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FIVE MINUTE RECORDINGS OF HEART RATE VARIABILITY IN PHYSICALLY ACTIVE STUDENTS: RELIABILITY AND GENDER CHARACTERISTICS

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ABSTRACT

Introduction

Heart rate variability (HRV) is regarded as a useful, non-invasive method for investigating the autonomic nervous system (ANS). Over the past decade there has been an increase in the number of HRV investigations in the disciplines of medical, sport and exercise science. Despite the extensive use of HRV in investigations of ANS functioning, there are questions relating to the reliability of the technique. Therefore, HRV reliability studies for different population groups have been advocated. Furthermore, research on gender differences in HRV is contradictory. This has resulted in the need to investigate gender characteristics in HRV.

Objectives

The objective of this study was to evaluate the reliability of short-term (5min) recordings of HRV, and to determine the association between HRV and gender.

Methods

Forty four physically active students (n= 21, age= 21.17 (1.55) males and n=23, age= 19.75 (1.76) females) participated in the study. Heart rate variability parameters were determined from five minute recording of interbeat intervals (IBI) using a Suunto t6 heart rate monitor (HRM). Testing was repeated over 4 consecutive days under the same conditions. The following HRV time and frequency domain measures were calculated using Kubios HRV Software Version 2.0: mean heart rate (HR), standard deviation of normal to normal intervals (SDNN), root mean square of successive differences (RMSSD), percentage of beats that changed more than 50 ms from the previous beat (pNN50), low frequency in normalized units (LFnu), high frequency in normalized units (HFnu) and low frequency to high frequency ratio in normalized units (LF/HFnu ratio). The data was summarized using routine descriptive statistics. Relative reliability was calculated using interclass correlation coefficients (ICC) (ICC of >0.80 indicated good to excellent reliability) and absolute reliability using typical error of measurement (TEM) and TEM as a percentage of the mean score (TEM%). This statistical measures were computed for days 2 vs 3 (REL 1), 3 vs 4 (REL 2). Day 1 was used as a familiarization day. An unpaired T-test was used to determine whether there

were any differences between males and females for the above HRV parameters. Significance was set at $p \leq 0.05$.

Results

The ICCs for both REL 1 and REL 2 indicated good to excellent (ICC >0.8) reliability for IBIs and pNN50 for the time domain results. In general, the time domain results had a higher relative reliability than the frequency domain results. Males had an overall lower relative reliability than females for frequency domain parameters. Absolute reliability for REL 2 showed a slightly lower TEM value as compared to REL 1.The largest gender differences in TEM were seen in the frequency domain parameters. Specifically, for males, the TEM was higher than females for the LF/HFnu ratio (REL 2: 116%), the HFnu (REL 1: 90%) and the LFnu (REL 1: 68%). Overall the TEM% was relatively high in most HRV parameters specifically for LF/HFnu (REL 1: 31.4% females and 48.1% males; REL 2: 29.7% females and 40.4% males). These findings indicate that males have decreased absolute reliability compared to females and that random error is greater in men for the frequency domain parameters. Gender differences illustrated significant differences for resting HR (16% higher in females ($p < 0.0001$)), IBIs (21% higher in females ($p <0.0001$)) and LF/HFnu ratio (41% higher in males ($p = 0.003$)). The findings indicate that females have higher total HRV.

Conclusions

Short term recordings of HRV over consecutive days using the Suunto t6 HRM and Kubios custom HRV software are reliable depending on the HRV parameter being analysed. Overall, the relative reliability results suggest that HRV using the Suunto t6 and Kubios is good. However, the absolute reliability results suggest low reliability. In particular, males demonstrated a poorer absolute reliability (high TEM and TEM%) than females, suggesting a larger day to day random error in males. Furthermore, specific HRV measures differed between males and females demonstrating that females have higher parasympathetic modulation compared to men. The overall higher HRV in females could explain the possible cardio-protective mechanism observed in premenopausal women.

Key words: Heart rate variability, Parasympathetic, Reliability, Interbeat Intervals

DECLARATION

I, Takshita Sookan, declare that the work on which this project is based is original and my own work (except where acknowledgements indicate to the contrary) and that neither the whole work nor part thereof has been, is presently, or is to be submitted for another degree at this or any other university.

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Takshita Sookan

Durban

May 2011

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LIST OF ABBREVIATIONS

1. Autonomic Nervous System – ANS
2. Sympathetic Nervous System - SNS
3. Parasympathetic Nervous System – PNS
4. Heart Rate - HR
5. Cardiovascular – CV
6. Heart Rate Variability – HRV
7. Electrocardiagram – ECG
8. Interbeat-Intervals - IBI
9. Coronary Heart Disease - CHD
10. Sinoatrial Node – SA node
11. Cardiovascular Disease – CVD
12. Blood Pressure – BP
13. Standard Deviation of NN all Normal RR Intervals – SDNN
14. Percentage of adjacent RR intervals that varied by more than 50ms - pNN50)
15. The root mean square of the difference between the coupling intervals of adjacent RR intervals – RMSSD
16. Mean Heart Rate – Mean HR
17. Resting Heart Rate - RHR
18. Triangular Interpolation of NN – TINN
19. Power Spectral Density – PSD
20. Very Low Frequency – VLF
21. Low Frequency – LF
22. High Frequency – HF
23. Low Frequency to High Frequency ratio – LF/HF ratio
24. Normalized Units – n.u.
25. Hertz - Hz
26. Absolute Values of Power - ms^2
27. Low Frequency in Normalized Units – LFnu
28. High Frequency in Normalized Units – Hfnu
29. Low Frequency to High Frequency Ratio in Normalized Units – LF/HFnu ratio
30. Respiratory Sinus Arrhythmia - RSA
31. Body Mass Index – BMI

32. International Physical Activity Questionaire – IPAQ
33. Kilo Calories – Kcal
34. Heart Rate Monitor - HRM
35. Human Performance Laboratory - HPL
36. Autoregressive Model – AR model
37. Typical Error of Measurement – TEM
38. Typical Error of Measurement Percentage – TEM%
39. Inter Class Correlations – ICC
40. Confidence Intervals – CI
41. Day 2 versus day 3 - REL 1
42. Day 3 versus day 4 - REL 2

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INTRODUCTION

The autonomic nervous system (ANS) governs many of the body's internal functions, through its two pathways, the sympathetic (SNS) and parasympathetic (PNS) nervous systems. The cardiovascular (CV) system, in particular the heart rate (HR), is mostly regulated by the ANS via the activity of these two pathways. (Borresen and Lambert, 2008) and (Aubert et al., 2003) acknowledge that the analysis of heart rate variability (HRV) provides information about the functioning of the ANS.

A number of different tools have been used to measure and monitor the CV system; they include electrocardiography (ECG), echocardiography, and HRV. The first two tools show electrical and structural changes, whilst HRV is a measure of the time intervals or interbeat intervals (IBIs) between each QRS complex (Berkoff et al., 2007). Heart rate variability may most simply be described as the frequency with which the IBIs changes in a set period of time (Tarvainen and Niskanen, 2008). The analysis is divided into time and frequency domains (Aubert et al., 2003). Changes in the domains of HRV reflect changes in autonomic modulation of the heart both within a single recording or over time (Nagai and Moritani, 2004).

The data obtained from the analysis of HRV provides information about the about ANS functioning, which has relevance for the understanding of physical, emotional, and mental functioning (Pinna et al., 2007) . High HRV is an adaptive quality in a healthy body (Algra et al., 1993), whilst low HRV has been linked to a number of disease states including coronary heart disease (CHD), diabetes (Keenan and Grossamn, 2006) and depression (Earnest et al., 2004) as well as overtraining and poor recovery in athletes (Seiler et al., 2007).

In a variety of circumstances, increased HRV is associated with lower mortality rate (Nagai and Moritani, 2004). It has been demonstrated that trained individuals have a higher HRV than sedentary individuals, suggesting that exercise training can increase

HRV in normal populations (Nagai and Moritani, 2004). The use of HRV to measure and monitor the impact of training, recovery, health and other related physiological events has the potential to improve health and performance (Sinnreich et al., 1998, Berkoff et al., 2007).

Scientific study of the variability in HR is fairly new. In recent years, the use of HRV as a clinical tool has increased and so too has research pertaining to its reliability (Pinna et al., 2007). Although such a broad use of this methodology would assume that the reliability of HRV measurements has been thoroughly evaluated, the evaluation has often been inadequate (Sandercock et al., 2007). Therefore, despite its extensive use in physiological and clinical research, the analysis of HRV is still poorly supported by thorough reliability studies (Pinna et al., 2007). Furthermore, gender characteristics discussed in the literature of HRV are contentious. Most of the research that includes differences between males and females has been inadvertent (Dietrich et al., 2006, Kristal-Boneh et al., 2002). The research on gender differences indicated that females may have a cardio-protective mechanism, which suggests that women would have a higher parasympathetic tone compared to males (Ramaekers et al., 1998). There is currently limited research worldwide and in South Africa relating to the reliability and gender aspects of HRV**.**

The aim of this study was therefore to evaluate the reliability of short-term recordings of HRV and the association between HRV parameters and gender.

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

An individual's adaptation to psychological and physical stressors is met in part by the autonomic nervous system's (ANS) control of the body's cardiovascular (CV) system (Tortora and Grabowski, 2000). The effect of the ANS can be understood through the measurement of heart rate variability (HRV). To determine whether HRV is a viable marker of ANS functioning, it is important that validity and reliability of the HRV parameters are established (Taskforce, 1996). Currently, there is limited research available examining the reliability of HRV measurements (Umetani et al., 1998), (Ramaekers et al., 1998, Pinna et al., 2007, Nunan et al., 2009, Sinnreich et al., 1998). In addition, gender influences HRV but research on this aspect is controversial (Sinnreich et al., 1998, Umetani et al., 1998, Ramaekers et al., 1998). This review will firstly, examine the physiological mechanisms underpinning the concept of HRV. Secondly, it will examine current HRV research, including its use in health and athletic/sport performance settings and the influence of gender and ageing on HRV. Finally, it will discuss the validity and reliability studies that have been performed in the area.

1.2 Autonomic Nervous System and Heart Rate Variability

The ANS is portrayed as the neurological 'pacemaker' of the CV system as it acts on the Sino-Atrial Node (SA Node) of the heart (Opie, 1991)**.** The CV system is mostly controlled by ANS regulation through the activity of the sympathetic (adrenergic) and parasympathetic (vagal or cholinergic) pathways. The sympathetic branch of the ANS augments HR while the parasympathetic branch decreases HR. The vagus nerve plays a role in mediating the parasympathetic pathway through the release of acetylcholine. The effect of any vagal impulse is brief because the acetylcholine is rapidly hydrolysed (Faulkner et al., 2003)**.** Acetylcholine results in slowing of both the activity at the SA node and cardiac impulses, which ultimately reduces HR. The SNS exerts its influence by releasing the neurotransmitter norepinephrine at the sympathetic nerve endings. This stimulation increases the rate of SA node discharge and accelerates atrioventricular

conduction of impulses (Lopes and White, 2006). Under resting conditions, in healthy individuals, parasympathetic tone overrides sympathetic tone (Lopes and White, 2006). Parasympathetic influences exceed sympathetic effects probably via two independent mechanisms: a cholinergically induced decline of norepinephrine released in response to sympathetic activity, and a cholinergic attenuation of the response to an adrenergic stimulus. The influence and balance that these two branches of the ANS exert on the heart has been termed the sympathovagal balance (Keenan and Grossamn, 2006). The measurement of HRV has been used as a non-invasive tool to examine sympathovagal balance (Sandercock et al., 2005).

1.3 Definition of Heart Rate Variability

Heart rate variability depicts the modulation of the CV system by the ANS and other physiological regulatory systems (Tortora and Grabowski, 2000). The numerous measures of HRV reflect the fluctuations or variability of the changes in the interval, or time between R-waves during a specified time of HR recording (Lopes and White, 2006). These R-wave time intervals are called RR intervals or interbeat intervals (IBIs) and are measured in milliseconds (Algra et al., 1993) (Figure 1.1). Breathing or respiration plays an important role in HRV. In a controlled atmosphere in which respiration is accounted for, IBIs lengthen with expiration and shorten with inspiration (Lopes and White, 2006).

Multiple biological rhythms overlay one another to produce the resultant pattern of HRV. Heart rate variability has relevance for understanding the effect of acute and chronic stressors on physical, emotional, and mental functioning (Taskforce, 1996). Increased variability in HR IBIs is an adaptive quality of a healthy body (Algra et al., 1993). Lower HRV has been reportedly linked to increased risk of cardiovascular disease (CVD) (Moodithaya and Avadhany, 2009). This decrease in variability has been proposed as a shifting of cardiac autonomic balance towards sympathetic dominance, compared to parasympathetic activity which prevails under normal, healthy, resting conditions (Moodithaya and Avadhany, 2009). Sympathetic hyperactivity has been linked to the development of atherosclerosis, CV hypertrophy, cardiac arrhythmia and sudden cardiac death (Earnest et al., 2008).

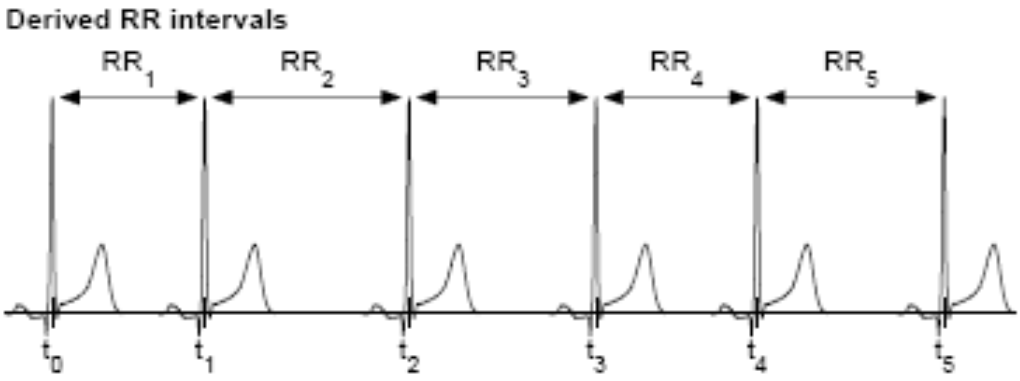

Figure 1.1: Diagram depicting RR Intervals (Tarvainen et al., 2008).

1.4 Mechanisms Regulating Heart Rate Variability

1.4.1 The Cardiovascular Neural Regulation Model

The regulation of the heart is under the control of the medulla oblongata (brain stem) which is in the CV centre. This CV centre has the ability to influence and direct suitable output by increasing or decreasing the frequency of nerve impulses via the two branches of the ANS (Tortora and Grabowski, 2000).

The neural regulation of circulatory functions is principally accomplished through the excitatory influences of sympathetic and parasympathetic outflows, which are modulated by at least three major factors: 1) central integration, 2) peripheral inhibitory baroreceptor vagal reflex mechanisms (with negative feedback characteristics), and 3) peripheral inhibitory excitatory sympathetic reflex mechanisms (with positive feedback characteristics) (Taskforce, 1996).

Figure 1.1 provides a schematic representation of the Cardiovascular Neural Regulation Model. From the figure it can be seen that opposite feed back mechanisms, originating in the brain, have an affect on the neural control of the CV system. The regulation of blood pressure (BP) controls the negative feed back mechanisms that results in the HRV

pattern (Lopes and White, 2006). Baroreceptors monitor changes in pressure and stretch in the walls of the blood vessels. Even if BP is normal, the distribution of blood flow may require adjustment. The baroreceptors detect an increase in BP which results in an increased parasympathetic stimulation via the vagus nerve and a decrease in sympathetic stimulation. This outcome decreases HR. Baroreceptor and vagal afferent fibers from the cardiopulmonary region mediate negative feedback mechanisms (exciting the vagal and inhibiting the sympathetic outflow). Conversely if BP rises, the baroreceptors are stretched less and send impulses at a decreased rate to the medulla oblongata which results in decreased parasympathetic activity and increased sympathetic activity. Sympathetic afferent fibers mediate positive feedback mechanisms (exciting the sympathetic and inhibiting the vagal flow) (Lopes and White, 2006, (Taskforce, 1996, Tortora and Grabowski, 2000).

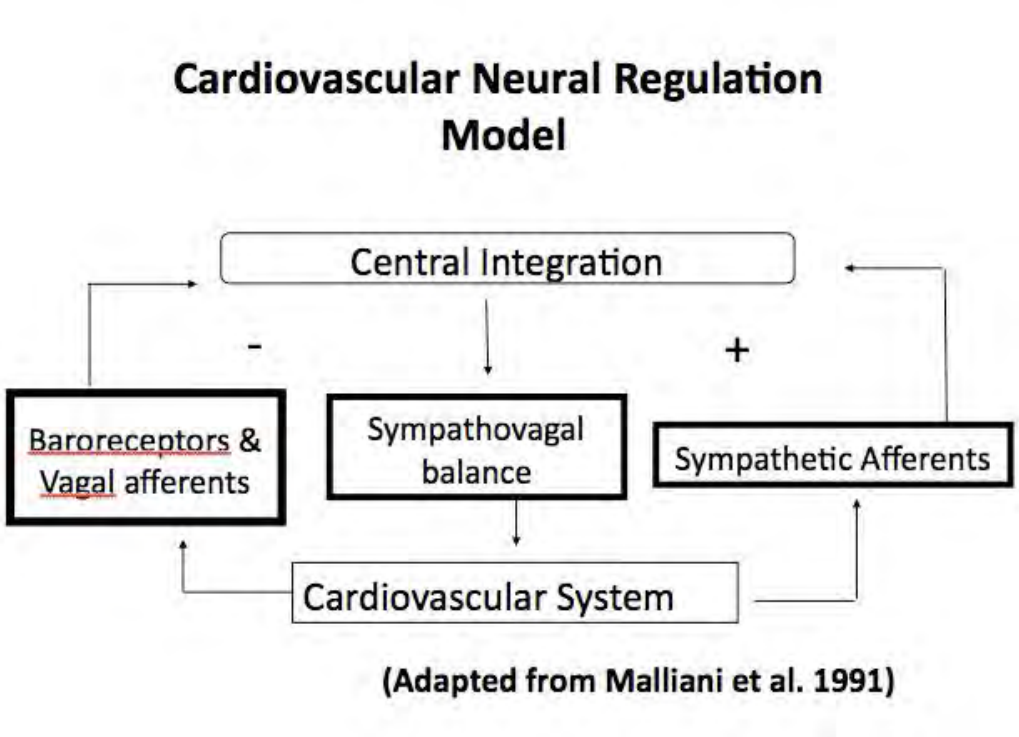

Figure 1.2: Diagrammatic Representation of Autonomic Regulation of the Heart (Lopes and White, 2006).

1.5 Heart Rate Variability Frequencies and Parameters

Variations in HR may be evaluated by a number of methods including time domain (statistical measures), geometric methods, frequency domain (spectral analysis) and nonlinear methods (Roy et al., 2009). Heart rate variability time domain parameters include the following: 1) standard deviation of NN (normal to normal) or normal IBIs (SDNN), 2) percentage of adjacent RR intervals that varied by more than 50ms (pNN50), and 3) the root mean square of the difference between the coupling intervals of adjacent IBIs (RMSSD) (Taskforce, 1996, Lopes and White, 2006). These may be divided into two classes, (a) those derived from direct measurements of the IBIs or instantaneous HR (e.g. SDNN), and (b) those derived from the differences between IBIs (e.g. RMSSD) (Taskforce, 1996).

Geometric methods are a result of series of NN intervals which are converted into geometric patterns, such as RR triangular index and triangular interpolation of NN (TINN) (Lopes and White, 2006). The major advantage of geometric methods lies in its comparative insensitivity to the investigative quality of the series of the NN intervals (Taskforce, 1996).

Spectral analysis or frequency domain methods for the study of tachograms have been used for many years. Power spectral density (PSD) examination provides an understanding of sympathetic and parasympathetic effects on HRV (Lopes and White, 2006) and presents basic information of how power distributes as a function of frequency (Taskforce, 1996). The advantage of the frequency domain measure over time domain is that the frequency measures provide more data and explanation of the contributions of sympathetic and parasympathetic activity (Lopes and White, 2006).

Heart rate variability is frequently estimated by means of spectral analysis in which power in three or four frequency bands is determined (Keenan and Grossamn, 2006). Three main spectral components are distinguished in a spectrum calculated from short term recordings of 2 to 5 minutes: very low frequency (VLF), low frequency (LF), and

high frequency (HF) components. The distribution of power and the central frequency of LF and HF are not fixed but may vary in relation to changes in autonomic modulations of the heart period. The physiological explanation of the VLF component is much less defined and the existence of a specific physiological process attributable to these heart period changes might even be questioned. VLF assessed from short-term recordings (e.g. <5 min) is an uncertain measure and should be avoided when interpreting the PSD of short-term ECGs (Taskforce, 1996).

Measurement of VLF, LF and HF power components is usually made in absolute values of power (ms^2), but LF and HF may also be measured in normalized units (n.u.) which represent the relative value of each power component in proportion to the total power minus the VLF component (Roy et al., 2009). The representation of LF and HF in n.u. emphasizes the controlled and balanced behavior of the two branches of the ANS. Moreover, normalization tends to minimize the effect on the values of LF and HF components of the changes in total power (Taskforce, 1996). Heart rate recordings of approximately 1 min are needed to assess the HF components of HRV while approximately 2 min are needed to address the LF component. In order to standardize different studies investigating short-term HRV, 5 minute recordings of a stationary system are preferred unless the nature of the study dictates another design (Taskforce, 1996).

Power spectral density provides basic information of how power or variance distributes as a function of frequency (Taskforce, 1996). Psychophysiological research suggests that the HRV frequency ranges reflect different biological influences (Lopes and White, 2006). The LF component has been proposed as reflecting both sympathetic and parasympathetic effects on the heart and generally occurs in a band between 0.04 Hz and 0.15 Hz. However, researchers noted that the LF peak is influenced by the baroreceptors mediated regulation of BP and reflects predominantly sympathetic activity (Moodithaya and Avadhany, 2009). The influence of vagal efferent modulation of the SA node can be seen in the HF band, loosely defined between 0.15 and 0.4 Hz and known as respiratory sinus arrhythmia (RSA) because it occurs at the respiratory frequency (Lopes and White, 2006). The ratio of power in the LF and HF components

(LF/HF ratio) has been used to provide an estimate of cardiac sympathovagal balance and can assess the fractional distribution of power (Aubert et al., 2003, Keenan and Grossamn, 2006). Chemoreceptor processes, thermoregulation, and the rennin-angiotensin system have been tied to VLF (Keenan and Grossamn, 2006). The VLF range is associated with sympathetic activation (Lopes and White, 2006).

Nonlinear analysis of HRV is a technique that makes fundamentally different assumptions about the data as compared to time and frequency domain analysis. These methods assume that much of the behaviour is irregular but still not random. They are not designed to assess the magnitude of variability but rather they estimate the correlation of properties and the complexity of HRV. Researchers use this analysis to investigate rapid dynamic changes (i.e. during exercise, hypertension) in ANS (Lopes and White, 2006). There are many techniques of nonlinear analysis but in the field of sport and exercise the Poincar´e plot is widely used. It provides a graphical depiction of CV dynamics which is in an elliptical shape. It is a scattergram in which each IBI is plotted as a function of the previous IBI. For example a healthy young individual at rest would demonstrate high HRV and this would be seen by a big ellipse as compared to an older individual who would have reduced HRV and therefore a reduced ellipse (Lopes and White, 2006).

1.6 Heart Rate Variability and Health

1.6.1 Cardiovascular Disease

Experimental evidence for a connection between a predisposition for lethal arrhythmias and signs of either reduced vagal activity or increased sympathetic activity encouraged the development of quantitative markers of autonomic activity (Taskforce, 1996). Heart rate variability measurements have been validated and represent one of the most promising markers of ANS function (Algra et al., 1993, Nunan et al., 2009).

The clinical relevance of HRV was first appreciated in 1965 when Hon and Lee noted that fetal distress was preceded by alterations in IBIs before any appreciable change

occurred in the HR itself (Taskforce, 1996). Research over the past few decades has demonstrated a significant relationship between the ANS and CV mortality, including sudden cardiac death (Taskforce, 1996) with HRV, and the quantification of its spectral components, being a predictor of CV morbidity and mortality (Kristal-Boneh et al., 2002).

After adjusting for known risk factors, reduced HRV was associated with progressing age and with an increased risk of cardiac events in clinically disease-free patients (Nagai and Moritani, 2004a). Lower HRV was proven to be associated with a greater risk for developing hypertension among normotensive men, and hypertension is one of the major risk factors of CHD. Acute myocardial infraction is accompanied by a decreased HRV, which is due to reduced vagal or increased sympathetic outflow to the heart (Lui et al., 2003). Reduced HRV in short-term recordings (2 hours) was shown to predict new cardiac events, hypertension and hyperglycaemia, in a middle-aged general population (Dietrich et al., 2006). Dietrich et al (2006) found associations between social position, behavioural factors, components of the metabolic syndrome, and HRV. In contrast, lower mortality rate is associated with increased HRV (Nagai and Moritani, 2004).

1.6.2 Obesity and Diabetes

Decreased values of HRV has been shown to be indirectly proportional to BP, body mass index (BMI) and hyperinsulinemia in obese adults and young patients without clinical symptoms of CVD, diabetes or damage of the target organ (Keenan and Grossamn, 2006).

Autonomic nervous system dysfunction has been documented in obese adults but there is limited information relating to ANS dysfunction in obese children. In one study, that investigated ANS function in childhood (7±13 years of age) obesity, there was normal activity of the SNS, and hypoactivity of the PNS. The authors concluded that PNS dysfunction is a risk factor in childhood obesity (Yakincia et al., 2000). Furthermore,

Nagai et al. (2004) showed that obese children possess decreased SNS activity as well as PNS activity compared to lean children who have similar physical activity levels. This ANS activity reduction, associated with the amount of body fat in inactive state, could be an etiological factor for the onset or development of childhood obesity. Regular physical activity has also been shown to have a favorable influence on parasympathetic activity (Gutin et al., 2000). Overall ANS activity in both lean and obese children has been shown to be improved with physical activity. The findings of Nagai et al. (2004) demonstrated that regular physical activity might be effective in preventing and treating obesity associated ANS dysfunction in children.

Research has suggested that factors such as physical activity level, may lead to a different profile of sympathovagal activity in obese individuals. The findings of several cross-sectional and interventional research studies have shown that increased physical activity is associated with higher levels of overall ANS activity in adults (Nagai and Moritani, 2004).

1.6.3 Mental Health, Stress and Depression

Studies have also shown that clinical depression may be linked to decreased HRV (Earnest et al., 2008). Heart rate variability measurements can also be sensitive to stress and can be seen to decrease with age, which has been attributed to a decrease in efferent vagal tone (Keenan and Grossamn, 2006). Effective emotional regulation depends on being able to flexibly adjust your physiological response to a changing environment. Increased stress has been linked to a greater risk of developing chronic diseases (Tortora and Grabowski, 2000). The body's stress response includes an increase HR which is a result of increased sympathetic activity (Taskforce, 1996). This stress causes increased hormone release, which enhances cardiac contractility resulting in tachycardia at rest (Tortora and Grabowski, 2000).

1.7 Heart Rate Variability, Exercise and Performance

Regular exercise training can lead to positive CV changes (Berkoff et al., 2007). Different types of exercise training (aerobic, resistance training) have different physiological and physical impacts on the ANS.

Research has shown that trained athletes have a higher HRV than sedentary individuals and that exercise training can increase HRV in normal populations (Nagai and Moritani, 2004). Overtraining is characterized by reduced performance and marked fatigue and is a result of increased training load coupled with decreased quality recovery (Lopes and White, 2006). Heart rate variability has been shown to be reduced in athletes with the overtraining syndrome (Sandercock et al., 2005). Sandercock et al. (2005) examined ANS activity in several middle distance runners and found that heavy training shifted the cardiac autonomic balance towards a predominance of the sympathetic over parasympathetic drive. When recorded during the night, HRV appeared to be a better tool than resting HR to evaluate cumulated physical fatigue, because it magnified the induced changes in ANS activity (Sandercock et al., 2005).

ANS recovery is more rapid in highly trained than in trained subjects after high intensity exercise (Seiler et al., 2007). The high frequency training ensures that these adaptive effects are cumulative. Conversely, incomplete recovery from frequent training can make the stress related side effects cumulative as well. The day to day distribution of training intensity may be a crucial variable to effectively balance negative stress effects and positive adaptive effects so that performance development is achieved without stagnation or overtraining (Seiler et al., 2007).

Exercise training enhances total HRV in normal older adults. The most predominant alterations are in nocturnal HR (Nagai and Moritani, 2004). Studies have shown that endurance athletes have an elevated level of parasympathetic tone in comparison to sedentary people. The effect of resistance training on autonomic tone is less clear. Elite athletes have been shown to have higher parasympathetic tone than recreational athletes

and non athletes (Berkoff et al., 2007). Persons who are more physically active show a wider range between their maximal and minimal HR. Chronic exercise training produces a resting bradycardia that is thought to be due partly to enhanced parasympathetic modulation (Nagai and Moritani, 2004).

The assessment of HRV in postmenopausal women is essential because CVD typically occurs later in women than in men, with CVD being one of the two leading causes of death in women (Ribeiro et al., 2001). Changes in lifestyle and the inclusion of physical activity has a significant affect on the metabolic processes surrounding CVD (Earnest et al., 2008).

Earnest et al. (2008) conducted research on postmenopausal women participating in exercise training over a 6 month period. Their primary findings were that moderate intensity exercise training improves HRV characteristics in previously sedentary, overweight or obese postmenopausal women. It has been reported that normal aging influences indices related to CV function, including the adrenoreceptor responsiveness and baroflex sensitivity which results in decreased parasympathetic activity (Tortora and Grabowski, 2000), (Earnest et al., 2008). Although there is evidence that vagal tone is generally higher in women than men (Ribeiro et al., 2001), (Sinnreich et al., 1998) progressive aging reduces the difference between the two genders.

A key feature of physical exercise training is an enhancement of the CV system's interactions, which includes the improvement in the neural regulatory aspects of cardiac regulation through the SA node (Tortora and Grabowski, 2000). Earnest et al. (2008) found that physical activity facilitated improvements in HRV in older women and that the intensity of exercise training needs to be moderate in nature to bring about these improvements.

In general, the research pertaining to physical activity and its effects on HRV provides strong evidence that exercise training has beneficial effects in increasing HRV in all population groups if minimum guidelines are adhered to.

1.8 Gender, Ageing and Heart Rate Variability

The association between gender and HRV characteristics is controversial (Umetani et al., 1998, Sinnreich et al., 1998, Ryan et al., 1994, Ramaekers et al., 1998).When using HRV in clinical settings for predictive purposes, researchers have to take into account the impact of various important physiological factors. Two of these are prominent: gender and age. Research on age is widespread and it has been shown that HRV decreases with normal aging and is linked with increased risk of mortality (Freitas et al., 1997).

Heart rate variability is constant over a 1-year period in older adults who do not alter their activity level (Dietrich et al., 2006). Results of a study performed by Umetani et al. (1998) demonstrated that HRV decreases with aging. Importantly, it was found that this age related decline can affect the predictive value of HRV, principally in the aging population, by making it difficult to distinguish low HRV due to disease from that due to normal aging. Research has demonstrated that HVR decline occurs at a different rate in male and female subjects (Umetani et al., 1998).

Therefore, gender also influences HRV but the research on gender differences is limited. Most of the data acquired has been incidental reports in studies that focused on other concerns (Dietrich et al., 2006, Kristal-Boneh et al., 2002, Lui et al., 2003). Available reports are controversial with some reporting a higher HRV for female (Sinnreich et al., 1998, Ramaekers et al., 1998) than for male subjects and others reporting the converse (Umetani et al., 1998). Some literature suggests that gender differences and age could have more prognostic traits (Ribeiro et al., 2001). The aging process results in HRV decreases placing individuals at a greater risk of developing CVD (Dietrich et al., 2006). Gender differences may also aid in diagnosis and

identifying risk factors for CVD. Research predominately suggests females have an overall higher HRV than males (Lui et al., 2003).

Umetani et al. (1998) observed that gender differences are most pronounced in young (30 years) subjects. The HRV of young male subjects were significantly greater than that of age matched female subjects, indicating greater parasympathetic tone in males. However, differences disappear by age fifty (Umetani et al., 1998). Conversely, Ryan et al. (1994) found that vagal HF power was higher in females. They found that this difference is most apparent for young (20-39) and middle aged (40-64) females. Raemaeker et al. (1998) noted specifically that LFnu and the LF/HFnu ratio were higher in males, however HFnu showed no difference between the genders. Sinnreich et al. (1998) observed that the RMSSD and HF components (measures that reflect predominantly vagal activity) were small and non significant, but that the LF/HF ratio (suggested to reflect sympathovagal balance) differed substantially due to great VLF and LF power found in the male participants. They concluded that their results illustrated relatively higher sympathetic activity in men compared to women (Sinnreich et al., 1998).

Gender differences in the association between duration of type 2 diabetes and HRV have also been investigated (Nolan et al., 2004). Among males, duration of diabetes was independently and inversely associated with vagal HR modulation and total RR variability but this was not apparent in females. In contrast, HF was inversely associated with age of diabetes diagnosis and 10-year absolute risk for CVD among female subjects. Longitudinal research is needed to establish whether risk factors for early cardiac autonomic impairment differ among men and women with type 2 diabetes (Nolan et al., 2004).

Cardiovascular disease is a leading cause of death in many developed countries. The relation between oestrogen and CVD has been discussed in many epidemiological studies. Research surrounding CVD has suggested that premenopausal females have a

reduced risk of CVD compared to males and postmenopausal women (Lui et al., 2003). Evidence shows that the incidence of CVD increases after menopause in women and that the CV mortality rate of postmenopausal women with oestrogen replacement therapy is lower than that of women who do not take it. To explain the mechanism of this phenomenon, the effects of oestrogen on the lipid profile and vascular activity have been discussed and established in studies (Lui et al., 2003). Moodithaya et al. (2009) examined the effect of oestrogen as a possible reason for young females having higher HRV and a cardio-protective mechanism compared to older females and/or their male counterparts. The authors demonstrated that postmenopausal women have a significantly reduced overall fluctuation in autonomic input to the heart and vagal index of HRV. This was reflected by postmenopausal women having a lower total power and HF in absolute power compared to young women which was associated with oestrogen levels (Moodithaya and Avadhany, 2009, Ribeiro et al., 2002). Additionally, it was demonstrated that oestrogen administration in premenopausal women attenuated vasoconstrictor responses to norepinephrine and reduced total body norepinephrine spillover, which is an index of sympathetic neural activity (Lui et al., 2003).

In conclusion most of the evidence provided strongly suggests that females have a higher HRV before they reach menopause. This finding supports research indicating that women have a cardio-protective mechanism due to higher oestrogen levels premenopause. Progressive aging has been widely reported to decrease HRV. Therefore taking gender and age into account facilitates a more accurate demarcation of the normal range of HRV and HR, which could help to improve the diagnostic and predictive usefulness of HRV in clinical settings (Umetani et al., 1998).

1.9 Reliability and Validity of Heart Rate Variability Measurements

Reliability can be defined as the ability to replicate a measurement from day to day by the same observer in identical conditions thus rendering it reliable (Petrie and Sabin, 2000). The reliability of HRV has been frequently investigated yet remains poorly quantified (Taskforce, 1996). Assessing the reliability of a measure that evaluates dynamic physiological processes is a complex task (Sandercock et al., 2007). It has also

been recommended that precision of measurements of all new commercially available HRV technologies be assessed to validate the instrument (Nunan et al., 2009, Taskforce, 1996). Too establish the use of HRV, standardization of HRV protocols and parameters are required. Repeatability studies can assist in the process of standardizing HRV protocols and parameters. Few studies have examined the repeatable characteristics of HRV with regards to day to day readings in different population groups (Taskforce, 1996).

Pinna et al. (2007) performed an in-depth assessment of absolute and relative reliability of standard indexes of short-term laboratory HRV recordings in subject with spontaneous and paced breathing. These researchers found that HRV parameters are subject to large day to day random variations depicting decreased repeatability qualities. They concluded that random error of HRV parameters was in part due to sampling variability of the estimated parameters. Observed differences between individuals mostly reflected differences in a subject's true value rather than random error (Pinna et al., 2007, Sandercock et al., 2007). Sinnreich et al. (1998) found that short term recordings of HRV had a high reliability coefficient and low coefficient of variation (which includes biological variability and method error) that indicated a considerable consistency with time.

Sandercock et al. (2005) undertook a review of literature on the reliability of short term measurements of HRV. This review reported that statistical analysis plays an important role in reporting reliability and therefore the authors only included studies that used appropriate statistics. Furthermore, methodology and sample size were also controlled for due to the sensitivity of HRV (Sandercock et al., 2007). The aim of this literature review was to determine whether HRV measures made at rest or during specific interventions are reliable in healthy subjects and clinical populations. Short-term (5 min) recordings were reported to have a number of advantages, including testing under various conditions which allows researchers to compare recordings so that they can observe autonomic responses to different stimuli or interventions. These recordings were shown to be well controlled, easy and quick to perform and analyse (Sandercock et al., 2005). The review concluded that there is a small effect of test–retest duration on

reliability and no single HRV measurement appears less reliable than another. However, they suggested that describing HRV as a reliable measurement technique appears to be a generalization, as results of reliability studies are varied, and dependent on a number of factors (Sandercock et al., 2005).

A study examining a commercially available heart rate monitor (HRM), the Polar S810, provided information on the validity and reliability of this equipment for analysis of HRV. The findings of the study were that the Polar S810 provided a valid measure for most HRV parameters. The results from the Polar S810 demonstrated that this piece of equipment should not be used to detect small changes in HRV (Nunan et al., 2009).

Weippert et al. (2010) conducted research on the comparison of three mobile devices for measuring IBIs and HRV: two breast belt systems, the Polar S810i HRM and the Suunto t6 HRM, and an ambulatory ECG system. With respect to IBI measurements, it was found that the three instruments can be used interchangeably. In this study, spectral analysis techniques were shown to be uncomplicated for normalized HRV frequency measures but this was not the case when absolute values were used. The results concluded that the tested breast belt systems are reliable for long-term HRV analysis (Weippert et al., 2010). There was also minimal bias between short-term HRV measures obtained by the three instruments. The results showed that these devices were unreliable when testing individuals as compared to large groups and it was not recommended to use different devices for an intra-individual HRV analysis study (Weippert et al., 2010).

Many factors can affect HRV recordings such as mood, alertness and mental activity which are very difficult to control for in studies (Pinna et al., 2007). Other factors that can be controlled for should be noted. Time of day of HRV recording is important, early morning readings like those used in most repeatability studies are suggested (Pinna et al., 2007, Sinnreich et al., 1998). Eating should be avoided together with the intake of caffeine which can increase HR and affect HRV readings. Heavy physical activity that is done in conjunction with HRV testing may provide inaccurate readings of elevated

sympathetic activity due to insufficient recovery period (Lopes and White, 2006). Sleeping patterns also affect HRV recordings. A lack of sleep can inhibit parasympathetic activity, therefore increasing HR (Tortora and Grabowski, 2000). Intake of other stimulants like alcohol and smoking should also be avoided as it dilates vessels and increases contractility in the heart which is triggered by stimulation of sympathetic activity.

In conclusion, HRV has been shown to be under the influence of random error and within subject variation. These finding suggest that assessing treatment effects in individuals using HRV maybe complex (Pinna et al., 2007). Considering this factor, the assessment of performance changes maybe be difficult to calculate as HRV may not be able to detect minor alterations under different circumstances (Lamberts et al., 2009). Sample sizes and confounding factors should be more rigorously controlled for to aid reliability. Research surrounding reliability is still confounding due to inadequate methodology and statistical methods used for analysis. Therefore further reliability studies are advocated.

1.10 Conclusion

Research into HRV has increased steadily over the last few decades. This research has provided an insight to the control mechanisms of HRV and new techniques for testing HRV has made this process easier. However, despite well documented studies on HRV, certain aspects still require clarification as shown in this review of literature. Overtraining, gender and reliability of HRV is still poorly quantifiable. Conflicting research on these aspects makes standardization of HRV difficult, therefore decreasing the possible benefits of HRV recordings.

CHAPTER 2: SCIENTIFIC PAPER PUBLICATION

The following paper is published in the Cardiovascular Journal of Africa (CVJA) 2011 Jun 25;22:1-7. The Appendix VII provides a copy of the accepted article as it appears in the journal.

This study was undertaken in light of limited literature pertaining to the reliability of heart rate variability measurements and the contradictory information relating to gender differences in HRV.

HEART RATE VARIABILITY IN PHYSICALLY ACTIVE PARTICIPANTS: RELIABILITY AND GENDER CHARACTERISTICS.

2.1 Abstract

2.1.1 Purpose

To evaluate the reliability of short term recordings (5 mins) of heart rate variability (HRV) and the association between HRV and gender.

2.1.2 Methods

HRV time and frequency domain parameters were calculated in 44 physically active students (21 males and 23 females) over 4 consecutive days. A Suunto t6 heart rate monitor was used to obtain inter-beat intervals (IBI's) that were then transferred to Kubios HRV Analysis Software. The relative reliability (intraclass correlation (ICC)) and absolute reliability, (typical error of measurement, TEM and typical error or measurement as a percentage, TEM %) of the HRV parameters were then calculated for Day 2 vs. Day 3 and Day 3 vs. Day 4, with Day 1 being a familiarization day. The following HRV parameters were calculated; 1) Time domain: Resting heart rate (RHR), R-R intervals (IBI), standard deviation of Normal to Normal intervals (SDNN), root mean square differences of the standard deviation (RMSSD), percentage of beats that changed more than 50 ms from the previous beat (pNN50); 2) Frequency Domain: Low frequency normalized units (LFnu), high frequency normalized units (HFnu), low frequency to high frequency ratio in normalized units (LF/HFnu). An analysis of variance (ANOVA) with Tukey post-hoc testing was performed to compare HRV parameters in males and females. Significance was set at $p \leq 0.05$.

2.1.3 Results

The ICCs for both day 2 versus day 3 (REL 1) and day 3 versus day 4 (REL 2) indicated primarily good to excellent (>0.8) relative reliability. The lowest value was found in the

LF/HFnu ratio (ICC = 0.36) for males. Absolute reliability was low with TEM% greater than 10% for all HRV parameters, except IBI's. Females demonstrated better relative (higher ICC's) and absolute reliability (lower TEM and TEM%) compared to males for the frequency domain. The relative and absolute reliability for the time domains were similar except for SDNN where the absolute reliability was higher in males. ANOVA illustrated significant gender differences for the LF/HFnu ratio (41% higher in males, $p = 0.003$), HFnu (12% higher in females, $p = 0.02$) and IBI (21% higher in females, $p < 0.0001$).

2.1.4 Conclusions

Short term recordings of HRV over 3 consecutive days demonstrated a high relative reliability. However, a low absolute reliability indicated large day-to-day random variation in HRV which would make the detection of intervention effects using HRV difficult in individual participants. Females were shown to have a higher parasympathetic modulation of HRV which may indicate an underlying cardioprotective mechanism in females compared to males.

Key words: Heart rate variability, sympathovagal balance, reliability.

2.2 Introduction

Heart rate variability (HRV) is recognized as a versatile and promising, non-invasive marker of autonomic nervous system (ANS) modulation (Weippert et al., 2010). Research into the use of HRV has increased in both clinical and research environments and over a broad spectrum of disciplines (Nunan et al., 2009, Pinna et al., 2007). However, in the disciplines of sport and exercise science, there is limited information available on the reliability of HRV measures, in particular related to new, commercially available equipment that coaches and athletes have access to. This fact, together with the extensive amount of variables that alter HRV measures, makes it difficult to compare HRV studies and develop a universal standard (Nunan et al., 2009). While

most studies agree that age is inversely associated with HRV (10) research is less consistent on the impact of gender with studies demonstrating that HRV measures are either the same or differ considerably between the genders and may also be HRV parameter dependant (Ramaekers et al., 1998 , Ryan et al., 1994, Sinnreich et al., 1998, Umetani et al., 1998).

Heart rate variability reflects the changes in the interval between heart beats (R-waves) over time. The time between one R-wave and the next, in milliseconds, is termed the RR interval or the interbeat interval (IBI) (Algra et al., 1993). The ANS governs the IBI's via the sympathetic and parasympathetic pathways (Aubert et al., 2003). The relative dominance of either pathway over the other represents an alteration in the sympathovagal balance which is reflected in IBI changes (Keenan and Grossamn, 2006). Under normal resting conditions in healthy individuals it has been suggested that the parasympathetic pathway is dominant resulting in a high HRV (Algra et al., 1993), whilst lower HRV, and poor health, has been linked to increased sympathetic activity at rest (Lopes and White, 2006, Umetani et al., 1998). However, research has demonstrated that the age, physical activity status, gender and the HRV parameter examined are important factors to consider when examining HRV (Ramaekers et al., 1998, Ryan et al., 1994, Sinnreich et al., 1998, Umetani et al., 1998) .

Research has identified the potential use of HRV for identifying healthy and diseased states. In particular, a significant relationship between the ANS, low HRV and cardiovascular mortality, including sudden cardiac death, has been reported (Ribeiro et al., 2001, Taskforce, 1996). In addition, studies have shown that trained athletes have higher HRV compared to sedentary individuals, suggesting that exercise training can increase HRV in normal populations (Nagai and Moritani, 2004). The overtraining syndrome is assumed to be consequence of an imbalance between long-term inappropriate high training volume and too little time for regeneration (Borresen and Lambert, 2008, Brooks et al., 2005). Alterations in the ANS have been presented as a mechanism underlying the signs and symptoms of the overtraining syndrome (Hynynen et al., 2006). A study which examined ANS activity in several middle distance runners suggested that heavy training shifted the cardiac autonomic balance toward a predominance of the sympathetic over the parasympathetic drive which was represented by a decrease in HRV (Hynynen et al., 2006).

Heart rate variability has the potential to be a useful monitoring tool in the fields of health, fitness and sports performance. However, currently, there are issues regarding the standardisation and reproducibility of HRV measurement as there are many confounding factors that can influence HRV. These include factors such as mood, alertness, mental activity, gender and age (Freitas et al., 1997, Pinna et al., 2007, Ryan et al., 1994). Whilst the research relating to gender differences is controversial (Freitas et al., 1997, Ramaekers et al., 1998, Umetani et al., 1998) the relationship between age and HRV has been well documented (Dietrich et al., 2006, Ryan et al., 1994, Sinnreich et al., 1998, Umetani et al., 1998). Reduced HRV is associated with progressing age and with an increased risk of cardiac events in clinically disease-free patients (Nagai and Moritani, 2004).

The development of wireless heart rate monitoring equipment which has the ability to record IBIs has provided athletes, coaches, scientists and medical practitioners with mobile and easy to use systems that allow for the analysis of HRV. The commercially available Suunto t6 heart rate system (Suunto; Vantaa, Finland) is one such instrument that is widely used for monitoring heart rate during exercise. The validity of the Suunto t6 in measuring IBI for determining HRV has been reported recently (Weippert et al., 2010). Whilst previous studies have examined the reliability of other commercially available devices (Polar S810) for measuring HRV (Nunan et al., 2009, Weippert et al., 2010), there is limited information on the reliability of the Suunto t6 for HRV measurement (Roy et al., 2009, Weippert et al., 2010). Analysis of the IBI's to determine HRV can be performed using custom software like Kubios Heart Rate Variability Software Version 2.0 (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) (Roy et al., 2009, Tarvainen and Niskanen, 2008). To date there are few publications reporting the use of both the Suunto t6 and Kubios Software together (Roy et al., 2009).

The primary aim of this study was therefore to investigate the reliability of Kubios Software HRV measures calculated from the Suunto t6 IBI's in physically active individuals. Furthermore we investigated whether there were any gender differences in HRV parameters.

2.3 Methods

2.3.1 Participants

This study was conducted on 50 physically active young adults (males n=25; females n=25), although data analysis was performed on 21 males and 23 females due to exclusion criteria discussed in the statistical analysis of the data (Table 2.1). There were significant differences between the genders in terms of height, mass and percent body fat, but no differences in age, BMI, waist to hip ratio or weekly physical activity levels (Table 2.1). Participation was voluntary, and written informed consent was obtained from all participants. The study was approved by the Institution's Biomedical Research Ethics Committee (REF: BE111/010). Participants were excluded from the study if they experienced a cold or feverish illness in the month leading up to the study, were smokers, had a pre –existing heart condition either current or in the past, were pregnant, diabetic, had congestive heart failure, or acute or chronic renal disease, if they have a pacemaker, or were taking type 1A antiarrhythmics (quinidine, procainamide, disopyramide, or moricizine).

Table 2.1: Mean (± *SD*) of demographic data by gender.

	Males (n=21)	Females (n=23)	*p*
Age (years)	21.17 (1.55)	19.75 (1.76)	0.45
Height (cm)	177.3 (9.09)	160.5 (6.49)	<0.0001*
Weight (kg)	76.19 (14.69)	60.41 (9.64)	0.0001*
BMI (kg/m^2)	24.03 (2.64)	23.41 (2.89)	0.467
Waist/hip ratio	0.88 (0.07)	0.85 (0.068)	0.123
Percent Body Fat	13.73 (4.53)	18.63 (3.77)	0.0004*
IPAQ (Kcals/week)	5828(3806)	5491 (3751)	0.789

IPAQ = International Physical Activity Questionnaire

2.3.2 Assessment of Physical Activity Status

The International Physical Activity Questionnaire (IPAQ) is a validated questionnaire primarily designed for population surveillance of physical activity among adults (age range 15-69 years (Hagstromer et al., 2006)). This questionnaire was used to classify the physical activity status of the participants in the week leading up to the first (Day 1) testing day. The IPAQ requires the summation of duration (in minutes) and frequency (days) for different categories of physical activity. Based on this information MET-minutes/week are calculated with the MET minute scores being equivalent to kilocalories expended per week. The participants were then classified into low, moderate or high physical activity levels. On average, the participants in the study were classified as being in the moderate category with an average of 5828 Kcals (Table 2.1) expended per week (833 kcal expended per day). Moderate is defined by the IPAQ guidelines as a pattern of activity done on 3 or more days, at least 20 minutes per day and described as vigorous-intensity activity or 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day.

2.3.3 Protocol

Testing was conducted within the Human Performance Laboratory (HPL) at our institution. The temperature in the HPL was maintained at 22^{o}C with 50% humidity. Participants were provided pre-test instructions the week before the testing to help control for factors that could alter heart rate variability readings. They were asked to avoid caffeine, eating, heavy physical activity, smoking, and alcohol intake for the 10 hours preceding each laboratory visit. Each participant attended 4 testing sessions for 4 days in a row at the same time of the day. Testing was performed between 7am-9am.

On testing Day 1, which counted as the familiarisation day, each participant read the information sheet provided on the study, signed a written informed consent, completed a pre-test questionnaire (IPAQ) and medical history questionnaire. The medical questionnaire examined cardiovascular, metabolic and respiratory disease history (personal and family) as well as risk factors and signs and symptoms for these diseases.

In addition, participants provided information on their current medication and supplement intake. Height, mass, waist and hip circumferences and 3 site skinfolds (females: tricep, supra-iliac and mid thigh; males: chest, abdominal and mid-thigh) were then measured. Height and weight were recorded using a calibrated medical height gauge and balance scale (Detecto, Webb City, USA). A Harpenden skin fold calliper was used for skin fold measurements to calculate percent body fat using the Jackson and Pollock equations (Jackson et al., 1978, 1980). Body mass index (BMI) (mass (kg)/height (m^2) and waist to hip ratio (waist:hip circumferences) were then calculated and the HRV measurement protocol was then followed.

2.3.4 Heart Rate Variability Measurement

The participants were fitted with the Suunto t6 heart rate monitor (HRM) (Suunto; Vantaa, Finland). The electrodes on the transmitter were wet with water and were placed on the chest against bare skin to ensure good skin contact. Participants were tested while lying supine with the total testing time lasting 20 minutes. This time was divided into 15 minutes of rest followed by a 5 minute measurement of IBI's. The IBI's were then transferred to a laptop (HP ProBook) computer where the data were stored in the Suunto Team Manager software programme (Firstbeat Technologies, Ltd; Jyvaskyla , Finland). The data were then exported as a text file to the HRV analysis software (Kubios Heart Rate Variability Software Version 2.0; Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) for analysis of the following HRV parameters 1) Time domain: Resting heart rate (RHR), R-R intervals (IBI), standard deviation of Normal to Normal intervals (SDNN), root mean square differences of the standard deviation (RMSSD), percentage of beats that changed more than 50 ms from the previous beat (pNN50); 2) Frequency Domain: Low frequency normalized units (LFnu), high frequency normalized units (HFnu), low frequency to high frequency ratio in normalized units (LF/HFnu).

The Suunto t6 HRM and Kubios programme (Roy et al., 2009) comply with guidelines recommended by the Taskforce of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology standards for measurement of HRV (Taskforce, 1996) . Before processing, the IBI's were manually corrected for ectopic/

missed beats. There is currently no universal method for identifying and editing ectopic beats. The amount and type of editing of IBI data has different effects on various HRV indices (Salo et al., 2001). In the present study, manual editing or interpolation (Salo et al., 2001) of the IBI's intervals was performed using the following guidelines: If a significantly higher IBI (representing an ectopic beat) was noted then that reading was deleted and average of the two adjacent IBI's replaced the deleted one. If a significantly lower value (representing a missed beat) was noted, that IBI was deleted and replace with the previous IBI. If the ectopic or missed beats exceeded 20% of a participant's overall 5 minute recording the participant was not included in the analysis (Nunan et al., 2009). This occurred in six participants (4 males and 2 females) with the result that only 44 participants were included in the final analysis (n = 21 males and n = 23 females).

Once the IBI's were imported into the Kubios programme the software automatically analyzed the HRV in both time and frequency domains. Power spectral analysis was performed using the Autoregressive (AR) algorithm in accordance with the recommendations (Taskforce, 1996) . The AR algorithm was used as it yields improved resolution especially for short term HRV measurements (Roy et al., 2009, Tarvainen and Niskanen, 2008). This algorithm creates a power spectral analysis with distinct frequency bands; namely high-frequency (HF), low-frequency (LF) and very low frequency (VLF). The LF component has been proposed as reflecting both sympathetic and parasympathetic effects on the heart and occurs in a band between 0.04 Hz and 0.15 Hz. However, researchers noted that the low frequency band is influenced by baroreceptor mediated regulation of blood pressure and reflects predominantly sympathetic activity (Moodithaya and Avadhany, 2009). The HF (0.15 Hz - 0.4 Hz) band corresponds with respiratory sinus arrhythmia (RSA) and is said to reflect parasympathetic activity (Moodithaya and Avadhany, 2009). Chemoreceptor processes, thermoregulation, and the rennin-angiotensin system have been linked with the VLF band. We did not use data collected from the VLF range for this study.

2.3.5 Statistical Analysis

The data were summarized using routine descriptive statistics (mean ± SD). Reliability measures were determined for day 2 versus day 3 and day 3 versus day 4. Absolute and

relative reliability of several HRV parameters was calculated using the procedures described by Hopkins (2000). Hopkins (2000) has argued that the statistical analysis used in reliability studies should include observed values and confidence limits of the typical error. These measures are sufficient to characterize the reliability of a measure and that they substantially enhance comparison of the reliability of tests, assays or equipment (Hopkins, 2000).

Absolute reliability is the degree to which repeated measurements vary for individuals. This type of reliability is expressed either in the actual units of measurement or as a proportion of the measured values (dimensionless ratio) (Atkinson and Nevill, 1998). Absolute reliability was calculated using the typical error of measurement (TEM) and TEM as a percentage (%), expressed as % of the mean score (Hopkins, 2000). Sport and exercise science reliability studies have rarely reported the separate analysis of homoscedastic and heteroscedastic data (Atkinson and Nevill, 1998). These parameters show how the measurement error relates to the magnitude of the measured variable. When the amount of random error increases as the measured values increase, the data are said to be heteroscedastic. When there is no relation between the error and the size of the measured value, the data are described as homoscedastic (Hopkins, 2000). Homoscedastic errors are expressed in the actual units of measurement (TEM) but heteroscedastic data are measured on a ratio scale. With homoscedastic errors, the raw data is analysed with conventional parametric analyses, but heteroscedastic data is transformed logarithmically before analysis or investigated with an analysis based on ranks (Atkinson and Nevill, 1998). In the present study the HRV data for each parameter was examined using the technique described by Hopkins (2000) and was found to be homoscedastic. TEM and TEM% were calculated with 90% CI for Day 2 vs. Day 3 and for Day 3 vs. Day 4, using a spreadsheet downloaded from http://www.newstats.org. The TEM is also known in statistical terms as the coefficient of variation (CV).

Furthermore, for reliability studies it has been suggested that relative reliability is presented together with absolute reliability (Atkinson and Nevill, 1998). Relative reliability is the degree to which individuals maintain their position in a sample with repeated measurements and is represented in the form of correlation coefficients. In this study we used Interclass correlations (ICC) with 95% CI (Hopkins, 2000). The main

advantage of ICC over Pearson's correlation is that it can be used when more than one retest is being compared with a test (Atkinson and Nevill, 1998). Various categories of reliability are based on the ICC. An ICC >0.8 is usually regarded as good to excellent reliability, whereas an ICC between 0.6 and 0.8 may be taken to represent substantial reliability (Pinna et al., 2007).

In order to determine whether there were any gender differences in the HRV parameters, the data for each HRV parameter for days 2, 3 and 4 were combined and analysis of variance (ANOVA), with a Tukey post-hoc testing, was performed to compare males versus females. The data were analysed with STATISTICA version 8.0 (Statsoft Inc, Tulsa, Oklahoma, USA) for any statistical significance ($p \leq 0.05$).

2.4 Results

A total of 44 participants with complete data sets were analysed. The outcomes of the reliability analysis are presented in Tables 2.2 and 2.3 for Day 2 vs. Day 3 and Day 3 vs. Day 4.

2.4.1 Typical error of measurement

The absolute reliability indices for the HRV parameters are shown in Table 2.2. The data presented here offers precision estimates for single measurements of Suunto t6 HRV and data for making decisions when monitoring changes or responses to interventions in individuals.

Overall for both males and females, the results show that for most HRV frequency domain parameters the second comparison Day 3 vs. Day 4 had lower TEM values compared to the first Day 2 vs. Day 3. This slight decrease in the typical error of measurement (TEM) could be due to a familiarisation effect (Aubert et al., 2003). Females demonstrated better absolute reliability in the HRV frequency domain as demonstrated by lower TEM's. Specifically, the TEM's for Day 3 vs. Day 4 were lower in females for the LF/HFnu ratio (116% lower), 90% lower for HFnu (Day 2 vs. Day 3) and 68% lower for LFnu (Day 2 vs. Day 3). The lowest typical error of measurement as

a percentage (TEM%), was 11.5% and was found in the females for HFnu (Day 2 vs. Day 3). Time domain results had a low TEM% for IBIs (Day 2 vs. Day 3: 4.8 males and females; Day 3 vs. Day 4: 4.9 females and 4.1 males). Overall the TEM% was relatively high in most HRV parameters specifically for LF/HFnu (Day 2 vs. Day 3: 31.4% females and 48.1% males; Day 3 vs. Day 4: 29.7% females and 40.4% males). The TEM was similar for males and females for the time domain parameters, specifically, RMSSD, pNN50 and the IBI's. However, the TEM for SDNN was 42% lower in the males (Day 3 vs. Day 4).

2.4.2 Interclass Correlations

The Interclass Correlations (ICCs) for REL 1 ranged between 0.36-0.88 for the AR frequency domains and 0.70-0.92 for the time domains (Table 2). For Day 3 vs. Day 4 the ICC's ranged between 0.72-0.86 for the AR frequency domains and 0.72-0.93 for the time domains. The ICCs for both Day 2 vs. Day 3 and Day 3 vs. Day 4 indicated good to excellent (>0.8) reliability correlations for IBI's and pNN50 from the time domain results. RMSSD reliability was good to excellent with the exception of the males for Day 3 vs. Day 4 that showed substantial reliability of 0.79. SDNN variable results depicted substantial reliability for both Day 2 vs Day 3 and Day 3 vs Day 4.

Reliability ICC's for the frequency domain were lower compared to the time domain. The ICCs in the frequency domain were between 0.36 and 0.88 with the lowest value for these correlations being the LF/HFnu ratio (ICC = 0.36) for males. It was found that overall, the male participants had lower ICC's when compared to females for the same HRV parameter in the frequency domain analysis. The ICC's for most time domain parameters were similar between the males and females with the exception of the IBI's. Males displayed high IBI ICC values (Day 2 vs. Day 3 = 0.90, Day 3 vs. Day 4 = 0.93) which indicated higher relative reliability compared to the females (Day 2 vs. Day 3 =0.83, Day 3 vs. Day 4 =0.79). Time domain ICC's that were similar between males and females were SDNN (Day 3 vs. Day 4), males (0.73) and females (0.72) and pNN50 (Day 2 vs. Day 3), males (0.86) and females (0.84).

Table 2.2: Reliability indexes for frequency and time domain HRV parameters

		Day 2 vs Day 3 (REL 1)			Day 3 vs Day 4 (REL 2)		
HRV parameters		**TEM**	**TEM (%)**	**ICC**	**TEM**	**TEM (%)**	**ICC**
LF/HF nu Ratio	**Female**	0.25 (0.20-0.36)	31.4 (25.1-45.2)	0.80 (0.59-0.91)	0.24 (0.19-0.35)	29.7 (23.5-43.4)	0.83 (0.63-0.92)
	Male	0.54 (0.41-0.78)	48.1 (36.5-69.5)	0.36 (0.08-0.69)	0.43 (0.33-0.62)	40.4 (31.0-58.3)	0.72 (0.41-0.88)
HFnu	**Females**	6.63 (5.13-9.38)	11.5 (8.9-16.2)	0.88 (0.74-0.94)	7.92 (6.13-11.21)	13.4 (10.3-18.9)	0.81 (0.61 - 0.92)
	Males	12.58 (9.62-18.17)	24.6 (18.8-35.5)	0.55 (0.17-0.79)	8.95 (6.85-12.93)	16.7 (12.8-24.2)	0.78 (0.53 - 0.90)
LFnu	**Female**	7.78 (6.26-10.38)	17.8 (14.3-23.8)	0.84 (0.70-0.92)	7.26 (5.84-9.69)	17.2 (13.9-23.0)	0.86 (0.74 - 0.93)
	Male	13.04 (10.41-17.70)	26.6 (21.2-36.1)	0.52 (0.19-0.74)	9.40 (7.50-12.76)	20.2 (16.11-27.4)	0.75 (0.54-0.88)
IBIs	**Females**	44.90 (35.21-61.98)	4.8 (3.8-6.6)	0.83 (0.67-0.91)	45.89 (35.99-63.34)	4.9 (3.9-6.9)	0.79 (0.60-0.89)
	Males	53.46 (40.90-77.20)	4.8 (3.6-6.9)	0.90 (0.79-0.95)	46.46 (35.54-67.09)	4.1 (3.2-5.9)	0.93 (0.85-0.96)
SDNN	**Females**	28.83 (22.30-40.80)	31.2 (24.1-44.1)	0.77 (0.54-0.90)	35.58 (27.52-50.36)	32.2 (28.8-52.7)	0.72 (0.45-0.87)
	Males	20.70 (15.83-29.89)	19.9 (15.2-28.8)	0.70 (0.40-0.87)	20.62 (15.78-29.78)	20.2 (15.4-29.1)	0.73 (0.45-0.88)
RMSSD	**Females**	17.29 (13.37-24.47)	20.5 (15.8-29)	0.92 (0.82-0.97)	20.12 (15.56-28.48)	22.6 (17.5-32)	0.91 (0.80-0.96)
	Males	19.12 (14.63-27.62)	18.7 (14.3-27.1)	0.83 (0.64-0.91)	20.53 (15.71-29.65)	20.3 (15.5-29.3)	0.79 (0.55-0.89)
pNN50	**Females**	9.56 (7.39-13.53)	19.1 (14.8-27)	0.84 (0.67-0.93)	8.80 (6.80-12.45)	18.7 (14.4-26.4)	0.87 (0.72-0.94)
	Males	7.55 (5.78-10.90)	14.2 (10.9-20.5)	0.86 (0.69-0.94)	7.07 (5.41-10.21)	13.2 (10.1-19.1)	0.87 (0.72-0.95)

Heart Rate Variability: Frequency Domain: Low frequency normalized units (LFnu), high frequency normalized units (HFnu), low frequency to high frequency ratio in normalized units (LF/HFnu). **Time domain**: R-R intervals (IBI), standard deviation of Normal to Normal intervals (SDNN), root mean square differences of the standard deviation (RMSSD), percentage of beats that changed more than 50 ms from the previous beat (pNN50**); Reliability:** Intraclass correlation (ICC) is expressed as mean (95% CI); Typical Error of Measurement (TEM) and Typical Error of Measurement as a percentage (TEM %) are expressed mean (90% CI).

2.4.3 Gender Differences

There were significant differences between males and females for resting HR ($p < 0.0001$), R-R intervals ($p < 0.0001$) and for the frequency domain HRV parameters, HFnu ($p = 0.020$) and LF/HFnu ratio ($p = 0.003$) (Table 2.3). The female resting heart rate was 16% higher than the males ($p < 0.0001$) while the IBI's were 21% higher in the males ($p < 0.0001$). The LFnu ($p = 0.087$) and LF/HFnu ratio ($p = 0.003$) was 13% and 41% higher in the males respectively while the HFnu ($p = 0.02$) was 12% higher in the females. There were no significant differences between males and females in the HRV time domain parameters except for IBIs.

Table 2.3: Comparison of mean (± *SD*) of Days 2, 3 & 4 of heart rate variability data by gender.

	Men	Women	*p*
Resting HR (b/min)	55.09 (8.1)	65.84 (7.2)	<0.0001*
IBIs (milliseconds)	1126 (169)	927.7 (101)	<0.0001*
LFnu	47.95 (17.93)	42.48 (18.43)	0.087
HFnu	52.02 (17.73)	59.17 (17.16)	0.020*
LF/HF nu Ratio	1.114 (0.710)	0.792 (0.522)	0.003*
SDNN	103.1 (36.13)	93.39 (57.55)	0.252
RMSSD	101.3 (43.75)	87.57 (59.44)	0.137
pNN50	53.35 (18.71)	48.46 (22.77)	0.182

LFnu = low-frequency nu; HFnu = high-frequency nu; LHnu = low-frequency nu to high-frequency ratio; SDNN = standard deviation of Normal to Normal interval, RMSSD = root mean squared differences of the standard deviation; pNN50 = percentage of beats that changed more than 50 milliseconds from the previous beat.

2.5 Discussion

Despite extensive use and research in both clinical and physiological settings, HRV analysis is still poorly supported by rigorous reliability studies (Pinna et al., 2007). The purpose of the present study was to examine the reliability and gender characteristics of standard parameters of HRV from short-term (5 minutes) laboratory recordings in physically active individuals. The main findings of this study were that the reliability of HRV recording over consecutive days varies depending on the specific reliability index used, the HRV parameter examined as well as the gender of the population. A comparison of HRV frequency domain parameters for males and females demonstrated significant gender differences in the sympathovagal balance.

Although the definition of a categorical rating of relative reliability based on ICC's is still controversial (Pinna et al., 2007), the results from the present study demonstrate substantial to excellent relative reliability for the majority of time and frequency domain HRV parameters when comparing measurements obtained from Days 3 and 4. For most measurements, the ICC was >0.80 ('good') (Atkinson and Nevill, 1998) indicating that the repeated measurements reflect mostly the true value of HRV parameters relative to random error. Females demonstrated a higher relative reliability than males for all frequency domain parameters. The high ICC of these short term recordings indicate a considerable consistency with time similar to previous studies (Pinna et al., 2007, Sinnreich et al., 1998).

However, absolute reliability, indicated by the TEM and TEM%, revealed the presence of a large random error in all HRV parameters, particularly for the males. Females demonstrate better absolute reliability (lower TEM) than males for all parameters particularly those in the frequency domain. The TEM% also indicated a low absolute reliability due to the high values found specifically for the LF/HFnu ratio. These findings are similar to those of Pinna et al (2007) who found a greater random error (TEM) in frequency domain measures (Pinna et al., 2007). These results might place doubts for the use of HRV indices in assessing interventions or treatment affects in individual participants specifically when examining male participants or clinical populations (Pinna et al., 2007). Furthermore, these results place doubt in the use of HRV measurement for monitoring performance changes in well trained athletes. The

high TEM and TEM% indicated that HRV would be unable to detect small but significant changes (≤ 1%) in performance (Lamberts et al., 2009). Considering the absolute reliability is low (high TEM and TEM% values), the large random error found in HRV would require improved changes in HRV to occur to deem them meaningful (Lamberts et al., 2009).

2.5.1 Gender Differences

Findings of research examining HRV gender differences in healthy individuals are conflicting (Ramaekers et al., 1998, Ryan et al., 1994, Sinnreich et al., 1998, Umetani et al., 1998). Research has demonstrated that HRV measures are either the same or differ considerably between the genders and may also be HRV parameter or age dependant (Ramaekers et al., 1998 , Ryan et al., 1994, Sinnreich et al., 1998, Umetani et al., 1998) and therefore, further research has been advocated (Aubert et al., 2003). Umetani et al. (1998) has shown that HRV (for all measures) is significantly lower in "young" (10-29 yrs) females compared to their age-matched male counterparts. The gender differences subsequently decreased and then disappeared with age and at different rates for the HRV parameters. Their findings suggested a higher level of parasympathetic activity in males (Umetani et al., 1998). Conversely, Ryan et al. (1994) concluded that vagal high frequency power was higher in females. They suggested this difference is most apparent for young (20-39) and middle aged (40-64) females. Raemaeker et al. (1998) noted that the LFnu (sympathetic dominance) was higher in females however, HFnu (parasympathetic dominance) was not significantly different between the genders (Ramaekers et al., 1998). Sinnreich et al. (1998) observed that RMSSD and HF component (measures reflecting predominantly vagal activity) were small and non significant, but that the LF/HF ratio (suggested to reflect sympathetic/parasympathetic balance) differed substantially due to greater VLF and LF power found in the male participants. They concluded that their results illustrated relatively higher sympathetic activity in men compared to women. Our findings demonstrate a similar gender distinction to that of Ryan et al. (1994) and Sinnreich et al. (1998). A higher level of parasympathetic activity was found in the female participants compared to the males. This was demonstrated by a lower LF/HFnu ratio and higher HF value in the females.

The finding of a higher HRV and contribution of the parasympathetic nervous system to HRV in females may help explain the overall protection of premenopausal females from coronary heart disease (CHD), coronary mortality and sudden cardiac death, compared to males in this age group (Ribeiro et al., 2001, Sinnreich et al., 1998). Research has demonstrated that a high HRV is associated with improved cardiovascular health. Although not measured in the present study, the effect of oestrogen on HRV and parasympathetic activity in females may be a key factor in this finding. Research examining HRV in pre and postmenopausal women found a significant difference in HRV (Moodithaya and Avadhany, 2009). Post menopausal women had a significantly reduced HRV, as demonstrated by a higher relative power of LF and LF/HF ratio, which was related to a decline in the level of oestrogen. The authors concluded that a decline in the level of oestrogen from premenopausal to postmenopausal status favours the shifting of autonomic balance towards sympathetic dominance (Moodithaya and Avadhany, 2009). In support of this finding, research has demonstrated that physiological levels of oestrogen increase vagal tone and suppresses sympathetic modulation of heart rate in females (Lui et al., 2003).

A limitation of this study was that respiratory rate was not controlled when measuring the IBI's over the five minute recording period. Research has shown that the reliability of HRV measures is increased when respiratory rate is regulated (Lopes and White, 2006). Furthermore, part of intra-subject variability is also due to the natural change of HRV parameters that occurs under the influences of factors such as mood, alertness and mental activity, which is very difficult to control for in any study (Pinna et al., 2007).

2.6 Conclusion

Heart rate variability measures are a popular, non-invasive tool used to monitor autonomic function. Short term recordings are easy to perform and are suitable for both clinical and physiological research. However, the findings of the present study have demonstrated a high relative reliability but low absolute reliability for HRV. In particular, HRV random error was higher in males. For clinical or sport/exercise science practice, these results place in doubt the use of HRV indices for assessing small (< 5%) intervention or treatment affects, specifically when examining male or clinical

populations (Pinna et al., 2007). Furthermore, these results place doubt on the use of HRV measurement for monitoring performance changes in well trained or elite athletes which typically require assessment techniques that can detect physiological or performance changes that are < 1% (29). Finally, specific HRV parameters differed between males and females indicating a greater parasympathetic modulation of HRV in females. This finding suggests a possible mechanism for why premenopausal females have a lower incidence of coronary heart disease compared to males in the same age group as well as compared to postmenopausal women.

CONCLUSION

The findings of the present study indicate a large day to day random variation in repeated measurements when using the Suunto t6 HRM and Kubios custom HRV software to assess short-term HRV parameters in healthy, physically active individuals. The statistical analysis performed on the numerous HRV parameters suggested good to excellent relative reliability (ICC). However, both the absolute reliability indices, TEM and TEM%, were high, indicating a large within subject variation, particularly in males in the frequency domain parameters. This high absolute reliability questions the use of HRV for intervention studies or for the investigation of changes, as small alterations would not be detected (Pinna et al., 2007).

Furthermore, in this study significant gender differences were found suggesting that women have a higher overall HRV compared to men. Higher LFnu and the higher LF/HFnu ratio which we have been observed in our male population can be attributed to higher sympathetic activity in males, this result is similar to findings by Ramaekers et al. (1998). Their observation on gender differences provided evidence that premenopausal women are at less risk of CHD and that females develop CV illness at a later age than males. Higher sympathetic activity has been linked to the development of CHD and a higher inclination to sudden cardiac death. At age 50 gender differences disappear (Umetani et al., 1998) and the rate of CHD in woman increase (Ramaekers et al., 1998). This observation suggests that younger premenopausal women have a cardio-protective mechanism which maybe linked to increased levels in the hormone oestrogen (Ramaekers et al., 1998 , Lui et al., 2003).

The results of the present study can provide a guideline when comparing clinical and normal population groups in this age category. However exercise interventions including the affect of different types of training (i.e. resistance training) necessitate further investigation on HRV and ANS. Exercise training has been shown to have a positive effect on HRV and therefore can improve health status of the population as well as monitor changes in athletes and prevent incidences such as overtraining

syndrome (Berkoff et al., 2007, Seileret al., 2007). Furthermore our research into the statistical analysis of HRV has revealed that both relative and absolute reliability has to be calculated to provide adequate basis for reproducibility. Standardization of methodology of HRV and calculation of sample size is a crucial aspect of HRV reliability studies (Sandercock et al., 2007).

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APPENDIX

Appendix I

Information Sheet

Five minute recordings of heart rate variability in physically active students: reliability and gender characteristics

INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?

This project is being undertaken as part of the requirements for a postgraduate degree in Biokinetics. The aim is to evaluate the stability of short recordings of heart rate variability (HRV) with time, and the association of HRV with age and sex.

What Type of Participants are Needed?

This study requires participants aged 19 to 24years. The age, gender, and racial characteristics of the volunteer group will be matched and volunteers will be excluded if they are or may have been pregnant; if they have diagnosed diabetes, congestive heart failure, or acute or chronic renal disease; if they have a pacemaker; or if they are currently taking type 1A antiarrhythmics (quinidine, procainamide, disopyramide, or moricizine).

What will Participants be Asked to Do?

Should you agree to take part in this project, you will be asked to fill out forms and be involved in a testing protocol. Written informed consent will be obtained from all participants, and the study will be approved by the University of KwaZulu-Natal Research Ethics Committee.

We will examine participants in the Human Performance Laboratory situated in the Discipline of Sport Science at the University of KwaZulu-Natal. Each participant will attend 5 visits, each approximately 30 minutes long, for 5 days in a row at the same time of the day (7-9am).

The following tests and evaluations will be performed:

a) **Personal/Medical History Form:** you will be required to complete the enclosed personal/medical history form. The purpose of completing this form is to ensure that you meet the requirements to be included in the study, and that the researcher obtains necessary information about your lifestyle (including physical activity history)

b) **Testing Protocol:** you will have your height, weight, BMI, waist and hip circumferences, 3 site skin folds and HRV reading over a 5 minute period will be recorded.

Possible risks and discomforts:

Resting Measures: Height, weight, and circumference measurements will produce no physical discomfort.

Heart Rate Variability Monitoring: The participant will be required to wear a heart rate monitor across his/her chest, this should bring about minimal or no discomfort.

Potential benefits of this study:

This Study aims will provide baseline data for future use of HRV as a reliable tool in assessing various conditions.

Please be aware that you may decide not to take part in the project without any disadvantage to yourself of any kind.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What Data or Information will be collected and What Use will be made of it?

The information will evaluate stability of short recordings of heart rate variability (HRV) with time, and the association of HRV with age and sex.

The information obtained will be used to provide baseline normative data. This will primarily allow the researcher to determine whether the use of HRV is a reliable and valid tool.

Results of this project may be published but any data included will in no way be linked to any specific participant.

You are most welcome to request a copy of the results of the project should you wish.

The data collected will be securely stored in such a way that only those mentioned above will be able to gain access to it. At the end of the project any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.

What do participants have to avoid prior to testing?

We will ask participants to avoid caffeine, eating, heavy physical activity, smoking, and alcohol intake for the 10 hours preceding each laboratory visit. We will identify individuals who have not been fasting for 10 hours or who smoked in the morning, but will not exclude protocol violators from participating in the study.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:-

Takshita Sookan or Professor Andrew McKune

Department of Sport Science Department of Sport Science

Telephone Number: 0826390875 University Telephone Number: 031 2607394

This project has been reviewed and approved by the Faculty Ethics Committeeof the UNIVERSITY OF KWAZULU-NATAL

Appendix II

Informed Consent Sheet

Consent to Participate in Research

Dear _____

You have been invited by Masters Candidate Takshita Sookan and Associate Professor Andrew McKune from the Discipline of Sport Science, University of KwaZulu-Natal, to participate in a study examiningfive minute recordings of heart rate variability in physically active students: repeatability and age–sex characteristics.

The researcher will recruit 50 participants aged 19 to 24 years. The age, gender, and racial characteristics of the volunteer group will be matched and volunteers will be excluded if they are or may have been pregnant; if they have diagnosed diabetes, congestive heart failure, or acute or chronic renal disease; if they have a pacemaker; or if they are currently taking type 1A antiarrhythmics (quinidine, procainamide, disopyramide, or moricizine).

PLAN AND PROCEDURES:

Data

(a) Contact Details: I agree to give basic information and contact details about myself to the researcher including my name, age (date of birth), address and phone number.

(b) Medical History/Physical Activity Form: I agree to give information about my medical history and physical activity. The purpose of completing this form is to ensure that I meet the medical requirements to be included in this study and that the researcher obtains information to declare me “apparently healthy” for inclusion as a participant.

(c) Baseline Data Collection: I agree to have my height, weight, waist and hip circumference, skinfold measurements, resting heart rate and heart rate variability readings for 5 minutes.

RISKS AND DISCOMFORTS

Baseline Measurements: Measurement of height, weight, waist and hip circumference, heart rate, will produce no physical discomfort. Skinfold (testing body fat %) may produce slight discomfort for a few seconds when the skin is pinched to get a measurement but will disappear immediately after releasing skin.

POTENTIAL BENEFITS

To evaluate the stability of short recordings of heart rate variability (HRV) with time, and the association of HRV with age and sex.

TERMINATION OF PARTICIPATION

I understand that if the screening and data collection procedures provide evidence that the tests or activities cannot be safely performed , or if I have a pre-existing condition which will not allow me to participate in the study, I will be informed at that time and will not be included in the study. I understand that the investigator will explain the reason for the exclusion to me.

COSTS/COMPENSTAION

The policy of the University of Kwa-Zulu Natal does not provide for compensation or medical treatment to participants who are injured as a result of this research study. However, every effort will be made to make the tests and activities as safe as possible, with little risk of injury.

CONFIDENTIALITY

All data and information collected in this study will be maintained in complete confidence and privacy will be protected. I will not be identified in any report or presentation by name as a result of this study.

You have been informed about the study in detail by_____.

You may contact the investigators in this study Masters candidate Takshita Sookan (0826390875), Associate Prof Andrew McKune (031-260-7985), any time if you have questions about the research or if you are injured as a result of the research.

You may contact the **Biomedical Research Ethics Office** on **031-260 4769 or 260 1074** if you have questions about your rights as a research participant.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to stop at any time.

If you agree to participate, you will be given a signed copy of this document and the participant information sheet which is a written summary of the research.

The research study, including the above information, has been described to me orally. I understand what my involvement in the study means and I voluntarily agree to participate. I have been given an opportunity to ask any questions that I might have about participation in the study.

_____ _____

Signature of Participant **Date**

_____ _____

Signature of Witness **Date**

Appendix III

Medical History Questionnaire

Name_____Sex_____Age_____Date of Birth_____
Sport(s)_____Phone_____E-mail Address_____
In case of emergency, contact
Name_____Relationship_____Phone(H)_____(W)_____

Explain "Yes" answers on second page	Y	N
1. Has a doctor even denied or restricted your participation in sports for any reason?	☐	☐
2. Do you have an ongoing medical condition (like diabetes or asthma)?	☐	☐
3. Are you currently taking any prescription or nonprescription (over-the-counter) medicines or pills?	☐	☐
4. Do you have allergies to medicines, pollens, foods, or stinging insects?	☐	☐
5. Have you ever passed out or nearly passed out DURING exercise?	☐	☐
6. Have you ever passed out or nearly passed out AFTER exercise?	☐	☐
7. Have you ever had discomfort, pain, or pressure in your chest during exercise?	☐	☐
8. Does your heart race or skip beats during exercise?	☐	☐
9. Has a doctor ever told you that you have (check all that applies)? ☐ High Blood Pressure ☐ High Cholesterol ☐ A heart murmur ☐ A heart infection	☐	☐
10. Has a doctor ever ordered a test for your heart? (for example, ECG, echocardiogram)	☐	☐
11. Has anyone in your family died for no apparent reason?	☐	☐
12. Does anyone in your family have a heart problem?	☐	☐
13. Has any family member or relative died of heart problems or of sudden death before age 50?	☐	☐
14. Does anyone in your family have Marfan's syndrome?	☐	☐
15. Have you ever spent the night in a hospital?	☐	☐
16. Have you ever had surgery?	☐	☐
17. Have you ever used an inhaler or taken asthma medicine?	☐	☐
18. Were you born without or are you missing a kidney, an eye, a testicle, or any other organ?	☐	☐
19. Have you had infectious mononucleosis (mono) within the last month?	☐	☐
20. Do you have any rashes, pressure sores, or other skin problems?	☐	☐
21. Have you had a herpes skin infection?	☐	☐
22. Have you ever had a head injury or concussion?	☐	☐
23. Have you been hit in the head or been confused or lost your memory?	☐	☐
24. Have you ever had a seizure?	☐	☐
25. Do you have headaches with exercise?	☐	☐
26. Have you ever had numbness, tingling, or weakness in your arms or legs after being hit or falling?	☐	☐
27. Have you ever been unable to move your arms or legs after being hit or falling?	☐	☐
28. When exercising in the heat do you have severe muscle cramps or become ill?	☐	☐
29. Has a doctor told you that you or someone in your family has sickle trait or sickle cell disease?	☐	☐
30. Have you had any problems with your eyes or vision?	☐	☐
31. Do you wear glasses or contact lenses?	☐	☐
32. Do you wear protective eyewear, such as goggles or a face shield?	☐	☐
33. Are you happy with your weight?	☐	☐
34. Are you trying to gain or lose weight?	☐	☐
35. Has anyone recommended you change your weight or eating habits?	☐	☐

36. Have you ever had a stress fracture?	☐ ☐	42. Do you limit or carefully control what you eat?	☐ ☐
37. Have you been told that you have or have you had an x-ray for atlantoaxial (neck)?	☐ ☐	43. Do you have any concerns that you would like to discuss with a doctor?	☐ ☐
38. Do you regularly use a brace or assistive device?	☐ ☐	**FEMALES ONLY**	
39. Has a doctor ever told you that you have asthma or allergies?	☐ ☐	44. Have you ever had a menstrual period?	☐ ☐
40. Do you cough, wheeze, or have difficulty breathing during or after exercise?	☐ ☐	45. How old were you when you had your first menstrual period?	_____
41. Is there anyone in your family who has asthma?	☐ ☐	46. How many periods have you had in the last year?	_____

Explain "Yes" answers from previous page here:_____

_____

_____

_____

_____

_____

List all *previous injuries* and *approximate dates.* Check N/A if not applicable

N/A ☐ Shoulder/Elbow (dislocation, rotator cuff, AC separation):_____ Date:_____

N/A ☐ Arm/Wrist/Hand (fractures):_____ Date:_____

N/A ☐ Neck (burners, pinched nerve):_____ Date:_____

N/A ☐ Ribs/Abdomen:_____ Date:_____

N/A ☐ Low back pan (herniated disc):_____ Date:_____

N/A ☐ Leg (quadriceps, hamstring strain):_____ Date:_____

N/A ☐ Knee (ligament, meniscus, patella):_____ Date:_____

N/A ☐ Lower leg (shin splints, calf strain):_____ Date:_____

N/A ☐ Ankle/Calf/Foot (sprain, Achilles):_____ Date:_____

N/A ☐ Stress Fractures:_____ Date:_____

N/A ☐ Concussions:_____ Date:_____

If yes, have you ever been knocked out (unconscious)? Yes: ☐ No: ☐

How many times?_____

How long were you unconscious?_____

Have you ever lost your memory? Yes: ☐ No: ☐

How many times?_____

Did you have problems in the days afterward (confusion, headache, concentration)?

Yes: ☐ No: ☐

How long did it take you to recover?_____

Are you still having problems? Yes: ☐ No: ☐

Do you have any unhealed or chronic injuries? Yes: ☐ No: ☐

Please list:_____

_____

_____

I hereby state that, to the best of my knowledge, my answers to the above questions are complete and correct.

Signature of athlete_____ Date_____

Signature of parent/guardian_____ Date_____

Appendix IV

Data Recording Sheet.

Basic Information and Contact Details:

Date:_____

Name:_____

STUDY ID#:_____

Gender:_____

DOB (dd/mm/yyyy):_____

Age:_____

Phone Number:_____

Address:_____

Measurements

Height (m):_____

Weight (kg):_____

BMI:_____

Waist Circumference (cm):_____

Hip Circumference(cm):_____

Waist to Hip Ratio_____

3 Site Skinfold

Females Males

1.	Triceps: _____mm	1) Pectoral:_____mm
2.	Suprailiac:_____mm	2) Abdomen:_____mm
3.	Mid Thigh: _____mm	3) Mid Thigh:_____mm

Appendix V

International Physical Activity Questionnaire.

(October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please

consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**

_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**

_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**

_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days walking from place to place?

_____ **hours per day**

_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**

_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**

_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

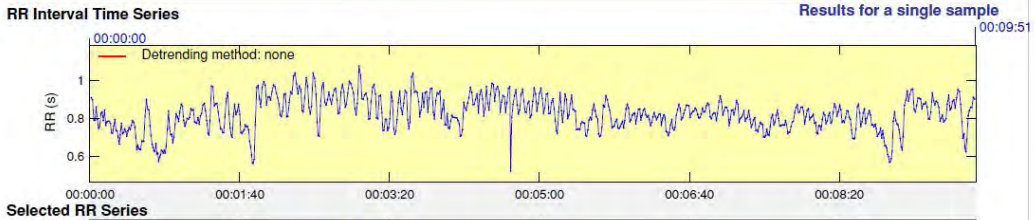
Appendix VI

Kubios Custom Heart Rate Variability Software Document.

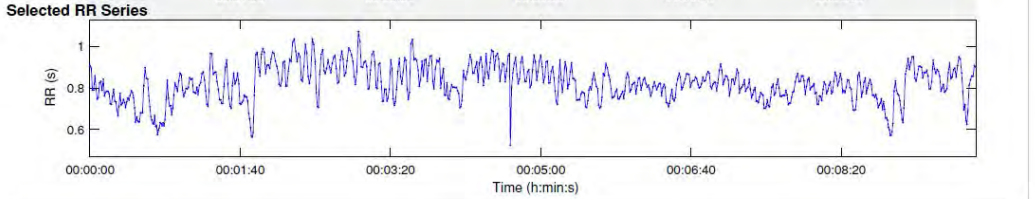
HRV Analysis Results

Taks Resting 2009.txt – xx/xx/xx – xx:xx:xx
Page 1/1

RR Interval Time Series — **Results for a single sample**



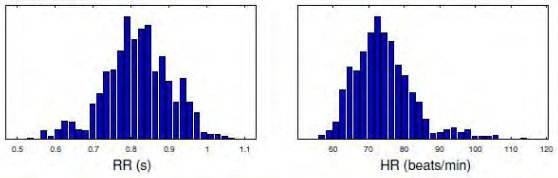
Selected RR Series



Time–Domain Results

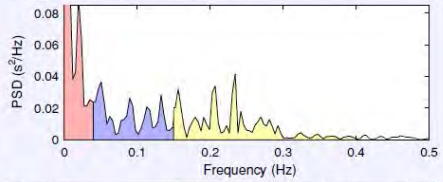
Variable	Units	Value
Mean RR*	(ms)	817.6
STD RR (SDNN)	(ms)	87.5
Mean HR*	(1/min)	74.28
STD HR	(1/min)	8.48
RMSSD	(ms)	55.8
NN50	(count)	208
pNN50	(%)	28.8
RR triangular index		21.235
TINN	(ms)	410.0

Distributions*



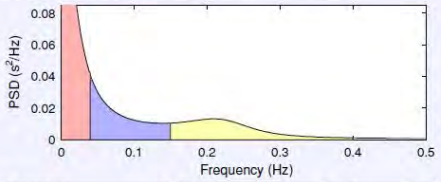
Frequency–Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms²)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	3129	46.6	
LF (0.04–0.15 Hz)	0.0508	1608	23.9	44.8
HF (0.15–0.4 Hz)	0.2344	1980	29.5	55.2
Total		6716		
LF/HF		0.812		

AR Spectrum (AR model order = 16, not factorized)

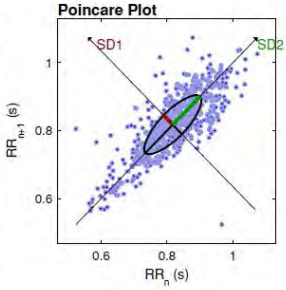


Frequency Band	Peak (Hz)	Power (ms²)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	3610	51.1	
LF (0.04–0.15 Hz)	0.0430	1819	25.7	52.6
HF (0.15–0.4 Hz)	0.2109	1640	23.2	47.4
Total		7069		
LF/HF		1.109		

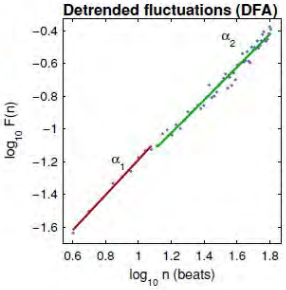
Nonlinear Results*

Variable	Units	Value
Poincare plot		
SD1	(ms)	39.5
SD2	(ms)	117.3
Recurrence plot		
Mean line length (Lmean)	(beats)	13.51
Max line length (Lmax)	(beats)	373
Recurrence rate (REC)	(%)	34.89
Determinism (DET)	(%)	98.91
Shannon Entropy (ShanEn)		3.411
Other		
Approximate entropy (ApEn)		1.294
Sample entropy (SampEn)		1.420
Detrended fluctuations (DFA): α1		1.076
Detrended fluctuations (DFA): α2		1.005
Correlation dimension (D2)		3.623

Poincare Plot



Detrended fluctuations (DFA)



*Results are calculated from the non–detrended selected RR series.

29–Jul–2009 10:47:10
Andrew McKune
Sport Science, Human Performance Lab, University of KwaZulu–Natal

Kubios HRV, version 2.0
Department of Physics
University of Kuopio, Finland

Appendix VII

AFRICA CARDIOVASCULAR JOURNAL OF AFRICA • Advance Online Publication, June 2011 1

Cardiovascular Topics

Heart rate variability in physically active individuals: reliability and gender characteristics

TAKSHITA SOOKAN, ANDREW J MCKUNE

Abstract

Purpose: To evaluate the reliability of short-term recordings (five minutes) of heart rate variability (HRV) and the association between HRV and gender.

Methods: HRV time- and frequency-domain parameters were calculated in 44 physically active students (21 males and 23 females) over four consecutive days. A Suunto t6 heart rate monitor was used to obtain inter-beat intervals (IBIs) that were then transferred to Kubios HRV analysis software. The relative reliability [intra-class correlation (ICC)] and absolute reliability, [typical error of measurement (TEM) and typical error of measurement as a percentage (TEM%)] of the HRV parameters were then calculated for day 2 versus day 3 and day 3 versus day 4, with day 1 being a familiarisation day. The following HRV parameters were calculated: (1) time domain: resting heart rate (RHR), R–R intervals (IBI), standard deviation of normal-to-normal intervals (SDNN), root mean square differences of the standard deviation (RMSSD), percentage of beats that changed more than 50 ms from the previous beat (pNN50); and (2) frequency domain: low-frequency normalised units (LFnu), high-frequency normalised units (HFnu), low-frequency to high-frequency ratio in normalised units (LF/HFnu). An analysis of variance (ANOVA) with Tukey *post-hoc* testing was performed to compare HRV parameters in males and females. Significance was set at $p \leq 0.05$.

Results: The ICCs for both relationship 1 and 2 indicated primarily good to excellent (> 0.8) relative reliability. The lowest value was found in the LF/HFnu ratio (ICC = 0.36) for males. Absolute reliability was low with TEM% greater than 10% for all HRV parameters, except IBIs. Females demonstrated better relative (higher ICCs) and absolute reliability (lower TEM and TEM%) compared to males for the frequency domain. The relative and absolute reliability for the time domains were similar except for SDNN where the absolute reliability was higher in males. ANOVA illustrated significant gender differences for the LF/HFnu ratio (41% higher in males, $p = 0.003$), HFnu (12% higher in females, $p = 0.02$) and IBI (21% lower in females, $p < 0.0001$).

Conclusions: Short-term recordings of HRV over three consecutive days demonstrated a high relative reliability. However, a low absolute reliability indicated large day-to-day random variation in HRV, which would make the detection of intervention effects using HRV difficult in individual participants. Females were shown to have a higher parasympathetic modulation of HRV, which may indicate an underlying cardioprotective mechanism in females compared to males.

Keywords: heart rate variability, sympathovagal balance, reliability

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Heart rate variability (HRV) is recognised as a versatile and promising non-invasive marker of autonomic nervous system (ANS) modulation.[1] Research into the use of HRV has increased in both clinical and research environments and over a broad spectrum of disciplines.[2,3] However, in the disciplines of sport and exercise science, there is limited information available on the reliability of HRV measures, in particular related to new, commercially available equipment that coaches and athletes have access to. This fact, together with the extensive number of variables that alter HRV measures, make it difficult to compare HRV studies and develop a universal standard.[2] While most studies agree that age is inversely associated with HRV,[4] research is less consistent on the impact of gender, with studies demonstrating that HRV measures are either the same or differ considerably between the genders and may also be HRV parameter dependant.[5,6]

Heart rate variability reflects the changes in the interval between heart beat (R waves) over time. The time between one R wave and the next, in milliseconds, is termed the R–R interval or the interbeat interval (IBI).[1] The ANS governs the IBIs via the sympathetic and parasympathetic pathways.[6] The relative dominance of either pathway over the other represents an alteration in the sympathovagal balance which is reflected in IBI changes.[1] Under normal resting conditions in healthy individuals, it has been suggested that the parasympathetic pathway is dominant, resulting in a high HRV,[4] while lower HRV and poor health has been linked to increased sympathetic activity at rest.[3,7] However, research has demonstrated that the age, physical activity status, gender and the HRV parameter examined are important factors to consider when examining HRV.[5,6]

Research has identified the potential use of HRV for identifying healthy and diseased states. In particular, a significant relationship between the ANS, low HRV and cardiovascular mortality, including sudden cardiac death, has been reported.[8,13]

In addition, studies have shown that trained athletes have higher HRV compared to sedentary individuals, suggesting that exercise training can increase HRV in normal populations.[14] The over-training syndrome is assumed to be the consequence of an imbalance between long-term, inappropriate, high training volume and too little time for regeneration.[15,16] Alterations in the ANS have been presented as a mechanism underlying the signs and symptoms of the over-training syndrome.[17] A study that examined ANS activity in several middle-distance runners suggested that heavy training shifted the cardiac autonomic balance toward a predominance of the sympathetic over the parasympathetic drive, which was represented by a decrease in HRV.[17]

Heart rate variability has the potential to be a useful monitoring tool in the fields of health, fitness and sports performance. However, currently, there are issues regarding the standardisation and reproducibility of HRV measurement, as there are many confounding factors that can influence HRV. These include factors such as mood, alertness, mental activity, gender and age.[3,6,18] While the research relating to gender differences is controversial,[5,7,18] the relationship between age and HRV has been well documented.[5,6,8,19] Reduced HRV is associated with progressing age and with an increased risk of cardiac events in clinically disease-free patients.[14]

The development of wireless heart rate-monitoring equipment, which has the ability to record IBIs has provided athletes, coaches, scientists and medical practitioners with mobile and easy-to-use systems that allow for the analysis of HRV. The commercially available Suunto t6 heart rate system (Suunto; Vantaa, Finland) is one such instrument that is widely used for monitoring heart rate during exercise. The validity of the Suunto t6 in measuring IBI for determining HRV has been reported recently.[1]

While previous studies have examined the reliability of other commercially available devices (Polar S810) for measuring HRV,[1,2] there is limited information on the reliability of the Suunto t6 for HRV measurement.[1,20] Analysis of the IBIs to determine HRV can be performed using custom software such as Kubios heart rate variability software version 2.0 (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland).[20,21] To date, there are few publications reporting the use of both the Suunto t6 and Kubios software together.[20]

The primary aim of this study was therefore to investigate the reliability of Kubios software HRV measures calculated from the Suunto t-6 IBIs in physically active individuals. Furthermore, we investigated whether there were any gender differences in HRV parameters.

Methods

This study was conducted on 50 physically active young adults (males: n = 25; females: n = 25), although data analysis was performed on 21 males and 23 females due to exclusion criteria, discussed in the statistical analysis of the data (Table 1). There were significant differences between the genders in terms of height, mass and percentage body fat, but no differences in age, body mass index, waist-to-hip ratio or weekly physical activity levels (Table 1). Participation was voluntary, and written informed consent was obtained from all participants. The study was approved by the Institution's Biomedical Research Ethics Committee (REF: BE111/010).

Participants were excluded from the study if they had experienced a cold or feverish illness in the month leading up to the study, were smokers, had a pre-existing heart condition either current or in the past, were pregnant, diabetic, had congestive heart failure, or acute or chronic renal disease, if they have a pacemaker, or were taking type 1A anti-arrhythmics (quinidine, procainamide, disopyramide or moricizine).

Assessment of physical activity status

The International Physical Activity questionnaire (IPAQ) is a validated questionnaire primarily designed for population surveillance of physical activity among adults (age range 15–69 years).[22] This questionnaire was used to classify the physical activity status of the participants in the week leading up to the first (day 1) testing day. The IPAQ requires the summation of duration (in minutes) and frequency (days) for different categories of physical activity.

Based on this information, MET-minutes/week are calculated with the MET minute scores being equivalent to kilocalories expended per week. The participants were then classified into low, moderate or high physical activity levels. On average, the participants in the study were classified as being in the moderate category, with an average of 5 828 kcals (Table 1) expended per week (833 kcal expended per day). Moderate is defined by the IPAQ guidelines as a pattern of activity done on three or more days, at least 20 minutes per day and described as vigorous-intensity activity, or five or more days of moderate-intensity activity and/or walking at least 30 minutes per day.

Protocol

Testing was conducted within the human performance laboratory (HPL) at our institution. The temperature in the HPL was maintained at 22°C with 50% humidity. Participants were provided pre-test instructions the week before the testing to help control for factors that could alter heart rate variability readings. They were asked to avoid caffeine, eating, heavy physical activity, smoking and alcohol intake for the 10 hours preceding each laboratory visit. Each participant attended four testing sessions for four days in a row at the same time of the day. Testing was performed between 07:00 and 21:00.

On testing day 1, which counted as the familiarisation day, each participant read the information sheet provided on the study, signed a written informed consent, completed a pre-test

TABLE 1. DESCRIPTIVE DATA, PHYSICAL CHARACTERISTICS AND PHYSICAL ACTIVITY LEVEL OF MALES AND FEMALES (MEAN ± SD)

	Males (n = 21)	*Females (n = 23)*	*p*
Age (years)	21.17 (1.55)	19.75 (1.76)	0.45
Height (cm)	177.3 (9.09)	160.5 (6.49)	< 0.0001*
Mass (kg)	76.19 (14.69)	60.41 (9.64)	0.0001*
BMI (kg/m²)	24.03 (2.64)	23.41 (2.89)	0.467
Waist/hip ratio	0.88 (0.07)	0.85 (0.068)	0.123
Percent body fat	13.73 (4.53)	18.63 (3.77)	0.0004*
IPAQ (Kcals/week)	5828 (3806)	5491 (3751)	0.789

IPAQ = International Physical Activity questionnaire.

questionnaire (IPAQ) and medical history questionnaire. The medical questionnaire examined cardiovascular, metabolic and respiratory disease history (personal and family) as well as risk factors and signs and symptoms for these diseases. In addition, participants provided information on their current medication and supplement intake.

Height, mass, waist and hip circumferences and three site skinfolds (females: tricep, supra-iliac and mid-thigh; males: chest, abdominal and mid-thigh) were then measured. Height and weight were recorded using a calibrated medical height gauge and balance scale (Detecto, Webb City, USA). A Harpenden skinfold calliper was used for skinfold measurements to calculate percentage body fat using the Jackson and Pollock equations.[23,24] Body mass index (BMI) [mass (kg)/height (m^2)] and waist-to-hip ratio (waist:hip circumferences) were calculated and the HRV measurement protocol was then followed.

Heart rate variability measurement

The participants were fitted with the Suunto t6 heart rate monitor (HRM) (Suunto; Vantaa, Finland). The electrodes on the transmitter were wet with water and were placed on the chest against bare skin to ensure good skin contact. Participants were tested while lying supine with the total testing time lasting 20 minutes. This time was divided into 15 minutes of rest followed by a five-minute measurement of IBIs. The IBIs were then transferred to a laptop (HP ProBook) computer where the data were stored in the Suunto team manager software program (Firstbeat Technologies, Ltd; Jyvaskyla , Finland).

The data were then exported as a text file to the HRV analysis software (Kubios heart rate variability software version 2.0; Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) for analysis of the following HRV parameters (1) time domain: resting heart rate (RHR), R–R intervals (IBI), standard deviation of normal-to-normal intervals (SDNN), root mean square differences of the standard deviation (RMSSD), percentage of beats that changed more than 50 ms from the previous beat (pNN50); and (2) frequency domain: low-frequency normaliszed units (LFnu), high-frequency normalised units (HFnu), low-frequency to high-frequency ratio in normalised units (LF/HFnu).

The Suunto t6 HRM and Kubios program[20] comply with guidelines recommended by the Taskforce of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology standards for measurement of HRV.[4] Before processing, the IBIs were manually corrected for ectopic/missed beats. There is currently no universal method for identifying and editing ectopic beats. The amount and type of editing of IBI data has different effects on various HRV indices.[25] In the present study, manual editing or interpolation[25] of the IBI intervals was performed using the following guidelines: if a significantly higher IBI (representing an ectopic beat) was noted, then that reading was deleted and the average of the two adjacent IBIs replaced the deleted one. If a significantly lower value (representing a missed beat) was noted, that IBI was deleted and replaced with the previous IBI. If the ectopic or missed beats exceeded 20% of a participant's overall five-minute recording, the participant was not included in the analysis.[3] This occurred in six participants (four males and two females), with the result that only 44 participants were included in the final analysis (n = 21 males and n = 23 females).

Once the IBIs were imported into the Kubios program, the software automatically analysed the HRV in both time and frequency domains. Power spectral analysis was performed using the autoregressive (AR) algorithm in accordance with the recommendations.[4] The AR algorithm was used as it yields improved resolution, especially for short-term HRV measurements.[20,21] This algorithm creates a power spectral analysis with distinct frequency bands, namely high frequency (HF), low frequency (LF) and very low frequency (VLF).

The LF component has been proposed as reflecting both sympathetic and parasympathetic effects on the heart and occurs in a band between 0.04 and 0.15 Hz. However, researchers noted that the low-frequency band is influenced by baroreceptor-mediated regulation of blood pressure and reflects predominantly sympathetic activity.[26] The HF (0.15–0.4 Hz) band corresponds with respiratory sinus arrhythmia (RSA) and is said to reflect parasympathetic activity.[26] Chemoreceptor processes, thermoregulation, and the renin–angiotensin system have been linked with the VLF band. We did not use data collected from the VLF range for this study.

Statistical analysis

The data were summarised using routine descriptive statistics (mean ± SD). Reliability measures were determined for day 2 versus day 3 and day 3 versus day 4. Absolute and relative reliability of several HRV parameters was calculated using the procedures described by Hopkins.[27] Hopkins has argued that the statistical analysis used in reliability studies should include observed values and confidence limits of the typical error. These measures are sufficient to characterise the reliability of a measure and they substantially enhance comparison of the reliability of tests, assays or equipment.[27]

Absolute reliability is the degree to which repeated measurements vary for individuals. This type of reliability is expressed either in the actual units of measurement or as a proportion of the measured values (dimensionless ratio).[28] Absolute reliability was calculated using the typical error of measurement (TEM) and TEM as a percentage (TEM%), expressed as percentage of the mean score.[27]

Sport and exercise science reliability studies have rarely reported the separate analysis of homoscedastic and heteroscedastic data.[28] These parameters show how the measurement error relates to the magnitude of the measured variable. When the amount of random error increases as the measured values increase, the data are said to be heteroscedastic. When there is no relation between the error and the size of the measured value, the data are described as homoscedastic.[27] Homoscedastic errors are expressed in the actual units of measurement (TEM) but heteroscedastic data are measured on a ratio scale. With homoscedastic errors, the raw data are analysed with conventional parametric analyses, but heteroscedastic data are transformed logarithmically before analysis or investigated with an analysis based on ranks.[28]

In the present study, the HRV data for each parameter were examined using the technique described by Hopkins[27] and were found to be homoscedastic. TEM and TEM% were calculated with 90% CI for day 2 versus day 3 and for day 3 versus day 4, using a spreadsheet downloaded from http://www.newstats.org.

The TEM is also known in statistical terms as the coefficient of variation (CV).

Furthermore, for reliability studies, it has been suggested that relative reliability is presented together with absolute reliability.[26] Relative reliability is the degree to which individuals maintain their position in a sample with repeated measurements and is represented in the form of correlation coefficients. In this study we used interclass correlations (ICC) with 95% CI.[27] The main advantage of ICC over Pearson's correlation is that it can be used when more than one retest is being compared with a test.[28] Various categories of reliability are based on the ICC. An ICC above 0.8 is usually regarded as good to excellent reliability, whereas an ICC between 0.6 and 0.8 may be taken to represent substantial reliability.[3]

In order to determine whether there were any gender differences in the HRV parameters, the data for each HRV parameter for days 2, 3 and 4 were combined and analysis of variance (ANOVA), with a Tukey *post-hoc* testing was performed to compare males with females. The data were analysed with STATISTICA version 8.0 (Statsoft Inc, Tulsa, Oklahoma, USA) for any statistical significance ($p \leq 0.05$).

Results

A total of 44 participants with complete data sets were analysed. The outcomes of the reliability analysis are presented in Table 2 for day 2 versus day 3 and day 3 versus day 4.

Typical error of measurement

The absolute reliability indices for the HRV parameters are shown in Table 2. The data presented here offer precision estimates for single measurements of Suunto t6 HRV and data for making decisions when monitoring changes or responses to interventions in individuals.

Overall for both males and females, the results show that for most HRV frequency-domain parameters, the second comparison day, day 3 versus day 4 had lower TEM values compared to the first day, day 2 versus day 3. This slight decrease in the TEM could be due to a familiarisation effect.[3] Females demonstrated better absolute reliability in the HRV frequency domain as demonstrated by lower TEMs. Specifically, the TEMs for day 3 versus day 4 were lower in females for the LF/HFnu ratio (116% lower), 90% lower for HFnu (day 2 vs day 3) and 68% lower for LFnu (day 2 vs day 3). The lowest typical error of measurement as a percentage (TEM%) was 11.5% and was found in the females for Hfnu (day 2 vs day 3).

Time-domain results had a low TEM% for IBIs (day 2 vs day 3: 4.8 males and females; day 3 vs day 4: 4.9 females and 4.1 males). Overall, the TEM% was relatively high in most HRV parameters, specifically for LF/HFnu (day 2 vs day 3: 31.4% females and 48.1% males; day 3 vs day 4: 29.7% females and 40.4% males). The TEM was similar for males and females for the time-domain parameters, specifically, RMSSD, pNN50 and the IBIs. However, the TEM for SDNN was 42% lower in the males (day 3 vs day 4).

Interclass correlations

The interclass correlations (ICCs) for relationship 1 ranged between 0.36 and 0.88 for the AR frequency domains and 0.70–0.92 for the time domains (Table 2). For day 3 versus day 4, the ICCs ranged between 0.72 and 0.86 for the AR frequency domains and 0.72–0.93 for the time domains. The ICCs for both day 2 versus day 3 and day 3 versus day 4 indicated good to excellent (> 0.8) reliability correlations for IBIs and pNN50 from the time-domain results. RMSSD reliability was good to excellent with the exception of the males for day 3 versus day 4, and showed substantial reliability of 0.79. SDNN variable results depicted substantial reliability for both day 2 versus day 3 and day 3 versus day 4.

TABLE 2. RELIABILITY INDICES FOR FREQUENCY AND TIME DOMAIN HRV PARAMETERS

HRV parameters		*Day 2 vs day 3*			*Day 3 vs day 4*		
		TEM	*TEM (%)*	*ICC*	*TEM*	*TEM (%)*	*ICC*
LF/HFnu	Female	0.25 (0.20–0.36)	31.4 (25.1–45.2)	0.80 (0.59–0.91)	0.24 (0.19–0.35)	29.7 (23.5–43.4)	0.83 (0.63–0.92)
	Male	0.54 (0.41–0.78)	48.1 (36.5–69.5)	0.36 (0.08–0.69)	0.43 (0.33–0.62)	40.4 (31.0–58.3)	0.72 (0.41–0.88)
HFnu	Females	6.63 (5.13–9.38)	11.5 (8.9–16.2)	0.88 (0.74–0.94)	7.92 (6.13–11.21)	13.4 (10.3–18.9)	0.81 (0.61–0.92)
	Males	12.58 (9.62–18.17)	24.6 (18.8–35.5)	0.55 (0.17–0.79)	8.95 (6.85–12.93)	16.7 (12.8–24.2)	0.78 (0.53–0.90)
LFnu	Female	7.78 (6.26–10.38)	17.8 (14.3–23.8)	0.84 (0.70–0.92)	7.26 (5.84–9.69)	17.2 (13.9–23.0)	0.86 (0.74–0.93)
	Male	13.04 (10.41–17.70)	26.6 (21.2–36.1)	0.52 (0.19–0.74)	9.40 (7.50–12.76)	20.2 (16.11–27.4)	0.75 (0.54–0.88)
IBIs (ms)	Females	44.90 (35.21–61.98)	4.8 (3.8–6.6)	0.83 (0.67–0.91)	45.89 (35.99–63.34)	4.9 (3.9–6.9)	0.79 (0.60–0.89)
	Males	53.46 (40.90–77.20)	4.8 (3.6–6.9)	0.90 (0.79–0.95)	46.46 (35.54–67.09)	4.1 (3.2–5.9)	0.93 (0.85–0.96)
SDNN	Females	28.83 (22.30–40.80)	31.2 (24.1–44.1)	0.77 (0.54–0.90)	35.58 (27.52–50.36)	32.2 (28.8–52.7)	0.72 (0.45–0.87)
	Males	20.70 (15.83-29.89)	19.9 (15.2–28.8)	0.70 (0.40–0.87)	20.62 (15.78–29.78)	20.2 (15.4–29.1)	0.73 (0.45–0.88)
RMSSD	Females	17.29 (13.37–24.47)	20.5 (15.8–29)	0.92 (0.82–0.97)	20.12 (15.56–28.48)	22.6 (17.5–32)	0.91 (0.80–0.96)
	Males	19.12 (14.63–27.62)	18.7 (14.3–27.1)	0.83 (0.64–0.91)	20.53 (15.71–29.65)	20.3 (15.5–29.3)	0.79 (0.55–0.89)
pNN50	Females	9.56 (7.39–13.53)	19.1 (14.8–27)	0.84 (0.67–0.93)	8.80 (6.80–12.45)	18.7 (14.4–26.4)	0.87 (0.72–0.94)
	Males	7.55 (5.78–10.90)	14.2 (10.9–20.5)	0.86 (0.69–0.94)	7.07 (5.41–10.21)	13.2 (10.1–19.1)	0.87 (0.72–0.95)

HRV = heart rate variability.
Frequency domain: LFnu = low-frequency normalised units, HFnu = high-frequency normalised units, LF/HFnu = low-frequency to high-frequency ratio in normalised units.
Time domain: IBI = R–R intervals, SDNN = standard deviation of normal-to-normal intervals, RMSSD = root mean squared differences of the standard deviation, pNN50 = percentage of beats that changed more than 50 ms from the previous beat.
Reliability: ICC = intra-class correlation and is expressed as a mean (95% CI), TEM = typical error of measurement, TEM% = typical error of measurement as a percentage, and both are expressed as means (90% CI).

Reliability ICCs for the frequency domain were lower compared to the time domain. The ICCs in the frequency domain were between 0.36 and 0.88, with the lowest value for these correlations being the LF/HFnu ratio (ICC = 0.36) for males. It was found that overall the male participants had lower ICCs when compared to females for the same HRV parameter in the frequency-domain analysis.

The ICCs for most time-domain parameters were similar between the males and females, with the exception of the IBIs. Males displayed high IBI ICC values (day 2 vs day 3 = 0.90, day 3 vs day 4 = 0.93), which indicated higher relative reliability compared to the females (day 2 vs day 3 = 0.83, day 3 vs day 4 = 0.79). Time-domain ICCs that were similar between males and females were SDNN (day 3 vs day 4), males (0.73) and females (0.72) and pNN50 (day 2 vs day 3), males (0.86) and females (0.84).

Gender differences

There were significant differences between males and females for resting HR ($p < 0.0001$), R–R intervals ($p < 0.0001$) and for the frequency-domain HRV parameters, HFnu ($p = 0.020$) and LF/HFnu ratio ($p = 0.003$) (Table 3). The female resting heart rate was 16% higher than that of the males ($p < 0.0001$), while the IBIs were 21% higher in the males ($p < 0.0001$). The LFnu ($p = 0.087$) and LF/HFnu ratio ($p = 0.003$) were 13 and 41% higher in the males, respectively, while the HFnu ($p = 0.02$) was 12% higher in the females. There were no significant differences between males and females in the HRV time-domain parameters.

Discussion

Despite extensive use and research in both clinical and physiological settings, HRV analysis is still poorly supported by rigorous reliability studies.[3] The purpose of the present study was to examine the reliability and gender characteristics of standard parameters of HRV from short-term (five-minute) laboratory recordings in physically active individuals. The main findings of this study were that the reliability of HRV recording over consecutive days varied depending on the specific reliability index used, the HRV parameter examined as well as the gender of the population. A comparison of HRV frequency-domain parameters for males and females demonstrated significant gender differences in the sympathovagal balance.

TABLE 3. COMPARISON OF MEAN (± SD) OF DAYS 2, 3 AND 4 OF HEART RATE VARIABILITY DATA BY GENDER

	Men	*Women*	*p*
Resting HR (b/min)	55.09 (8.1)	65.84 (7.2)	< 0.0001*
IBIs (ms)	1126 (169)	927.7 (101)	< 0.0001*
LFnu	47.95 (17.93)	42.48 (18.43)	0.087
HFnu	52.02 (17.73)	59.17 (17.16)	0.020*
LF/HFnu	1.114 (0.710)	0.792 (0.522)	0.003*
SDNN	103.1 (36.13)	93.39 (57.55)	0.252
RMSSD	101.3 (43.75)	87.57 (59.44)	0.137
pNN50	53.35 (18.71)	48.46 (22.77)	0.182

IBI = R–R intervals, LFnu = low-frequency normalised units; HFnu = high-frequency normalised units; LF/HFnu = low-frequency to high-frequency ratio in normalised units; SDNN = standard deviation of normal-to-normal interval, RMSSD = root mean squared differences of the standard deviation; pNN50 = percentage of beats that changed more than 50 ms from the previous beat.

Although the definition of a categorical rating of relative reliability based on ICCs is still controversial,[3] the results from the present study demonstrate substantial to excellent relative reliability for the majority of time- and frequency-domain HRV parameters when comparing measurements obtained from days 3 and 4. For most measurements, the ICC was above 0.80 ('good'),[28] indicating that the repeated measurements reflect mostly the true value of HRV parameters relative to random error. Females demonstrated a higher relative reliability than males for all frequency-domain parameters. The high ICC of these short-term recordings indicate a considerable consistency with time, similar to previous studies.[3,8]

However, absolute reliability, indicated by the TEM and TEM%, revealed the presence of a large random error in all HRV parameters, particularly for the males. Females demonstrated better absolute reliability (lower TEM) than males for all parameters, particularly those in the frequency domain. The TEM% also indicated a low absolute reliability due to the high values found specifically for the LF/HFnu ratio. These findings are similar to those of Pinna *et al.* who found a greater random error (TEM) in frequency domain measures.[3]

These results might place doubts on the use of HRV indices in assessing interventions or treatment effects in individual participants, specifically when examining male participants or clinical populations.[3] Furthermore, these results place doubt on the use of HRV measurement for monitoring performance changes in well-trained athletes. The high TEM and TEM% indicated that HRV would be unable to detect small but significant changes (≤ 1%) in performance.[29] Considering the absolute reliability was low (high TEM and TEM% values), the large random error found in HRV would require significant changes in HRV to occur to deem them meaningful.[29]

Gender differences

Findings of research examining HRV gender differences in healthy individuals are conflicting.[5-8] Research has demonstrated that HRV measures are either the same or differ considerably between the genders and may also be HRV parameter or age dependant,[5-8] and therefore, further research has been advocated.[3] Umetani *et al.*[5] has shown that HRV (for all measures) is significantly lower in 'young' (10–29 years) females compared to their age-matched male counterparts. The gender differences subsequently decreased and then disappeared with age and at different rates for the HRV parameters. Their findings suggested a higher level of parasympathetic activity in males.[5]

Conversely, Ryan *et al.*[6] concluded that vagal high-frequency power was higher in females. They suggested this difference was most apparent for young (20–39) and middle aged (40–64) females. Raemaeker *et al.* noted that the LFnu (sympathetic dominance) was higher in females, however, HFnu (parasympathetic dominance) was not significantly different between the genders.[7] Sinnreich *et al.*[8] observed that RMSSD and HF components (measures reflecting predominantly vagal activity) were small and non-significant, but that the LF/HF ratio (suggested to reflect sympathetic/parasympathetic balance) differed substantially due to greater VLF and LF power found in the male participants. They concluded that their results illustrated relatively higher sympathetic activity in men compared to women.

Our findings demonstrate a similar gender distinction to that of Ryan *et al.*[6] and Sinnreich *et al.*[8] A higher level of parasympathetic activity was found in the female participants compared to the males. This was demonstrated by a lower LF/HFnu ratio and higher HF value in the females.

The finding of a higher HRV and contribution of the parasympathetic nervous system to HRV in females may help explain the overall protection of pre-menopausal women from coronary heart disease (CHD), coronary mortality and sudden cardiac death, compared to males in this age group.[8,13] Research has demonstrated that a high HRV is associated with significant cardiovascular health. Although not measured in the present study, the effect of oestrogen on HRV and parasympathetic activity in females may be a key factor in this finding.

Research examining HRV in pre- and postmenopausal women found a significant difference in HRV.[26] Postmenopausal women had a significantly reduced HRV, as demonstrated by a higher relative power of LF and LF/HF ratio, which was related to a decline in the level of oestrogen. The authors concluded that a decline in the level of oestrogen from pre-menopausal to postmenopausal status favours the shifting of autonomic balance towards sympathetic dominance.[26] In support of this finding, research has demonstrated that physiological levels of oestrogen increase vagal tone and suppress sympathetic modulation of heart rate in females.[30]

A limitation of this study was that respiratory rate was not controlled when measuring the IBIs over the five-minute recording period. Research has shown that the reliability of HRV measures was increased when respiratory rate was regulated.[12] Furthermore, part of intra-subject variability was also due to the natural change of HRV parameters that occurs under the influence of factors such as mood, alertness and mental activity, which is very difficult to control for in any study.[5]

Conclusion

Heart rate variability measures are a popular, non-invasive tool used to monitor autonomic function. Short-term recordings are easy to perform and are suitable for both clinical and physiological research. However, the findings of the present study have demonstrated a high relative reliability but low absolute reliability for HRV. In particular, HRV random error was higher in males. For clinical or sport/exercise science practice, these results place in doubt the use of HRV indices for assessing small (< 5%) intervention or treatment effects, specifically when examining male or clinical populations.[5]

Furthermore, these results place doubt on the use of HRV measurement for monitoring performance changes in well-trained or elite athletes, who typically require assessment techniques that can detect physiological or performance changes below 1%.[29] Finally, specific HRV parameters differed between males and females, indicating a greater parasympathetic modulation of HRV in females. This finding suggests a possible mechanism for why pre-menopausal females have a lower incidence of coronary heart disease compared to males in the same age group, as well as compared to postmenopausal women.

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Appendix VIII

Biomedical Ethics Approval.

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23 July 2010

Ms. Takshita Sookan
Discipline of Sport Science
Westville Campus
University of KwaZulu- Natal

PROTOCOL: Heart rate variability in physically active individuals: repeatability and gender characteristics. REF: BE111/010

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application dated 12 May 2010.

The study was approved pending appropriate responses to queries raised. Your responses dated 06 July 2010 to queries raised on 21 June 2010 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 23 July 2010.

This approval is valid for one year from **23 July 2010.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/ResearchEthics11415.aspx.