing cardiac contractility (Aubert, et al., 2003; Makivic, et al., 2013; Sarmiento, et al., 2013; Dong, 2016; Gisselman, et al., 2016). Our findings suggest that after an ultra-endurance event there is some form of continued sympathetic stimulation and that the sympathetic drive continues well into the recovery phase (Pierpont, et al., 2000). Hence the continued tachycardia despite the parasympathetic activation (Pierpont, et al., 2000). Thus, based on the results of this study it can be deduced that there is increased cardiac contractility at rest as from after day one up to 24-hours of recovery. Therefore, athletes and healthcare professionals should be aware that the body is continuously under sympathetic dominance during rest as well as during each day of racing, meaning that each race day can be considered as a stress to the body, which in turn causes a disturbance in homeostasis and increase ANS dysfunction.

7.2 Effects on Time domain measures

As previously stated, the simplest method to evaluate HRV is through the measurement of time-domain measures during which the intervals between successive normal complexes are determined. In the present study, the measurements of the time domain measures (RR-variability, SDNN, NN50, and RMSSD) on HRV showed a significant decrease in HRV in post-event measures after day one, two and three.

Our results show a decreased vagal tone and increase in sympathetic tone at rest after each race day which significantly changed/decreased up to the final day. Two other studies reported a decrease in RR intervals, SDNN, NN50 and RMSSD during endurance events, which was indicative of an increase in sympathetic activity and decreased parasympathetic activity (Ramos-Camp, et al., 2016; Vallverdú, et al., 2017) – which is indeed in line with the results of this study.

The time-domain index of RMSSD is associated with the parasympathetic activity, which decreased slightly after day one although without any statistical significance compared to baseline. On days two and three, however, significant differences could be measured compared to baseline. The intensity (a time-trial pro-log) on day one could be considered higher compared to the other days and the duration of day one was shorter compared to day two and three. Herewith the duration factor plays a bigger role and that RMSSD is more influenced by the duration of the activity compared to the intensity of the activity (Perkins, et al., 2016; Vellverduú, et al., 2017).

By analysing the time domain measures, it reflected a decreased vagal tone and increase in sympathetic tone during post-event measures. In our study the mean HR and R-R variability was significantly ($p<0.05$, Table 6.2) affected as from day one, two and three which remind significantly up to 24-hours of recovery compared to baseline values.

7.3 Effects on Frequency domain measures

In our study, the measurements of the frequency domain measures showed a significant decrease in absolute and relative power in the different frequency bands of HRV. Knowing that frequency domain measures provides simple data of how power distributes as a function of frequency. The results of our study show a decrease in the frequency of the HRV spectrum.

Measurements of the TP of overall RR-variability reflects the overall power of the autonomic activity. It has been reported that changes in TP suggest changes in vagal modulation at rest (Task Force of the European Society of Cardiology, 1996). Our study shows a significant decrease in TP over the three days of the event. As previously discussed, the HR at post-event resting remained higher compared to that of the baseline values. Due to the work rate and accumulation of work across the three days of cycling, the post-event resting HR increased over the three days of the event. Thus, the resulting tachycardia is due to an increase in sympathetic drive resulting in a reduction in TP, leading to a significant decrease in HRV across the three days of the event.

A lot of controversies currently exists regarding the measurement of the VLF component and that the physiological explanation is much less defined, for mechanisms affecting the VLF include body temperature regulation, plasma renin

fluctuations and endothelial processes (Task Force of the European Society of Cardiology, 1996). Nonetheless, during our study, significant changes were observed after each day of the event compared to baseline but VLF returned to baseline levels after 24-hours of recovery. Low VLF power not only predicts autonomic dysfunction but also is indicative of an increase in inflammation (Lampert, et al., 2009).

This increase in inflammation is often seen due to the sympathetic response, and therefore it promotes the repairing process of exercise-induced myocardial and muscle-skeletal damage (Lampert, et al., 2009). In our study one notices the change after day two where there is a small change from sympathetic dominance towards parasympathetic change. The sympathetic system increases metabolic function to cope with challenges from outside the body, while the parasympathetic (vagal) system will start to take over to increases functions which are associated with growth and repair in the body. For example, due to an increase in sympathetic nervous system activity due to an increase in heart rate the heart rate should at a point decrease by means of an increase in vagal activity which will thereby decreasing heart rate and restoring homeostasis.

Heart rate variability is known to be a reliable measure for cardiac vagal activity and is inversely associated with levels of inflammatory markers (Cooper, et al., 2015). Cooper et al. (2015) showed that a decrease in LF to be associated with an increase in levels of C-reactive protein.

Therefore, although there was a decrease in power and the power started to plateau one can link this to the increase in the inflammation from the VLF power. During which the inflammation starts to promote the healing and repair process of exercise-induced myocardial and muscle-skeletal damage as from day two and three.

The Task Force of the European Society of Cardiology (1996) reported that over trained athletes might show a significant drop in both time domain measure (SDNN and RMSSD) and frequency domain measures (LF, HF, and TP). Our study, although showing a significant decrease in LF after day one, did not show significant changes on days two and three.

Although our study showed an overall decrease in LF, it is indicative that the sympathetic stimulation is not completely blunted but starts to recover on day two and three, but more so on day three.

The LF/HF ratios from a five-minute ECG recording in a supine position measuring greater than 2.0 is an expression of the sympatho-vagal balance and prevalence of increased sympathetic activity (Cataldo, et al., 2018). Our study showed a disturbance and significant increased sympathetic activity at rest (after day 1; 6,23±6,73, day two; 8,24±6,93, day three; 5,15±4,37) which is all depended on the stress of the event conditions. Which results in a decrease in performance and an increase in inflammation, which is, indicate by ANS dysfunction and imbalances – therefore adequate recovery is necessary to promote the repair and healing processes and improve performance. Our study showed that the greatest disturbance occurred after day two (8,24±6,93) and after day three (5,15±4,37) the ANS started to improve but remained blunted. This is indicative that although the participants were at rest their ANS had an increase in the sympathetic drive.

The HF component is mediated by the parasympathetic nervous system (Task Force of the European Society of Cardiology, 1996). A decrease in HF power in our study is indicative of decreased vagal activity and thereby reflecting an increase in an

undesirable stimulus due to the extreme event conditions and the duration and intensity of the ultra-endurance event.

Contrary to the studies of Kaikkonen et al. (2007b) and Kaikkonen et al. (2009); Perkins et al. (2015), we found a significant decrease in HF from after day one, which remained significantly decreased until after day three and at 24-hours of recovery HF recovered to baseline values.

This is indicative of a decreased vagal activity due to the increased exercise stimuli, which in our study the duration of exercise stimuli accumulatively increased and the exercise intensity during the three days varied between low to moderate to high.

Herewith through the observed changes in sympathetic and parasympathetic markers, we have shown through HRV measures that endurance exercise during extreme conditions and different exercise intensities increases stress on the autonomic nervous system. Furthermore, asides from mean HR and RR variability all other variables of HRV recovered to baseline values after 24-hours, showing that the autonomic nervous system may require more than 24-hours to fully recover HRV from a single ultra-endurance event.

7.4 Recovery time and HRV

In our study, it is shown that 24-hours did not seem to allow for adequate recovery of the ANS back to baseline values after completing an ultra-endurance event, as shown by the lack of return to baseline of the mean HR and RR variability. As described before and with evidence of other researchers (Bernardi, et al., 1996; Hautala, et al.,

2001; Gratze, et al., 2005) it is therefore shown and recommended that more than 24-hour recovery time would be more appropriate to completely asses the state of the ANS after an ultra-endurance event and for cardiac autonomic recovery.

Both Hautala et al. (2001) and Dong, (2006) have addressed the implication that may arise during persistent abnormal changes in autonomic regulation. That there is an increased risk in sudden cardiac death in athletes who present with a diminished vagal activity, which is indicated by a decrease in HRV with the variability that becomes more constant (Dong, 2016).

Hence, our study shows that after an ultra-endurance event there was a diminished vagal activity and a decrease in HRV with the variability becoming more constant. These changes only remained significantly mostly up to after day two. This may suggest that those at risk of sudden cardiac arrest/death are at the highest risk on the second day of a three-day event such as the one studied.

CHAPTER 8

8. LIMITATIONS

The main limitation of this study would be the assessment up to 24-hours post-event and not beyond; which in our study showed that the vagal activity remained blunted up to at least 24-hours after recovery. Bernardi et al. (1996); Hautala et al. (2001) and Gratze et al. (2005) showed that cardiac parasympathetic activity returned to baseline levels after 24-hours of recovery and that complete cardiac autonomic recovery can take up to 48-hours of recovery (Stanley, et al., 2013).

Secondly, it is known that there are other known parameters such as symptoms and physiological assessments that may have been done to determine recovery time after an ultra-endurance race and that HRV measure to assess the ANS alone is merely one measure that can be used but along with other known parameters would hold greater significance. Makivic & Bauer (2015) reported that a variety of parameters exist where one can measure post-exercise recovery these parameters include VO2max testing, measurements of creatine kinase, C-reactive protein, plasma cortisol, blood leukocyte and many more. However, all these methods are mostly invasive, expensive, time-consuming and unavailable to the general population.

Thirdly, the time period as to when HRV was measured for post-event measures have no relevance due to that all participants underwent a pre-reading stabilization period within two-minutes after crossing the finishing line for five-minute rest to ensure that heart rate levelled before recording HRV. Whereas the analysis of HRV has become a widely accepted method tha is non-invasive and can accurately assess the ANS modulation (Makivic & Bauer, 2015).

Lastly, there are additional factors that can be used to assess recovery after an endurance race other than assessing the ANS alone with HRV. Changes in the ANS along with peripheral fatigue, which includes general muscle and joint stiffness (subjective factors), should also be assessed to determine the appropriate recovery time. Botek et al. (2008) showed that an athlete's cardiac autonomic recovery took up to 44-hours after running a marathon and that subjective factors took 58-hours of recovery. After 67-hours of recovery, the athlete only presented with persisted fatigue and painful walking.

CHAPTER 9

9. CONCLUSION

The present study is particularly unique because the accurate HRV data obtained is the first to show the influence of a three-day day ultra-endurance mountain bike event on the ANS. This study compares baseline measures with changes across three days of racing and 24-hour recovery post-event in trained healthy athletes. During the ultra-endurance event cyclists were exposed to diverse conditions of heat, exercise intensity level, fatigue level, and terrain.

The primary findings of this study were that across the three-day event, measurements of the HRV spectrum, except LF, were significantly altered until after day three. The data are an indication that after an ultra-endurance event a withdrawal of vagal predominance occurs, shifting to sympathetic dominance.

This data also indicates that all dependant variables except for mean HR and RR variability returned to baseline value after 24-hours of recovery, therefore the ANS changes and imbalances persisted and that further recovery is needed. This recovery is needed to decrease the resting HR, blood pressure, cardiac force contraction and conduction velocity at rest, which in our study shows an increase in sympathetic activity at rest.

Our study confirmed the hypothesis that there are significant variations in HRV during the three days of competing in an ultra-endurance mountain biking event and HRV did not fully recover to baseline values within 24-hours.

CHAPTER 10

10. RECOMMENDATIONS

Our study provides the following information regarding HRV and ultra-endurance event:

- That HRV has significant value in ultra-endurance sport. It provides insight for sports practitioners, athletes, and coaches on the implication of an ultra-endurance event and the effect thereof on HRV. The data provided is supportive that 24-hours is not adequate for the full recovery of the ANS after an ultra-endurance event. Therefore, it would be wise when assessing recovery after a single ultra-endurance event that one should assess recovery beyond 48-hours.

- That HRV can be used to analyse the stress of the ultra-endurance event on the ANS, which in our study showed to be significant after day one, two and three of the event. Our study provides valuable statistics, which has a clinical significance of the impact on the ANS from an ultra-endurance event, and that the athletes did present with diminished vagal activity after each day of racing.

- Our study found significant changes in HRV parameters (decreased RMSSD, NN50, HF, TP and an increase in LH/HF) all of which reflected an increase in sympathetic activity at rest after each day of racing. This data also indicates that the mean HR and RR variability variables did not returned to baseline value after 24-hours of recovery, showing ANS dysfunction and imbalances persisted up to at least 24-hours of post-event recovery.

- The evidence from our study is that there is a continued sympathetic activity during and 24-hours after the event, leading to cardiovascular effects that require longer recovery before embarking on further exercise stress.

- The ANS affects correlating to sudden cardiac arrest suggests that healthcare and emergency personnel should be particularly aware of increased risk on day-two of an ultra-endurance event.

Therefore, we recommend making use of HRV a measuring tool to assess cardiac autonomic activity, during which sports practitioners, athletes, and coaches can assess the stress of ultra-endurance events on the ANS and plan for correct recovery strategies after a single ultra-endurance event. Furthermore, we recommend that HRV should not only be used to asses recovery but should be used as a screening tool where sports practitioners can assess the state of the ANS before an ultra-endurance event. This is because should the ANS be in a state of dysfunction, susceptible athletes might be at risk for a cardiovascular event. Further research is needed to determine how HRV measurements may be used in reducing cardiac events in individuals with an increased risk (Hautala, et al., 2001).

CHAPTER 11

11. REFERENCES

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CHAPTER 12

12. APPENDIX

Appendix 1

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Letter for Event Permission

Date 8 January 2018

Standard Bank Klein-Aus Vista MTB Challenge
Windhoek
Namibia

Attention: Mr. Piet Swiegers (Chief Race Official and Organiser)

Klein-Aus Vista CC hereby grants authority to Mr Anton Swart
(Name of Organization) (Name of Researcher/ Student)

to use the Standard Bank Klein-Aus Vista MTB three day stage event on the 3, 4th and 5th of May 2018 as a platform to conduct a research study and in so doing invite all full-marathon cyclists (Male/Female) taking part in the full-marathon event to volunteer to participate in the following Research Study:

The heart of an endurance athlete: impact of and recovery after an ultra-endurance event.

Respectfully,

Signature

Piet Swiegers (Chief Race Official)
Cell: +264 81 407 0691
info@klein-aus-vista.com
18/01/18

Signature

Mr. Anton Swart (Researcher/ Student)
Cell: +264 81 659 4708
ntnswrt@yahoo.com
19/01/18

Appendix 2

Invitation to participate in a voluntarily

Research Study

Centre for Exercise Science and Sport Medicine, School of Therapeutic Science, Faculty of Health Science, University of the Witwatersrand, Johannesburg

The evidence is that the impact of exercise leads to healthy physiological adaptations and that the majority of endurance athletes benefit from it (George, *et al.*, 2012). On the other hand, there is evidence that some endurance athletes develop a patho-physiological cascade. Athletes and clinicians should be mindful of it and react adequately to this patho-physiological phenomenon (George, *et al.*, 2012).

The aim of the study is to determine the effect of an ultra-endurance event (three-day mountain bike stage event covering a total distance of +-140km which is also highly technical and only advised for highly experienced riders) on the heart of athletes using heart rate variability (HRV) as a measuring tool.

Who is Eligible?

All riders (male and female) whom enter the three-day full-marathon event at the Standard Bank Klein-Aus Vista MTB Challenge will be able to volunteer to take part in the study.

What will you be ask to do?

Spend 5 minutes to take a Resting ECG two days before the event, immediate after the each day/stage of the event and 24-hours recovery measurement 24-hours after completing the event.

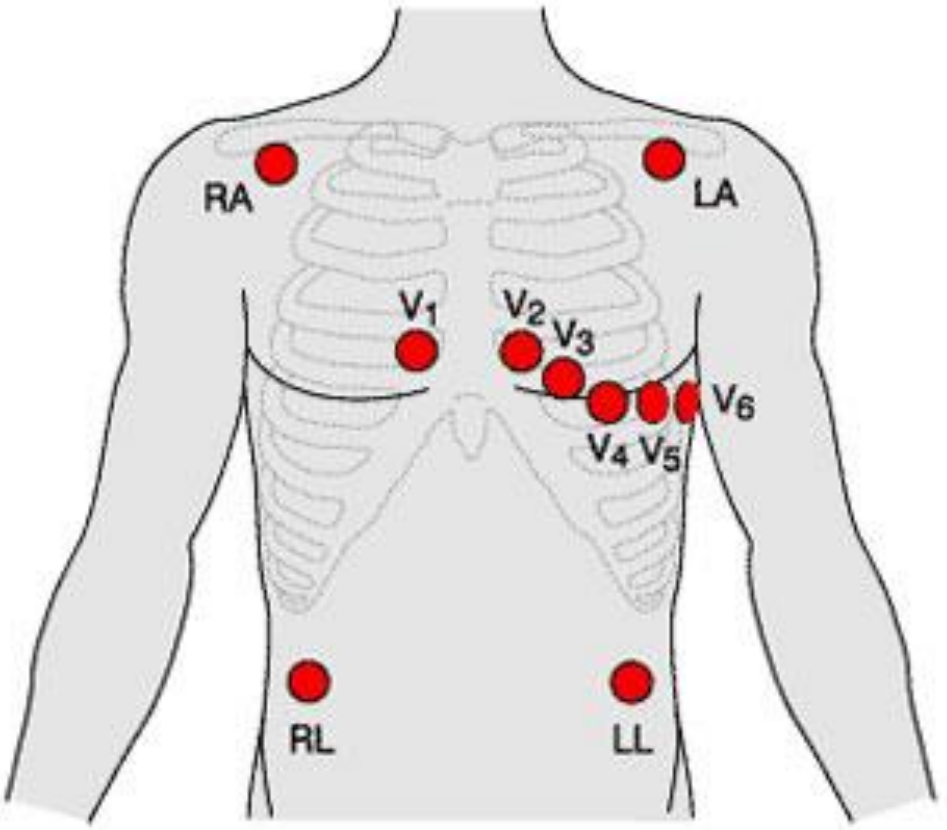
How to volunteer for participation in this study?

An information meeting will be held at Anton Swart Biokinetic Rehabilitation Practice on the 10th of April 2018 at 17:30. During which the necessary details regarding the study will be explained.

Please RSVP should you be interested in attending this meeting.
To take part in this research study or for more information, please contact Cell +264 81 650 4708 or email biopractice@iway.na

The principal researcher for this study is Mr. Anton Swart

Appendix 3



Appendix 4

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Consent Form: Use of Clinical Information for Research

A research proposal submitted to the Faculty of Health Sciences, University of the

Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree MSc Med in Biokinetics

Mr. Anton Swart

Student no. 510652

Centre for Exercise Science and Sport Medicine, School of Therapeutic Science, Faculty of Health Science, University of the Witwatersrand, Johannesburg

Supervisors: Prof Demitri Constantinou (MBBCh, BSc Med Hons, MSc Med, MPhil, FFIMS, FACSM) and Dr. Mamosilo Lichaba (MBChB, MPhil)

Johannesburg 2019

Consent Form: Use of Clinical Information for Research

Dear Participant,

You are hereby acknowledging participation in the research study "The heart of an endurance athlete: impact of and recovery after an ultra-endurance event". The nature and the purpose of this study is to assess whether there is a significant impact on the heart of endurance athletes during and after completing a three-day mountain bike event and assessing the recovery status after 24-hours of recovery after the event by using heart rate variability (HRV) as a measuring tool. Furthermore, to assess what guidelines can be used for athletes before returning to high intensity (greater than six metabolic equivalents) training after such an ultra-endurance event. The study actively involves conducting research aimed to answer the research question.

The use of such information is subject to the following:

1. Approval from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand.
2. The identity of a participant from whose file information is extracted will never be revealed to anyone but the researcher unless specific consent is obtained to do so. The information gathered does not contain the name of the patient but only a coded number in order to maintain anonymity.

We would like to obtain your consent to use information from your file for the purpose of research, subject to the above-mentioned event. If at any time, you choose to withdraw consent, you are free to do so and will not be prejudiced in any way.

Should you wish to contact us at any stage regarding consent, contact details are as follows:

Contact details of the researcher:

Anton Swart, Cell: +264 81 659 4708,

Email: ntnswrt@yahoo.com/biopractice@iway.na

The research will be supervised by:

1. Prof. Demitri Constantinou (MBBCh, BSc Med Hons, MSc Med, MPhil, FFIMS);

   Adjunct Professor: Sport and Exercise Medicine;

   Director: Centre for Exercise Science and Sport Medicine, School of Therapeutic Science, Faculty of Health Science, University of the Witwatersrand, Johannesburg; Email: Demitri.Constantinou@wits.ac.za.
2. Dr. Mamosilo Lichaba (MBChB, MPhil); Lecturer at Centre for Exercise Science and Sport Medicine, School of Therapeutic Science, Faculty of Health Science,

University of the Witwatersrand, Johannesburg; Sports Physician; Email: mamosilo.lichaba@wits.ac.za.

Should any participant have any queries, concerns or complaints regarding the ethical activities surrounding the study, the chair of the Wits Human Research Ethics Committee (Medical) can be contacted. Chairperson, Prof P. Cleaton Jones, Tel +27 (011) 717 2301, email peter.cleaton-jones1@wits.ac.za. Administrators, Ms Z Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng, Tel +27 (011) 717 2700/2656/1234/1252, email zanele.ndlovu@wits.ac.za; Rhulani.mkansi@wits.ac.za; and Lebo.moeng@wits.ac.za.

Consent Given

I_____ confirm I have read and understood the information on the study and have had the opportunity for any questions to be answered by the researcher. I hereby give consent to participate in the study.

PARTICIPANT: _____ DATE: _____

Appendix 5

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Participant information sheet

The heart of an endurance athlete: impact of and recovery after an ultra-endurance event.

A research proposal submitted to the Faculty of Health Sciences, University of the

Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree MSc Med in Biokinetics

Mr. Anton Swart

Student no. 510652

Centre for Exercise Science and Sport Medicine, School of Therapeutic Science, Faculty of Health Science, University of the Witwatersrand, Johannesburg

Supervisors: Prof Demitri Constantinou (MBBCh, BSc Med Hons, MSc Med, MPhil, FFIMS, FACSM) and Dr. Mamosilo Lichaba (MBChB, MPhil)

Johannesburg 2019

Participant information sheet

Title of Study: "The heart of an endurance athlete: impact of and recovery after an ultra-endurance event".

Introduction:

My name is Anton Swart. I am post-graduate student at the University of Witwatersrand at the Centre for Exercise Science and Sport Medicine, School of Therapeutic Science and Faculty of Health Science. Research is the process to learn the answer to a question and this research task is in partial fulfilment of the requirements for the degree MSc Med in Biokinetics. During the study, the goal is to assess whether there is a significant impact on the heart of endurance athletes during an ultra-endurance mountain bike event.

Invitation to participate:

I would like to invite you to participate in the following study, "The heart of an endurance athlete: impact of and recovery after an ultra-endurance event". The nature and the purpose of this study is to assess whether there is a significant impact on the heart of endurance athletes during and after completing a three-day mountain bike event and assessing the recovery status after 24-hours of recovery after the event by using heart rate variability (HRV) as a measuring tool. Furthermore, to assess what guidelines can be used for athletes that are returning to high intensity training after such an ultra-endurance event.

Thus, the potential use of the results from this study would be to advise endurance athletes on the impact of an ultra-endurance event on the heart and the recovery needed after an ultra-endurance event.

What is involved in participating in the study?

Study Design:

Data collection will be done in Namibia during the Standard Bank Klein-Aus Vista MTB Challenge during the full marathon event, which is a three-day mountain bike stage race which is considered highly technical and only advised for highly experienced riders. The event takes place in Aus, which is a small town in the South of Namibia in the desert. Anecdotally the event is seen as the toughest three-day stage event in Namibia. The event starts on the 3rd of May and continues the 4th and 5th May.

What are required from the participants?

- A resting Electrocardiography (ECG) on each participant in the supine position (flat on one's back) for five minutes to provide baseline (pre-event) values. Baseline testing will take place at Anton Swart Biokinetic Rehabilitation Practice in Windhoek two days prior to the event.
- Post-event measure will be taken after each stage/day, which starts on day one to day three. After the participants have completed each stage/day, a resting ECG on each participant provides post-event measures. Participants will undergo a pre-reading stabilization period of five minutes before each recording to ensure that

heart rate levelled out before recording HRV. Participants should move to the communicated area once crossing the finishing line for the post-event measurement procedure.

- A 24-hour recovery measurement will take place at Klein-Aus Vista Lodge the day after the participants have completed the three-day stage race. Usually the participants do not travel back to their destination on the same day of the race due to the great distance that needs to be covered by car and due to that the participant has just completed a three-day ultra-endurance mountain bike event.

Thus, all participants are expected to be involved in the study over a period of 6 days (starting two days before the event and ending the day after finishing the event). Participants will experience no discomfort or pain during these measurements.

Risks/Benefits for participating in the study:

There are no risks what so ever nor benefits for any participant during the testing procedures and measurements.

Participation:

Participants:

- May choose to withdraw from the study at any stage at no risk, penalty, loss of benefit or care to which the participant is otherwise entitled;
- Will not receive any compensation for participating in this study.

Confidentiality/ Anonymity:

For the duration of retention of records, each participant's health records and measurements will be kept in accordance with the guidelines of the Health Professional Council of South Africa (HPCSA) and Health Professional Council of Namibia (HPCNA). All hard copy records will be stored in a safe place with electronic copies uploaded to online cloud storage, safeguarded by a password. All records and measurements will be stored for a period of not less than six years and only the researcher will have access to these records. Indirect identifiers (Numbers) replace all obvious identifiers (Name and Surnames) of participants in the main data set to retain confidentiality. A separate data set known as the "Key" will hold the indirect identifiers with the obvious identifiers.

Contact Details:

Contact details of the researcher are as follows:

Anton Swart, Cell +264 81 659 4708,

Email: ntnswrt@yahoo.com/biopractice@iway.na

The research will be supervised by:

Prof. Demitri Constantinou (MBBCh, BSc Med Hons, MSc Med, MPhil, FFIMS);
Adjunct Professor: Sport and Exercise Medicine;
Director: Centre for Exercise Science and Sport Medicine, School of Therapeutic Science, Faculty of Health Science, University of the Witwatersrand, Johannesburg;
Email: Demitri.Constantinou@wits.ac.za.

Dr. Mamosilo Lichaba (MBChB, MPhil); Lecturer at Centre for Exercise Science and Sport Medicine, School of Therapeutic Science, Faculty of Health Science, University of the Witwatersrand, Johannesburg; Sports Physician;
Email: mamosilo.lichaba@wits.ac.za.

Participants may contact the researcher or supervisors regarding any queries relating to the study at any point in time.

Should any participant have any queries, concerns or complaints regarding the ethical activities surrounding the study, the chair of the Wits Human Research Ethics Committee (Medical) can be contacted. Chairperson, Prof P. Cleaton Jones, Tel +27 (011) 717 2301, email peter.cleaton-jones1@wits.ac.za. Administrators, Ms Z Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng, Tel +27 (011) 717 2700/2656/1234/1252, email zanele.ndlovu@wits.ac.za; Rhulani.mkansi@wits.ac.za; and Lebo.moeng@wits.ac.za. Or the chair of the Biomedical Research Ethics Committee (BREC) and Research Management Committee (RMC) of Namibia can

be contacted. Chairperson, Dr. H. Nangombe, Tel +264 612032562, email hnangombe@gmail.com / hnangombe@mhss.gov.na.

Thank you for taking the time to read this information sheet. Your assistance and participation will be highly appreciated and can make a meaningful contribution to future research.

Kind Regards,

Mr. Anton Swart

Post-graduate student

MSc Med in Biokinetics

Email: ntnswrt@yahoo.com / biopractice@iway.na

Cell: +264 81 659 4708

Appendix 6

REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198
Windhoek
Namibia

Ministerial Building
Harvey Street
Windhoek

Tel: 061 – 2032537
Fax: 061 – 222558
Email: shimenghipangelwa71@gmail.com

OFFICE OF THE PERMANENT SECRETARY

Ref: 18/3/3 AS
Enquiries: Mr. J. Nghipangelwa

Date 14/02/ 2018

Mr. Anton Swart
University of Witwatersrand
Johannesburg
South Africa

Dear Mr. Swart

RE: The heart endurance athlete: Impact of and recovery after an ultra-endurance event

Reference is made to your application to conduct the above-mentioned study.

1. The proposal has been evaluated and found to have merit.

2. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**

3.1 The data to be collected must only be used for academic purposes;

3.2 No other data should be collected other than the data stated in the proposal;

3.3 Stipulated ethical considerations in the protocol related to the protection of Human Subjects' should be observed and adhered to, any violation thereof will lead to termination of the study at any stage;

3.4 A quarterly report to be submitted to the Ministry's Research Unit;

3.5 Preliminary findings to be submitted upon completion of the study;

3.6 Final report to be submitted upon completion of the study;

3.7 Separate permission should be sought from the Ministry of Health and Social Services for the publication of the findings.

Yours sincerely,

PERMANENT SECRETARY
MINISTRY OF HEALTH & SOCIAL SERVICES

Ms. P Masabane
Acting Permanent Secretary

Appendix 7

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Mr Anton Swart et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M171037

NAME: (Principal Investigator)	Mr Anton Swart et al
DEPARTMENT:	School of Therapeutic Science Centre for Exercise Science and Sports Medicine Anton Swart Biokinetic Rehabilitation Practice, Windhoek - Namibia
PROJECT TITLE:	The Heart of an Endurance Athlete: Impact of and Recovery after an Ultra-Endurance Event
DATE CONSIDERED:	27/10/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Demitri Constantinou
APPROVED BY:	Prof C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	20/04/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in October and will therefore be due in the month of October each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

21/04/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES