

# **The Influence of Ultra-Endurance Exercise on the Cardiovascular and Related Physiological Systems**

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## General Abstract

**INTRODUCTION:** There is currently limited longitudinal data investigating the performance and health-related influence of ultra-endurance training and participation. Cross-sectional investigations have highlighted a potential for those performing most exercise to be at an increased risk of cardiovascular events. If such risks occur, they are likely to be due to a combination of the stress provided through training and events together.

**PURPOSE:** To assess the development of several physiological factors associated with exercise training and to gain a greater insight regarding the changes in cardiac electrical conductance from endurance training. A sub-study sought to investigate the short and longer-term influence of an iron-distance triathlon on indirect measures of arterial stiffness.

**METHOD:** Part 1: Seventy-six previously recreationally active participants underwent a 6 month endurance training programme in preparation for an iron-distance triathlon, consisting of a 3.86km swim, 180.25km cycle, and a 42.2km run. Multiple assessments were performed at months 0, 2, 4 and 6; including submaximal and exhaustive cycling tests, anthropometric measurements and 12-lead ECG's. Part 2: Eleven athletes from part 1 (TRI) and 10 recreational control participants (NOTRI) were assessed on 4 occasions with identical time intervals. Arterial stiffness and cardiovascular functional parameters were obtained 7 days prior (T1) to an iron-distance triathlon, 12–18 hours post-event, 7 days post-event, and 28 days post-event.

**RESULTS:** Part 1: Cardiorespiratory fitness and performance parameters increased over the training period, irrespective of age, with greatest improvements from month 0–2 and the least improvements from month 4–6. Additionally, a progressive increase was observed in the frequency of both training-related and training-unrelated ECG findings. Part 2: A significant difference in central arterial stiffness was found between TRI and NOTRI 12–18 hours post-event and 7 days post-event but not prior to or 28 days post-event. No differences were observed between groups for peripheral stiffness at any time-point. Additionally, no time effect was observed when the TRI group were treated separately.

**CONCLUSION:** Training caused significant improvements to fitness related physiological factors. In a minority of individuals, endurance training induced bioelectrical patterns of what is currently referred to as abnormal criteria, which may reflect a normal change to what was previously thought of as abnormal findings or, alternatively, be pathological manifestations in previously healthy individuals. Part 2 of this study showed a delayed central arterial stiffening may occur one day and one week following a single day ultra-endurance event. Importantly, all measurements were found to be similar one month post-event; implying only a transient exercise-induced elevation to arterial stiffness.

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## Selected Abbreviations and Acronyms

**8OHdG** = 8-hydroxydeoxyguanosine (biomarker of oxidative damage to DNA).

**aDBP** = aortic diastolic blood pressure

**ADP** = adenosine diphosphate

**AGE** = proteins or lipids that become glycosylated after exposure to sugar

**Aix** = augmentation index (AP/PP)

**Aix@HR75** = augmentation index corrected for a heart rate of 75 beats per minute.

**AMP** = adenosine monophosphate

**AP** = augmentation pressure

**aPWV** = aortic PWV

**ARVC** = arrhythmogenic right ventricular cardiomyopathy

**aSBP** = aortic systolic blood pressure

**ATP** = adenosine triphosphate

**AV** = atrioventricular

**baPWV** = brachial to ankle pulse wave velocity

**BBB** = bundle branch block

**BH<sub>3</sub>** = trihydrobiopterin

**BH<sub>4</sub>** = tetrahydrobiopterin

**CAD** = coronary artery disease

**cfPWV** = carotid to femoral pulse wave velocity

**cGMP** = cyclic guanosine monophosphate

**CHD** = coronary heart disease

**CLBBB** = complete left bundle branch block

**cNOS** = constitutive nitric oxide synthase

**CNS** = central nervous system

**CO<sub>2</sub>** = carbon dioxide

**CRBBB** = complete right bundle branch block

**cTn** = cardiac troponin

**cTnC** = cardiac troponin C

**cTnI** = cardiac troponin I

**cTnT** = cardiac troponin T

**CV** = cardiovascular

**CVD** = cardiovascular disease

**ECG** = electrocardiograph

**eNOS** = endothelium nitric oxide synthase

**ET-1** = endothelin 1

**FAC** = focal adhesion kinase

**FMD** = flow mediated dilation

**GC** = guanylate cyclase

**GTP** = guanosine triphosphate

**HR** = heart rate

**HR<sub>max</sub>** = maximal heart rate

**ICAM-1** = intercellular adhesion molecule 1

**iNOS** = inducible nitric oxide synthase

**LAD** = left axis deviation

**LAE** = left atrial enlargement

**LBBB** = left bundle branch block

**LDL** = low density lipoprotein

**LV** = left ventricle

**LVED** = left ventricular ejection duration

**LVH** = left ventricular hypertrophy

**MMP's** = matrix metalloproteinases

**MRI** = magnetic resonance imaging

**NADP** = nicotinamide adenine dinucleotide

**NF $\kappa$ B** = nuclear factor kappa-light-chain-enhancer of activated B cells

**nNOS** = neuronal nitric oxide synthase

**NOS** = nitric oxide synthase enzyme

**NOTRI** = non ultra-endurance, recreationally active control participants

**NS** = non-supplemented ultra-endurance triathlon group

**NT-proBNP** = N-Terminal pro-brain natriuretic peptide

**O<sub>2</sub>** = oxygen

**O<sub>2</sub><sup>-</sup>** = superoxide

**ONOO<sup>-</sup>** = peroxynitrite

**pDBP** = peripheral diastolic blood pressure

**PNS** = Parasympathetic nervous system

**PP** = pulse pressure (systolic blood pressure - diastolic blood pressure).

**pSBP** = peripheral systolic blood pressure  
**PWV** = pulse wave velocity  
**RAD** = right axis deviation  
**RAE** = right atrial enlargement  
**RBBB** = right bundle branch block  
**rHR** = resting heart rate  
**RPE** = rating of perceived exertion  
**RPM** = revolutions per minute  
**RV** = right ventricle  
**RVH** = right ventricular hypertrophy  
**S** = supplemented ultra-endurance triathlon group  
**SA** = sinoatrial

**SCD** = sudden cardiac death  
**SNS** = Sympathetic nervous system  
**sRPE** = session rating of perceived exertion  
**TRI** = ultra-endurance triathletes  
**VCAM-1** = vascular cell adhesion protein 1  
**VEGF** = vascular endothelial growth factor  
 $\dot{V}O_2$  = volume of oxygen consumption  
 $\dot{V}O_{2max}$  = maximal volume of oxygen consumption  
 $\dot{V}O_{2peak}$  = peak volume of oxygen consumption  
 $\dot{V}O_{2plateau}$  = plateau in the volume  
 $\dot{W}_{max}$  = maximal power output

## Measuring Units

**%** = percentage  
**°** = degrees  
**°C** = degrees Celsius  
 $\mu\text{g}\cdot\text{g creatinine}^{-1}$  = micrograms per gram of creatinine  
 $\mu\text{l}\cdot\text{g creatinine}^{-1}$  = microlitres per gram of creatinine  
**AU** = arbitrary units  
 $\text{beats}\cdot\text{min}^{-1}$  = beats per minute  
**cm** = centimetre  
**dec mins** = decimal minutes  
**kg** = kilogram  
 $\text{kg}\cdot\text{m}\cdot\text{s}^{-2}$  = kilograms per metre per second squared  
**km** = kilometre  
**kPa** = kilopascals  
 $\text{m}\cdot\text{s}^{-1}$  = metres per second  
**ml** = millilitre

$\text{ml}/\text{mmHg}^{x10}$  = millilitres per millimetres of mercury to the power of 10 (volume x pressure)  
 $\text{ml}/\text{mmHg}^{x100}$  = millilitres per millimetres of mercury to the power of 100 (volume x pressure)  
 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{mins}^{-1}$  = millilitres per kilogram per minute  
**mm** = millimetre  
 $\text{mm}\cdot\text{s}^{-1}$  = millimetres per second  
**mmHg** = millimetres of mercury  
**mmol** = millimole  
 $\text{mmol}\cdot\text{L}^{-1}$  = 1 millimolar per litre  
**ms** = milliseconds  
**mV** = millivolts  
**s** = second  
**yr** = years  
 $\mu\text{l}$  = microliter

## Statistical units

**F** = statistic used to test the significance of a model  
**p** = significance level  
**r** = Pearson's correlation statistic  
**W** = Shapiro-Wilk statistic  
 $\eta\text{p}^2$  = partial eta squared  
 $\chi^2$  = chi squared

$\epsilon$  = epsilon  
**df** = degrees of freedom  
**M** = mean  
**SD** = standard deviation  
**SEM** = standard error of the mean

## Definition of Terms

**Afterload** = stress developed in the wall of the left ventricle during ejection. Defined as the end load against which the heart contracts to eject blood.

**Antioxidant** = molecule that inhibits the oxidation of other molecules.

**Arrhythmia** = an irregular rhythm of the heart beat

**Arteriosclerosis** = thickening and hardening of the walls of the arteries.

**Atherogenesis** = the process of forming atheromatic plaque in arteries.

**Atheroma** = an accumulation of material in the inner layer (tunica intima) of arterial walls. The material consists predominantly of macrophage cells and debris.

**Atherosclerosis** = a specific form of arteriosclerosis characterised by deposition of materials (plaques) in and on the artery walls, which can restrict blood flow.

**Atria** = two upper chambers of the heart which receive returning blood from the veins, and are responsible for passing blood to the ventricles

**Attenuate** = to reduce the force, effect, or thickness.

**Augment** = to increase or make something greater.

**Baroreflex** = one of the body's homeostatic mechanisms that contributes to blood pressure regulation at constant levels.

**Bradycardia** = slow heart rhythm

**Cardiomyocyte** = muscle cells that form the cardiac muscle

**Catheter** = a thin tube inserted into the body. In the current context this refers to vessels of the body.

**Chronotropic effects** = effects that change the heart rate (rhythm or rate). Positive chronotropes increase heart rate; negative chronotropes decrease heart rate

**Coagulate** = cause a fluid to change to a solid or semi-solid state.

**Conduit** = a channel for conveying fluid.

**Diastole** = the resting phase between contractions in the cardiac cycle, characterised by filling of the chambers of the heart

**Diastolic blood pressure** = the minimum arterial pressure during the relaxation/filling phase of the ventricles of the heart.

**Distensible** = capable of being dilated or expanded

**Fibrillation** = a type of arrhythmia associated with irregular and rapid contraction of cardiac muscle fibres, resulting in poor blood flow to the body

**Fidelity** = refers to the degree of exactness with which something is reproduced. High fidelity indicates very similar results to the original effect.

**Flow mediated dilation** = dilation of a vessel caused by blood flow through it. A measurement obtained to assess endothelial function, greater flow mediated dilation = greater endothelial function.

**Free radical** = an uncharged molecule having an unpaired electron. Characteristically highly reactive and short-lived. Free radicals form part of a chain reaction involving subsequent donations of unpaired electrons to other molecules, making them unstable and highly reactive.

**Glutathione** = endogenous antioxidant, suggested to be the most potent antioxidant produced by the body. Composed of cysteine, glutamic acid, and glycine.

**Glycation** = the result of covalent bonding of a protein or lipid with a sugar molecule without controlling action of an enzyme

**Haemodynamic** = the study of the movements of blood flow around the body

**Heterogeneous** = diverse in characteristics.

**Homeostasis** = Body's ability to maintain internal stability of physiological processes.

**Homogeneous** = of the same kind; for example, participants of a study sharing the same characteristics such as height, weight, ethnicity.

**Hyperglycaemia** = an excess of glucose in the bloodstream

**Hypervagotonia** = Elevated innervation of the vagus nerve

**Lipid peroxides** = biomarker of oxidative stress; indicator of oxidative damage to polyunsaturated fatty acids (PUFAs).

**Locomotion** = movement

**Lumen** = the cavity of a tubular organ i.e. the lumen of a blood vessel.

**Matrix metalloproteases** = proteases capable of degrading extracellular matrix proteins (in the blood vessel context the relevant proteins refer to collagen and elastin). These proteases have a major role in angiogenesis, apoptosis and differentiation.

**Morphology** = the study of the form and structure of a biological component.

**Muscle Metaboreflex** = negative feedback reflex raising blood pressure and flow. Working muscles create metabolic by-products under environments of inadequate nutritional supply leading to neural stimulation of increased sympathetic tone.

**Myocyte** = type of cell found in muscle tissue. A cardiac myocyte is a heart muscle cell.

**Occlusion** = the blockage of a blood vessel.

**Oxidant** = the molecule in an oxidation-reduction (redox) reaction that accepts an electron from another species.

**Oxidative Stress** = an imbalance of oxidants and antioxidants in favour of oxidant production

**Parasympathetic nervous system** = part of the nervous system regulating the functions of the body's involuntary organs which activates what is

known as the 'rest and digest' response. PNS functions with actions that do not require immediate reaction.

**Parasympathetic tone** = degree of parasympathetic nervous system activation, usually up-regulated at times of rest.

**Preload** = end diastolic pressure that stretches the right or left ventricle of the heart. Defined as the initial stretching of the cardiac myocytes prior to contraction.

**Pulse pressure** = the difference between systolic and diastolic blood pressure values. Represents the force that the heart generates each time it contracts.

**Redox** = combination of reduction and oxidation

**Redox balance** = a state of balance between oxidative stress and antioxidant processes.

**Sensitivity** = the true positive rate, measures the proportion of actual positives which are correctly identified as such

**Specificity** = the true negative rate, measures the proportion of negatives which are correctly identified as such

**Sympathetic nervous system** = part of the nervous system regulating the functions of the body's involuntary organs which activates what is known as the 'fight or flight' response. Causes vasoconstriction of blood vessels.

**Sympathetic tone** = degree of sympathetic nervous system activation, usually up-regulated in times of stress or emergency.

**Systole** = the contraction phase of the cardiac cycle resulting in the ejection of blood from the chambers of the heart

**Systolic blood pressure** = the highest arterial blood pressure occurring immediately after the contraction/ejection phase of the ventricles of the heart.

**Tachyarrhythmias** = fast and irregular heart rhythm

**Tetrahydrobiopterin** = cofactor in NO synthesis from L-arginine by NOS within endothelial cells

**Thrombosis** = formation of a blood clot in a blood vessel, obstructing flow.

**Training load** = the sum of the intensity, volume and frequency of training sessions

**Transient** = lasting only for a short time.

**Triglycerides** = glycerol with three fatty acid groups

**Vascular tone** = the degree of constriction of a vessel relative to its maximally dilated state

**Ventricle** = two main chambers of the heart responsible for pumping blood to the lungs (right ventricle) and the rest of the circulation (left ventricle).

**Viscoelastic** = the property of a substance to exhibit both viscous and elastic characteristics under the application of temporary deformation.

# **Chapter One: Introduction**

## 1.1 Rationale

The ironman distance triathlon is a relatively new event which has dramatically increased in participation rates over recent years. The event requires participants to swim 3.86 km, cycle 180.25 km, and run 42.2 km. The purpose of this study was to assess the wide range of physiological adaptations and adaptive responses occurring in humans exposed to prolonged physical endurance load. The implications of this study are in relation to the importance of ethical safety in regards to ultra-endurance exercise.

Abundant research has highlighted the beneficial aspects of exercise in healthy and diseased individuals (Warburton, Nichol and Bredin, 2006), including cardiovascular (CV) health and longevity (Dai, Rabinovitch and Ungvari, 2012; Seals et al., 2009). However, the mechanisms underlying these effects are not fully known. Although moderate activity levels have received extensive praise, there is a necessary importance to ascertain whether excessive exercise induces harm to the human body. Studies have identified an increased relative risk of CV disease (CVD) in those performing most aerobic exercise, highlighting the possible adverse effects of exercise (Burr, et al., 2014; O'Keefe et al., 2012; Paffenbarger et al., 1986; Quinn et al., 1990; Schnohr, Marott, Lange & Jensen, 2013; Schnohr, O'Keefe, Marott, Lange & Jensen, in press). For this reason, ultra-endurance athletes may represent a group of individuals at an elevated risk of CV events. The majority of recent intervention studies suggest a purely transient reduction in cardiac health in response to a single bout of excessive endurance exercise (La Gerche et al., 2008; Shave et al., 2010). However, epidemiological studies suggest an accumulative detrimental response to long-term involvement in excessive endurance exercise (Lee, Pate, Lavie & Blair, 2012; O'Keefe et al., 2012). Participation rates in ultra-endurance events have increased greatly within recent decades (Hoffman, Ong, & Wang, 2010; Knechtle, Knechtle & Lepers, 2011) therefore, the need to assess the health risks associated with such events has become more important. Studies investigating the cardiac and vasculature responses to excessive endurance exercise are predominantly limited to individuals who have been competing in such events for a period of time previous to the study. For this reason, events may have been masked by previous exposure to the exercise stimuli. The purpose of this study was to investigate the CV adaptations of the 'athlete's heart' induced by a long-term incremental endurance training programme in previously recreationally active individuals.

The athletic heart is a non-pathological condition associated with normal cardiac adaptations to routine exercise, predominantly endurance. This concept was introduced to differentiate serious heart diseases from normal athletic hearts, creating a new set of parameters for those meeting the athletic criteria. The heart of an endurance athlete is more than likely to undergo physiological changes in

structure, function and electrical activity as training progresses. These changes may place the individual in the diagnostic 'grey zone', representing uncommon cardiac changes which may be caused by an underlying pathological disease (Baggish & Wood, 2011). In a small minority of individuals, endurance training may trigger a pathological cascade placing them at an increased risk of sudden cardiac death (SCD) during exercise (George et al., 2012). The characterisation of the athlete's heart has helped in the differentiation from normal adaptation of the heart and several inherited cardiac diseases, most prominently hypertrophic cardiomyopathy, which may predispose to SCD (Baggish & Wood, 2011; Drezner, 2012; Drezner et al., 2012). Providing an insight of the changes to the electrical activity of the heart over a long-term, high load, training programme will provide an understanding of normal adaptations of the 'athletes heart'.

Carotid to femoral (cf) PWV represents the speed at which the pressure pulse travels from the carotid artery site to the femoral artery site and is considered the gold standard measure of arterial stiffness. Stiffer arteries allow the pressure wave to travel faster through the artery. Aix is an index of wave reflections, representing a manifestation of systemic arterial stiffness. Larger elastic arteries buffer pulsations from the heart; muscular arteries can alter the speed of travel of waves, whilst arterioles serve as major reflecting sites. Aix changes therefore may represent an altered wave reflection from peripheral arteries, without changing central arterial stiffness, through adaptations in the muscular arteries or arterioles (Edwards and Lang, 2005). Equally, changes in PWV may represent an alteration in central arteries, without affecting peripheral arterial stiffness. The two measurements are not mutually exclusive and may differ depending on the internal adaptations occurring.

Repetitive episodes of cyclical arterial shear stress provide a stimulus promoting vascular adaptations through endothelium-dependent remodelling. Longer exposure to such stimuli however may compromise the system mimicking disease states, inducing pro-atherosclerotic processes and coronary calcification in otherwise healthy individuals (Möhlenkamp et al., 2008). Studies investigating the cardiac and vasculature adaptations/responses to excessive endurance training and ultra-endurance bouts are predominantly limited to individuals who have been training and competing in such events for a period of time previous to the study. For this reason, subsequent effects of aforementioned events may have been masked by previous exposure to the exercise stimuli. Evaluating these responses in individuals who are unexposed to ultra-endurance events may give a greater insight as to whether these transient changes are associated with tissue damage or are potentially adaptive processes, and further, what the long-term consequences might be. On the other hand, It has been hypothesised that the detrimental responses may in fact be mitigated in ultra-endurance athletes as a result of exercise-induced adaptations i.e. antioxidant defence and

attenuated reactive oxygen species (ROS) production and overall greater care for protecting their bodies (Knez, Coombes & Jenkins, 2006). The results of this study will provide evidence as to whether a single bout of ultra-endurance exercise influences the vascular system. This evidence will contribute to the ethical standpoint as to the safety of participating in such events for the general population.

## 1.2 Aims

The general aim of this study was to assess several cardiovascular-related physiological changes occurring over a 6 month progressively incremental training period with reference to normal adaptations. Additionally it was sought to investigate the incidence of training-related and unrelated electrocardiographic (ECG) findings in response to endurance training. A secondary general aim was to investigate athletic status, as well as the acute and longer term effects of a long-distance triathlon on markers of arterial stiffness in a subgroup of participants.

## 1.3 Objectives

- Measure physiological variables every 2 months for a 6 month period.
  - Perform multi-stage submaximal cycling tests and measure lactate threshold.
  - Perform exhaustive cycling test and measure maximal volume of oxygen consumption ( $\dot{V}O_{2max}$ ), maximal power output, maximal heart rate ( $HR_{max}$ ).
  - Measure body fat (BF) with use of bioelectrical impedance assessment equipment.
  - Measure resting 12-lead ECG activity.
- Obtain training diary information and produce training load assessments for individuals.
- Identify training-related and training-unrelated 12-lead ECG findings.
- Measure arterial stiffness markers in a control population of recreationally active individuals.
- Measure arterial stiffness markers before and after long-distance triathlon in trained population.

## 1.4 Hypotheses

### 1.4.1 Study 1: Part 1

**H<sub>0</sub>:** Endurance training will not result in a statistically significant ( $p < 0.05$ ) change to base variables: i)  $\dot{V}O_{2max}$ , ii) lactate threshold and iii) maximal cycling power production.

**H<sub>1</sub>:** Endurance training will result in a statistically significant ( $p < 0.05$ ) increase to base variables: i)  $\dot{V}O_{2max}$ , ii) lactate threshold and iii) maximal cycling power production.

**H<sub>0</sub>:** Endurance training will not result in a statistically significant ( $p < 0.05$ ) difference to variables: i)  $HR_{max}$  and ii) BF percentage.

**H<sub>2</sub>:** Endurance training will statistically significantly ( $p < 0.05$ ) decrease to variables: i)  $HR_{max}$  and ii) BF percentage.

#### 1.4.2 Study 1: Part 2

**H<sub>0</sub>:** Endurance training will not result in a statistically significant ( $p < 0.05$ ) difference in ECG variables: i) rHR, ii) QRS axis, iii) P wave axis, iv) T wave axis, v) QTc interval.

**H<sub>3</sub>:** There will be a statistically significant ( $p < 0.05$ ) change in ECG variables: i) rHR, ii) QRS axis, iii) P wave axis, iv) T wave axis, v) QTc interval.

**H<sub>0</sub>:** Endurance training will not result in a change in the frequency of individuals showing: i) group 1 abnormal, training-unrelated ECG findings and ii) group 2, training-related ECG findings.

**H<sub>4</sub>:** Endurance training will result in a change in the frequency of individuals showing: i) group 1 abnormal, training-unrelated ECG findings and ii) group 2, training-related ECG findings.

**H<sub>0</sub>:** Endurance training will not result in a change in the frequency of findings from i) group 1 abnormal, training-unrelated ECG criteria and ii) group 2, training-related ECG criteria.

**H<sub>5</sub>:** Endurance training will result in a change in the frequency findings from i) group 1 abnormal, training-unrelated ECG criteria and ii) group 2, training-related ECG criteria.

#### 1.4.3 Study 2

**H<sub>0</sub>:** There will be no statistically significant ( $p < 0.05$ ) differences in arterial stiffness variables: i) PWV ii) Aix and iii) Aix@HR75, at any time-points between TRI and NOTRI groups.

**H<sub>6</sub>:** There will be a statistically significant ( $p < 0.05$ ) difference in arterial stiffness variables: i) PWV ii) Aix and iii) Aix@HR75, at one or more time-points between TRI and NOTRI groups.

**H<sub>0</sub>:** There will be no statistically significant ( $p < 0.05$ ) difference in cardiovascular variables: i) rHR, ii) aSBP, iii) aDBP and iv) LVED, at one or more time-points between TRI and NOTRI conditions.

**H<sub>7</sub>:** There will be a statistically significant ( $p < 0.05$ ) difference in cardiovascular variables: i) rHR, ii) aSBP, iii) aDBP and iv) LVED, at one or more time-points between TRI and NOTRI conditions.

**H<sub>0</sub>:** There will be no statistically significant ( $p < 0.05$ ) differences in arterial stiffness variables: i) PWV ii) Aix and iii) Aix@HR75 between any time-points in TRI participants only.

**H<sub>8</sub>:** There will be a statistically significant ( $p < 0.05$ ) difference in arterial stiffness variables: i) PWV ii) Aix and iii) Aix@HR75 between any time-points in TRI participants only.

**H<sub>0</sub>:** There will be no statistically significant ( $p < 0.05$ ) difference in cardiovascular variables: i) rHR, ii) aSBP, iii) aDBP and iv) LVED, between time-points in TRI participants only.

**H<sub>9</sub>:** There will be a statistically significant ( $p < 0.05$ ) difference in cardiovascular variables: i) rHR, ii) aSBP, iii) aDBP and iv) LVED, between time-points in TRI participants only.

**H<sub>0</sub>:** There will be no statistically significant ( $p < 0.05$ ) differences between S and NS groups for urinary variables: i) lipid peroxide concentration, ii) 8-hydroxydeoxyguanosine concentration, iii) glutathione requirement scores.

**H<sub>10</sub>:** There will be statistically significant ( $p < 0.05$ ) differences between S and NS groups for urinary variables: i) lipid peroxide concentration, ii) 8-hydroxydeoxyguanosine concentration, iii) glutathione requirement scores.

**H<sub>0</sub>:** There will be no statistically significant ( $p < 0.05$ ) differences in arterial stiffness variables: i) PWV ii) Aix and iii) Aix@HR75 at any time-point between S and NS groups.

**H<sub>11</sub>:** There will be a statistically significant ( $p < 0.05$ ) difference in arterial stiffness variables: i) PWV ii) Aix and iii) Aix@HR75 at one or more time-points between S and NS groups.

**H<sub>0</sub>:** There will be no statistically significant ( $p < 0.05$ ) difference in cardiovascular variables: i) rHR, ii) aSBP, iii) aDBP and iv) LVED, at one or more time-points between S and NS groups.

**H<sub>12</sub>:** There will be a statistically significant ( $p < 0.05$ ) difference in cardiovascular variables: i) rHR, ii) aSBP, iii) aDBP and iv) LVED, at one or more time-points between TRI and NOTRI conditions.

## **Chapter Two: Literature Review**

## 2.1 Study 1

### 2.1.1 Background: Ultra-Endurance Exercise and the Associated Changes

Ultra-endurance events have become increasingly popular in recent years throughout the world (Knechtle, Knechtle & Lepers, 2011; Hoffman, Ong, & Wang, 2010). Long-distance triathlons represent one type of ultra-endurance sport, which consist of three components: swimming, cycling and running; the iron-distance triathlon is the most popular of which, involving a 3.8km swim, 180km cycle and a 42.2km run (Knechtle, Knechtle & Lepers, 2011). Few longitudinal studies have investigated the pattern of CV and performance-related physiological adaptations, which occur in recreationally active individuals deciding to train for ultra-endurance events. Furthermore, the volume of exercise training, and the extent of training-induced adaptations, which is necessary to develop individuals to a level whereby they are able to complete such events is not well recognised. Improvements in this area will help future individuals wishing to train for ultra-endurance events monitor their progress.

An important aspect of investigation pertains to the safety of undertaking large training volumes, and the risk of repeatedly competing in prolonged endurance exercise. Several cross-sectional studies have noticed a potential for excessive exercise to induce damaging effects on the CV system (Lee, Pate, Lavie & Blair, 2012; O'Keefe et al., 2012; Paffenberger et al., 1986; Schwartz et al., 2014). In a small minority of individuals, endurance training may trigger a pathological cascade placing them in an increased risk of sudden death (George et al., 2011; George et al., 2012). Advancements in the area of CV responses and adaptations to prolonged endurance exercise and endurance training will contribute to the management of athletes and the identification of at risk individuals.

### 2.1.2 Endurance Exercise-Induced Physiological Adaptations

Completing repeated exercise bouts over a period of time provides stimulus for multiple physiological adaptations resulting in better performance in that activity. The extent of physiological change is multifactorial, dependent on age, gender and genetics of individuals, as well as the specificity, dose and recovery period of the training schedule (Jones & Carter, 2000). Endurance exercise relies heavily on the aerobic energy production pathway; adequate delivery of oxygen and fuels such as carbohydrate and lipids are essential to success in endurance performance. As such, endurance training is followed by adaptations to cardiorespiratory fitness, improving oxygen delivery to mitochondria of cells in the body (Jones & Carter, 2000). Habitual endurance training provides a stimulus for numerous physiological adaptations that facilitate improved endurance exercise capacity; for example, being able to sustain a submaximal workload for a longer period, or being able to attain a higher power output over a specified distance (Hawley, 2002). A training programme can be broken

down to three components, volume, intensity, and frequency of exercise sessions. Chronic exercise adaptations occur as a result of repeated acute signalling responses due to exercise stimulus.

### **2.1.3 Performance-Related Adaptations of Endurance Training**

Multiple physiological adaptations occur with endurance training, resulting in an enhanced capacity to perform well. Joyner and Coyle, (2008) have provided a schematic of these interacting physiological factors, which combine as determinants of performance (figure 1). One such adaptation refers to the high proportion of type I (slow twitch) muscle fibres found in endurance athletes, which possess a higher capillary density and oxidative potential than type II muscle fibres (Coyle, Sidossis, Horowitz & Beltz, 1992). Furthermore, type I fibres are associated with a greater gross efficiency during exercise than type II (fast twitch) fibres (Joyner & Coyle, 2008). The capillary supply to skeletal muscle is increased with endurance training, resulting in a reduction in the diffusion distance for substrates and gaseous exchange (Hawley, 2002). Enzymatic activity of the mitochondrial electron transport chain is increased with endurance exercise, resulting in a greater oxidative potential (Hawley, 2002). This increased oxidative potential is at least in part responsible for the endurance training associated shift in substrate utilisation during submaximal exercise. This is demonstrated by a decreased reliance on plasma glucose production and oxidation, with proportional increases in fat oxidation (Jeukendrup, Saris & Wagenmakers, 1998; Martin et al., 1993; Venables & Jeukendrup, 2008). A reduced reliance on glucose as a primary fuel during exercise contributes in part to a reduction in cellular acidosis and increased aerobic energy production combined with increased lactate clearance (Robergs, Ghiasvand & Parker, 2004). A resultant outcome is a delayed lactate accumulation, which for reasons discussed in chapter 2.1.5, is concurrent with increased endurance performance (Faud, Kindermann & Meyer, 2009).

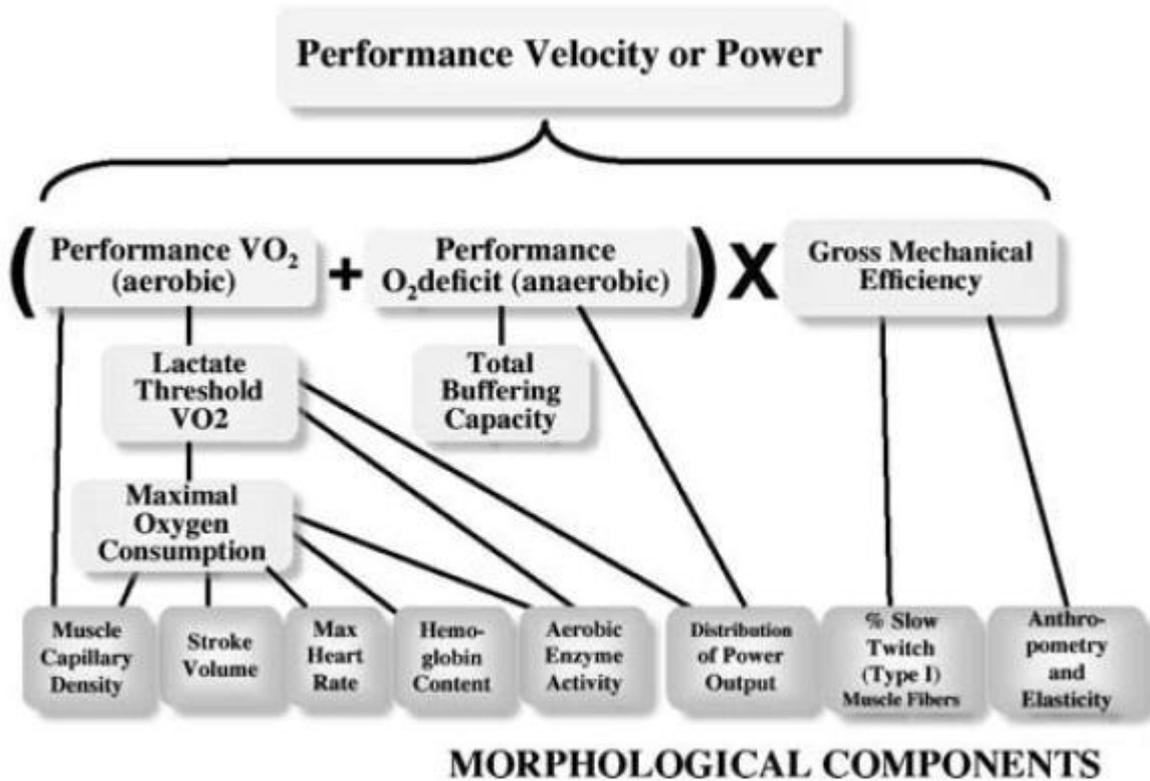


Figure 1. Schematic of several physiological factors interacting as determinants of performance. Taken from Joyner & Coyle (2008).

The response to exercise is not constant throughout the population. In fact, genetic polymorphisms have been identified to predict the extent of adaptation to exercise of fitness variables such as  $\dot{V}O_{2\max}$  (Bouchard et al., 2011; Ghosh et al., 2013). The complexity regarding responders and non-responders is exacerbated by the finding that individuals who show an attenuated training response in one parameter do not necessarily show the same low training response in other parameters (Scharhag-Rosenberger et al., 2012; Vollaard et al., 2009). Furthermore, the contribution of different factors is not uniform and improved performance may occur in the absence of some factors (Gibala et al., 2006).

#### 2.1.4 The Importance of Cardiorespiratory Fitness and Methodologies of Tests

$\dot{V}O_{2\max}$ , a measurement of the highest rate of oxygen consumption attained during maximal exercise, has long been associated with performance in endurance sports (Jones & Carter, 2000; Wilmore & Costill, 1999). Indeed,  $\dot{V}O_{2\max}$  is the most common parameter used to demonstrate a training effect, and is frequently used to indicate the cardiorespiratory fitness of an individual (Bassett & Howley, 2000). The major limitation to  $\dot{V}O_{2\max}$  is the ability of the cardiorespiratory system to deliver oxygen to exercising muscles (Bassett & Howley, 2000). The rate of ATP production that can be maintained during exercise is dependent on  $\dot{V}O_{2\max}$  (Bassett & Howley, 2000). As such,  $\dot{V}O_{2\max}$  sets the upper limit

for energy production during endurance events, but does not necessarily determine performance, since endurance events are not competed at 100% of  $\dot{V}O_{2max}$  (Jones & Carter, 2000). The product of stroke volume and HR comprise cardiac output, the volume of blood pumped out by the heart per minute; it is now considered that variations in  $\dot{V}O_{2max}$  are primarily due to differences in stroke volume, since  $HR_{max}$  is far less varied between individuals and delivery of oxygen is primarily facilitated by this mechanism (Bassett & Howley, 2000). A multitude of studies have recognised that the greatest change in  $\dot{V}O_{2max}$  occurs at the beginning of a training programme, followed by smaller adaptations with further enhanced endurance ability, and may eventually stabilise (Hickson, Hagberg, Ehsani & Holloszy, 1980; Isawaki et al., 2003; Scharhag-Rosenberger, Meyer, Walitzek & Kindermann, 2009). As such, further improvements in performance may be attributed to further improvements in factors such as exercise economy and lactate threshold (Jones & Carter, 2000).

In order to accurately measure  $\dot{V}O_{2max}$  an individual is usually expected to perform a graded physical exertion test for a sufficient duration which takes the individual to maximal effort whilst respiratory measurements are obtained.  $\dot{V}O_{2max}$  is attained when a plateau in oxygen consumption occurs.  $\dot{V}O_{2max}$  and  $\dot{V}O_{2peak}$  are often used interchangeably despite differences in definitions.  $\dot{V}O_{2peak}$  represents the highest value of  $\dot{V}O_2$  attained during a test. However, this value is determined independent of the athlete's effort, and so does not always reflect the highest value which is attainable by the individual (Day, Rossitier, Coat, Skasick & Whipp, 2003). Corroborative indices are used to ascertain whether a value reflects a sufficient effort given by the individual to produce an accurate  $\dot{V}O_{2max}$ . These include a maximum heart rate (HR) greater than 90% predicted; a respiratory exchange ratio above 1.10 and a blood lactate level greater than  $8\text{mmol}\cdot\text{L}^{-1}$  (Midgley, McNaughton, Polman & Marchant, 2007). A study by Astorino (2009) found sampling interval to largely affect the corresponding  $\dot{V}O_{2max}$  values. Sampling intervals of 60 seconds appeared to reduce the incidence of  $\dot{V}O_2$  plateau independent of fitness status as demonstrated by previous research (Astorino et al., 2000; Doherty, Nobbs & Noakes, 2003; Lucia et al., 2006).

Despite heavy criticisms to the current  $\dot{V}O_{2max}$  determination criteria (Midgley et al., 2007), an improved uniform set of criteria have not been validated. In contrast, Day et al. (2003) suggest that a plateau in oxygen consumption is not an essential prerequisite for ascertaining  $\dot{V}O_{2max}$  in maximal effort incremental ramp tests. In this study of 71 individuals,  $\dot{V}O_{2peak}$  attained from a ramp test did not differ from the plateau in the plot of  $\dot{V}O_2$  vs work rate (in constant-load tests). It is of particular importance that various studies have identified that  $\dot{V}O_2$  plateaus are rarely attained during ramp style maximal effort protocols (Day et al., 2003; Duncan, Howley & Johnson, 1997; Howley et al., 1995;

Rossiter, Kowalchuk & Whipp, 2005). Lactate threshold may be a firmer index of functional status than  $\dot{V}O_{2max}$ , since it is not confounded by the participant's subjective effort (Faud et al., 2009).

### **2.1.5 The Use of Lactate Threshold as a Functional Assessment of Endurance Performance**

As mentioned previously, exercise training can induce a change to the numbers and proportions of type I and II skeletal muscle fibres and also the detailed properties of individual muscle fibres (Coyle et al., 1992; Hawley, 2002). Changes in lactate accumulation occur secondary to this (Brooks, 2007; Joyner & Coyle, 2008). Endurance training can increase lactate clearance and reduce lactate production (Bergman et al., 1999). Since lactate production coincides with cellular acidosis, reducing its accumulation is associated with a delayed active muscular fatigue (Robergs et al., 2004). Hydrogen ( $H^+$ ) is produced during ATP break down, and used during mitochondrial respiration for energy production (Robergs et al., 2004). In the mitochondria,  $H^+$  molecules accumulate at high exercise intensities, where there is a shift towards a greater reliance on anaerobic respiration. In order to prevent acidosis and pyruvate accumulation,  $H^+$  binds with pyruvate to form lactate, providing an energy source during recovery from exercise and regenerating  $NAD^+$ . The coenzyme  $NAD^+$  is needed for glycolysis during anaerobic metabolism (Robergs et al., 2004).

Along with  $\dot{V}O_{2max}$ , the submaximal exercise intensity at the point by which blood lactate increases above resting levels is a strong predictor of endurance performance (Jones & Carter, 2000). An increased power output at lactate threshold allows a greater exercise intensity to be sustained in the absence of blood lactate accumulation. Blood lactate accumulation is concurrent with more rapid fatigue, due to the effects of metabolic acidosis on contractile function or depletion of muscle glycogen (Jones & Carter 2000; Robergs et al., 2004).

### **2.1.6 Health-Related Cardiovascular Adaptations of Endurance Training**

Abundant research has highlighted the beneficial aspects of exercise in healthy and diseased individuals (Warburton, Nichol and Bedin, 2006), including CV health and longevity (Dai, Rabinovitch and Ungvari, 2012; Seals et al., 2009). However, the mechanisms underlying these effects are not fully known. Increasing training volume and hence haemodynamic demand, influences the loading conditions of the heart and may provide sufficient stimulus for morphological changes to the heart.

#### **2.1.6.1 Endurance Athlete's Heart**

The athletic heart is a non-pathological condition associated with normal cardiac adaptations to routine exercise, which in current perspective predominantly relates to endurance type training (Scharhag, Löllgen & Kindermann, 2013). Training-induced adaptations to the heart include enlarged LV and RV volumes, increased LV wall thickness and cardiac mass. At least five hours of endurance training per week is suggested to be necessary to elicit dimensional changes to the heart (Scharhag,

2013). This concept was introduced to differentiate serious heart diseases from normal athletic hearts. New parameters have been set for those meeting the athletic criteria (Drezner et al., 2012). Normal training-related ECG findings and abnormal, training-unrelated findings are displayed in table 1. The heart of an endurance athlete is more than likely to undergo physiological changes in structure, function and electrical activity as training progresses. These changes may place the individual in the diagnostic 'grey zone', providing difficulty in the diagnosis of underlying pathological disease (Baggish & Wood, 2011). For the most part, these adaptations represent normal physiological adaptation, however accumulating evidence suggests that some of the remodelling may not be entirely benign (Ector et al., 2007, O'Keefe et al., 2012). The characterisation of the athlete's heart has helped in the differentiation from normal adaptation of the heart and several inherited cardiac diseases, most prominently hypertrophic cardiomyopathy, which may predispose to SCD (Baggish & Wood, 2011; Drezner, 2012; Drezner et al., 2012). Nevertheless, in a minority of individuals endurance training may trigger a pathological cascade, placing them in an increased risk of SCD (George et al., 2012). Providing an insight of the changes to the electrical activity of the heart over a long-term, high load, training programme will provide an understanding of normal adaptations of the 'athletes heart'.

Table 1. Group 1, abnormal training-unrelated and group 2, training-related ECG findings in athletes, adapted from Drezner et al. (2012).

ECG finding	Description
<b>Group 1 Abnormal, Training-Unrelated</b>	
T Wave inversion	> 1 mm in depth from baseline in 2 or more adjacent leads not including aVR or V1
ST segment depression	≥ 1 mm in depth in 2 or more adjacent leads
Pathological Q waves	> 3 mm in depth or > 0.04s in duration in 2 or more leads
Complete left bundle branch block	QRS > 120 ms, predominantly negative QRS complex in lead V1, and upright monophasic R wave in leads I and V6
Complete right bundle branch block	QRS > 120 ms, terminal R wave in lead V1, and wide terminal S wave in leads I and V6
Intraventricular conduction delay	Non-specific QRS > 120 ms
Left atrial enlargement	Prolonged P wave duration > 120ms in leads I or II with negative portion of the wave ≥ 1 mm in depth and ≥ 0.04s in duration in lead V1
Left axis deviation	QRS axis -30° to -90°
Right atrial enlargement	P wave ≥ 2.5 mm in leads II and III or V1
Right ventricular hypertrophy	Right QRS axis deviation ≥ 120° or voltage criteria > 10.5 mm (R in V1 + S in V5)
Mobitz type II 2° AV block	Intermittently non-conducted P waves not preceded by PR prolongation and not followed by PR shortening
3° AV block	Complete heart block

Ventricular pre-excitation	PR interval < 0.12 s with a delta wave
Long QT interval	QTc ≥ 470 ms (male) QTc ≥ 480 ms (female) QTc ≥ 500 ms (unequivocal LQTS)
Short QT interval	QTc ≤ 340 ms
Epsilon wave	Small negative deflection just beyond the QRS in V1 or V2
Profound sinus bradycardia	Resting heart rate < 30 beats·min <sup>-1</sup>
Atrial tachyarrhythmias	Supraventricular tachycardia, atrial-fibrillation, atrial flutter
Premature ventricular contractions	≥ 2 per tracing
Ventricular arrhythmias	Couplets, triplets or non-sustained ventricular tachycardia

**Group 2 Common, Training-Related**

Sinus bradycardia	Resting heart rate < 60 beats·min <sup>-1</sup>
First degree AV block	PR interval > 200 ms
Early repolarisation	J point 0.1 mV above isoelectric line in limb leads and 0.2 mV in precordial leads
Incomplete right bundle branch block	QRS duration > 100 ms and < 120 ms, terminal R wave in V1 & slurred S wave in leads I and V6
Isolated QRS voltage criteria for left ventricular hypertrophy	Sokolow-Lyon criteria > 3.5 mV (S in V1 + greatest R wave in V5 or V6)

Note: Isolated QRS voltage criteria for LVH does not involve non-voltage criteria for LVH such as left axis deviation, a 'strain' pattern of repolarisation, ST-segment depression, T-wave inversion, or pathological Q waves). Criteria for early repolarisation is one of many among literature and so results for which should be taken with caution.

### 2.1.6.2 Cardiovascular Risk and Possible Exercise-Induced Myocardial Injury

Although moderate activity levels have received extensive praise, there is a necessary importance to ascertain whether excessive exercise induces harm to the human body. Some studies have found an increased relative risk of CV disease in those performing most aerobic exercise, highlighting the possible adverse effects of exercise (Burr, et al., 2014; O'Keefe et al., 2012; Paffenbarger et al., 1986; Quinn et al., 1990; Schnohr, Marott, Lange & Jensen, 2013; Schnohr, O'Keefe, Marott, Lange & Jensen, in press). In the recent Copenhagen City Heart Study, a large scale longitudinal study, researchers observed a U-shaped curve for mortality risk relating to jogging quantity, frequency and speed (Schnohr et al., 2013). For this reason, ultra-endurance athletes may represent a group of individuals at an elevated risk of CV events. The majority of recent intervention studies suggest a purely transient reduction in cardiac health in response to a single bout of excessive endurance exercise (La Gerche et al., 2008; Shave et al. 2002; Shave et al., 2010). For example, Shave et al. (2002) found evidence of elevated cardiospecific biomarker cardiac troponin (cTn) T above the 99<sup>th</sup> percentile of 0.01 µg/L, but not above the clinical threshold for myocardial infarction of 0.1 µg/L, as a result of prolonged endurance exercise in 13 participants (50%). Additionally, systolic and diastolic cardiac dysfunction

following prolonged exercise is a common finding, which usually resolves shortly (~1 week) after participation (La Gerche et al., 2008; La Gerche et al., 2012). However, several epidemiological studies suggest an accumulative detrimental response to long-term involvement in excessive endurance exercise (Lee, Pate, Lavie & Blair, 2012; O'Keefe et al., 2012).

Several studies have reported elevations to myocardial injury biomarkers after endurance exercise, and it is now considered a common outcome with higher intensities and lesser athletic status (Neilan et al., 2006; Scherr et al., 2013; Shave et al., 2007; Shave et al., 2010). Two theories exist regarding the importance of post-endurance exercise elevations in cardiac injury biomarkers. Biomarkers such as creatine kinase and cTn used to determine cardiomyocyte damage remain controversial due to the kinetics of release. cTn appears rapidly in the bloodstream and likewise are cleared rapidly, opposing from the sustained release occurring with acute myocardial infarction. Some suggest this release may in fact represent normal adaptive physiological processes (Neumayr 2005; Shave et al., 2007). Normal physiologic skeletal muscle adaptation is preceded by protein breakdown, perhaps exercise-induced cardiac hypertrophy follows a similar adaptive process. The first hypothesis therefore is that myocardial injury provides a physiological stimulus for adaptation, leading to both an enhanced structure and function (Whyte, 2003). Contrasting this is the hypothesis that the observed myocardial damage is followed by scarring and fibrosis and increased risk of arrhythmogenic presentations (Whyte, 2003). Furthermore, the injury is suspected to become permanent as a result of inadequate recovery time between exercise bouts (Rowe, 1992). The postulation that exercise-induced myocardial injury leads to a deleterious and pathological process in the heart is supported by a limited number of studies.

There appears to be contrasting views regarding chronic training and CV health. On the one hand, those with the greatest endurance experience elicit the least cardiac damage biomarker response to prolonged exercise (Neilan et al., 2006; Shave et al., 2007). However, those training most ( $\geq 4$  times/week,  $>2/2.5$ h/week) show greatest signs of reduced CV health compared with those training less ( $\leq 3$  times/week,  $<1$ h) (O'Keefe et al., 2012; Schnohr et al., 2013; Schwartz et al., 2014). The reduction in biomarker release in experienced endurance athletes could be explained by greater myocardial scarring replacing normal cardiac tissue. Additionally, the potential for exercise-induced damage appears to increase with middle age and above (O'Keefe et al., 2012). Wen et al. (2011) found health benefits to accrue dose-dependently until around an hour per day of high intensity exercise, whereby further endurance training resulted in a much reduced benefit to all-cause mortality (figure 2). O'Keefe et al. (2012) have interpreted this data to show there exists a dose of exercise beyond which further CV benefits are not yielded. It has been suggested that exercise shares the same

principle as other factors, such as sleep and calorie intake, in that “just because some is good, more is not always better” (O’Keefe, Schnohr & Lavie, 2013, p.588).

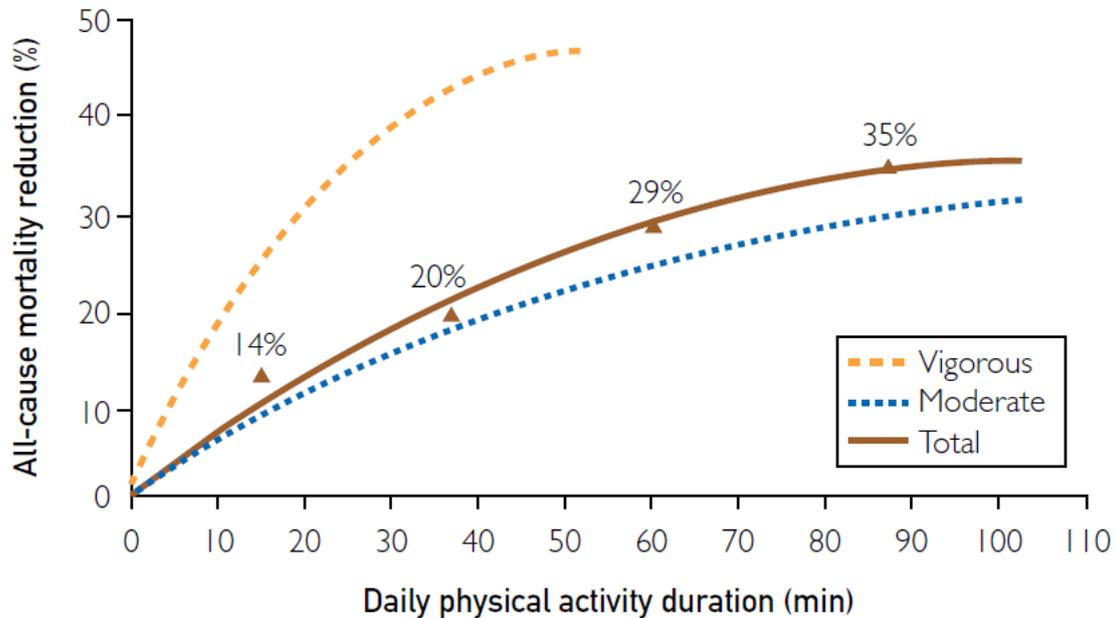


Figure 2. Relationship between volume and intensity of physical activity and all-cause mortality. Taken from Wen et al. (2011).

In further support of the potentially accumulative damaging impact of endurance exercise, recent studies have observed increases in myocardial scarring in older-aged endurance athletes. Breuckmann et al. (2009) found a 3-fold increase in patchy myocardial scarring in older age long-distance runners compared with age-matched controls. Similarly, Wilson et al., (2011) observed a high prevalence of patchy myocardial fibrosis of 50% of 12 lifelong veteran endurance athletes. No cases of cardiac fibrosis were found for age-matched controls and younger endurance athletes, indicating a link between habitual endurance exercise and myocardial fibrosis.

### 2.1.7 Endurance Training-Related Changes to the Cardiac Electrical Conductance

High endurance sports such as cycling, rowing, and cross country skiing have been shown to be significantly associated with a higher frequency and greater extent of ECG changes such as sinus bradycardia and increased QRS voltages compared to strength and speed type sports (Pellicia et al., 2000). ECG changes in athletes usually reflect electrical and structural remodelling as an adaptation to habitual exercise. This is likely to be related to the large cardiac output acquired during endurance training resulting in cardiac remodelling involving in part the LV and RV dimensions and wall thickness

(Maron & Pelliccia, 2006; Scharhag et al., 2002). For this reason, different ECG criteria are now used to identify underlying CVD in athletes compared to non-athletes. The changes associated with athletes are split into training-unrelated (group 1) and training-related (group 2) ECG changes (Drezner, 2012). Table 1 identifies these different criteria.

Recent investigation has found that training-unrelated ECG findings were more common in elite endurance trained athletes than non-endurance athletes (Brosnan et al., 2014). Right ventricular hypertrophy (RVH) (4.4% vs. 1.5%) and T wave inversion, particularly right precordial (14.3% vs. 4.7%), were most prevalent in endurance athletes. Importantly, cardiac imaging did not identify features of arrhythmogenic RV cardiomyopathy. This finding is supportive of non-pathological cardiac bioelectrical abnormalities in a small cohort of individuals, induced by endurance training.

### *2.1.7.1 Group 1 Training-Unrelated ECG Findings*

#### *2.1.7.1.1 Profound Sinus Bradycardia*

Profound sinus bradycardia, defined as a rHR below 30 beats·min<sup>-1</sup>, occurs in some highly trained athletes without the presence of pathological condition. However, this condition should be distinguished from sinoatrial dysfunction. Corrado (2010) notes the clarification can be made if: (i) there is an absence of dizziness or syncope, (ii) HR normalises during exercise with preservation of HR<sub>max</sub>, (iii) bradycardia reverses with detraining.

#### *2.1.7.1.2 Left Atrial Enlargement*

Left atrial enlargement (LAE) is currently considered an uncommon finding in athletes, which is prevalent in around 10-21% of HCM patients (Drezner et al., 2013). LAE is defined as a P wave duration > 120 ms in leads I and II with a negative portion ≥ 0.1 mV in depth and longer than 40 ms in V1. The clinical significance of left atrial remodelling in athletes has been questioned. Pelliccia et al. (2005) observed a relatively common (20%) prevalence of LAE in competitive athletes, largely without clinical consequence. Recently, Gati et al. (2013) found that of the 13% of group 2 changes present in 14 to 35 year olds, 42.6% were comprised of isolated left axis deviation (LAD) together with isolated LAE. Furthermore, echocardiographic evaluation failed to identify any structural or functional abnormalities of the 579 athletes displaying these patterns. Exclusion of isolated LAD and LAE improved specificity from 90-94% and only reduced sensitivity by 1.5%. LAE is unlikely to uncover underlying heart disease in competitive athletes and although current consensus recommends follow-up investigation, doing so may be unnecessary.

#### *2.1.7.1.3 Right Atrial Enlargement and Right Ventricular Hypertrophy*

Right atrial enlargement (RAE) and RV hypertrophy (RVH) are uncommon findings in athletes. A prevalence of 0.08% in athletes has been reported for RAE (P wave axis >70°) and 0.6% for right axis deviation (QRS axis >110°) (Pelliccia et al., 2000). Other investigators use a high P wave ≥ 0.25mV in

leads I and II or  $V_1$  to identify RAE (Drezner, 2012; Drezner et al., 2013). Sokolow-Lyon voltage criteria for RVH ( $R$  in  $VI + S$  in  $V5 \geq 1.5$  mV) identified just one of 172 professional soccer players, representing a prevalence of 0.6% (Sumauroo, Pyatt, Jackson, Perry and Ramsdale, 2001). According to Corrado et al. (2010) RAE and RVH do not represent a manifestation of exercise-induced cardiac remodelling. Instead, Corrado et al. (2010) suggested that underlying heart diseases associated with increased right atrial size and pathological RV dilation or hypertrophy should be excluded. Indeed, recent investigations have recognised the effects of prolonged endurance exercise on RV function after acute exercise and the enlarged RV dimensions resulting from endurance training (La Gerche, Connelly, Mooney, Maclsaac & Prior, 2007; Predel, 2014; Scharhag et al., 2002; Scharhag, Löllgen & Kindermann, 2013).

#### 2.1.7.1.4 ST-Segment Depression

ST-segment depression ( $\geq 0.1$ mV depression of ST segment below isoelectric line in two or more adjacent leads) is usually associated with T wave inversion, therefore true incidence rates are obscured (Corrado et al., 2010). In any case, this ECG pattern is a rare occurrence indicative of underlying heart disease. In a resting ECG, ST-segment depression should prompt follow-up investigation to eliminate the existence of heart disease (Drezner, 2012).

#### 2.1.7.1.5 T-wave Inversion

One of the most pertinent and easily recognisable ECG patterns for a potential CVD is T-wave inversion. T wave inversion  $> 0.1$  mV in two or more adjacent leads (excluding aVR and  $V_1$ ) is a common finding with HCM, and uncommon in healthy athletes (Drezner 2012, Drezner et al., 2013). The prevalence of T-wave inversion is similar among sedentary individuals and elite athletes and has been identified in around 2.7% and 4.4% of individuals (Pelliccia et al., 2000; Sharma et al., 1999). Discrepancy exists regarding the criteria for this ECG pattern, with some clinicians recommending 0.1mV inversions and some using 0.2mV criteria. Additionally, the location of the pattern, for example the leads associated with the pattern, is frequently different among different investigators (Corrado et al., 2010; Drezner, 2012; Pelliccia et al., 2000; Sharma et al., 1999). As with ST segment elevation, it is common for healthy Afrocaribbean athletes to develop a T-wave inversion in  $V_2$  to  $V_4$ , representing normal early repolarisation changes (Corrado et al., 2010).

#### 2.1.7.1.6 Intraventricular Conduction Delays

Intraventricular conduction delay, identified as a non-specific QRS duration  $\geq 120$  ms, is an uncommon finding affecting less than 2% of athletes and may represent a serious underlying CVD (Corrado et al., 2010). Likewise, LBBB and RBBB patterns have low incidence rates, identified in 1% and 0.8% of highly trained athletes (Pelliccia et al., 2006). Intraventricular delay is likely to occur in the myocardium

whereas bundle branch block may develop due to a degenerative lesion of the conducting tissue, or from various cardiac pathologies (Corrado et al., 2010).

#### 2.1.7.1.7 Ventricular Pre-Excitation

Ventricular pre-excitation (PR interval < 120 ms with a delta wave, slurred upstroke in the QRS complex) is another condition unrelated to training status. The prevalence of this ECG pattern in athletes and the general population is around 0.1% to 0.3% and most individuals are without symptoms (Corrado et al 2010). The condition occurs due to early activation of the ventricles resulting from impulses bypassing the AV node via an accessory pathway (Kulig & Koplán, 2010).

#### 2.1.7.1.8 QT Interval

The duration of the QT interval (the beginning of the QRS complex and the end of the T-wave) is influenced by HR, therefore calculations can be made to create a QT duration which is corrected for HR (QTc). Bazett's formula is considered the standard method of correcting for HR ( $QTc = QT/VRR$ ). However, corrections are not advised for HR below 40 and above 120 beats·min<sup>-1</sup> due to accuracy issues, which can be problematic for athletic individuals with sinus bradycardia (Corrado et al., 2010).

A QTc interval is considered long when it exceeds 470 ms in males and 480 ms in females. An individual presenting a QTc interval exceeding 500 ms is considered to have unequivocal long-QT syndrome (Drezner, 2012). Usually, athletes will have longer QTc intervals than less active controls due to the extent of sinus bradycardia and sinus arrhythmia. Transient causes associated with prolonged QTc include metabolic changes and electrolyte disorders; therefore stringent assessment of nutritional intake should be undertaken prior to conclusions being made (Corrado et al., 2010).

Short-QT syndrome ( $QTc < 380$  ms) is an inheritable ion-channel disease characterised by short cardiac repolarisation (Drezner, 2012). Rapid cardiac repolarisation predisposes individuals to life threatening atrial and ventricular fibrillation in the absence of structural disease (Corrado et al., 2010). Transient QT shortening may be caused by hypercalcaemia, hyperkalaemia, hyperthermia, acidosis, and some drugs. As with long QT interval, alternative assessments should be conducted to account for these confounding factors prior to making assumptions.

#### 2.1.7.1.9 Axis Deviation

The QRS axis represents the average direction in which the excitatory process spreads throughout the myocardium. Likewise vectors are obtained for the P and T waves. QRS axis deviation alone is not a specific finding of pathological condition, but should warrant further investigation as an abnormal finding in athletes (Drezner, 2012). Normal QRS axis is age dependent until 8 years old. At birth there is a right axis deviation which progressively shifts leftward to reach normal adult ranges. Thereafter QRS axis remains stable dependent of conductive tissue abnormalities apparent with older age

(Humes, 2014). As mentioned previously, LAD without structural or functional abnormalities is not as infrequent as formerly thought (Gati et al., 2013). Therefore the inclusion of such patterns as uncommon training-unrelated warranting further investigation is disputed and potentially unjustified.

### *2.1.7.2 Group 2 Training-Related ECG Findings*

#### *2.1.7.2.1 Sinus Bradycardia*

Resting sinus bradycardia, defined as a resting heart rate (rHR) less than 60 beats·min<sup>-1</sup>, is a common finding in athletes reported in up to 91% of endurance athletes (Drezner, 2012; Thompson, 2007). Sinus bradycardia is suggested to reflect the level of athletic condition (Corrado et al., 2010). The mechanisms underlying this adaptation are multifactorial including the autonomic nervous system, sinoatrial remodelling, cardiac hypertrophy, baroreflex resetting and genetic predispositions (Corrado et al., 2010; Matelot, Schnell, Kervio, Du Boullay & Carré, 2013). Alterations to HR<sub>max</sub> with endurance training likely involve similar mechanisms (Zavorsky, 2000).

#### *2.1.7.2.3 First-Degree and Mobitz Type I Second-Degree Atrioventricular Block*

First degree and Mobitz type I second-degree AV block are common findings in athletes, displayed in around 35% and 10% of athletes respectively (Corrado et al., 2010; Drezner, 2012). As with sinus bradycardia, up regulated parasympathetic tone and down regulated resting sympathetic tone largely mediates the slowing and block of AV electrical conduction (Corrado et al., 2010).

#### *2.1.7.2.4 Isolated Voltage Criteria for Left Ventricular Hypertrophy*

Morphologic cardiac changes including increased wall thickness and ventricular mass, associated with athletic conditioning, are reflected in the 12-lead ECG. Isolated increases of QRS amplitude (normal QRS axis, normal ST-segment and T wave repolarisation) are the usual product of LV Hypertrophy (LVH) of trained individuals. Isolated QRS amplitude assessed by the Sokolow-Lyon voltage criteria (S wave in V1 + maximal R wave in V5 or V6 > 3.5mV), accounted for around 60% of the abnormal ECG's in elite Italian athletes (Pellicia et al., 2000). In this study 40% of 1005 athletes ECG's were considered abnormal. Non-voltage ECG criteria for LVH such as atrial enlargement or left axis deviation, are normally absent in healthy athletes. On the other hand, an isolated increase of QRS voltage is an uncommon finding in hypertrophic cardiomyopathy (HCM) patients.

#### *2.1.7.2.5 Incomplete Right Bundle Branch Block*

Incomplete right bundle branch block (RBBB) is another adaptation which is highly associated with athletes, in particular those with an endurance type background (Drezner, 2012). This finding is identified by ECG criteria of a QRS duration <120 ms, with an RSR pattern in V1, V2 or V3 (figure 3). Studies estimate the prevalence of incomplete RBBB to range from 35-50% in athletes compared with less than 10% prevalence in healthy controls (Corrado et al., 2010). Arrhythmogenic RV cardiomyopathy (ARVC), a life threatening condition, is suspected when incomplete RBBB is

accompanied with (i) T-wave inversion included in leads V3 and V4, or (ii) premature ventricular beats with left bundle branch block (LBBB) morphology.



Figure 3. Characteristic electrocardiography tracing of an individual with incomplete right bundle branch block; RSR pattern in V1 and slurred S wave in V6 and QRS duration < 120 ms.

ECG tracing is from a participant of study 1, of which details are provided in chapter four.

#### 2.1.7.2.6 Early Repolarisation

Early repolarisation, defined as an elevation of the QRS-ST junction (J-point) of at least 0.1 mV from baseline accompanied by slurring of the terminal QRS complex. The early repolarisation pattern is generally considered a benign ECG finding in athletes, being prevalent in around 50-80% of athletic individuals (Corrado et al., 2010). This ECG finding is more frequent during resting conditions and disappears with deconditioning, reflecting the hypervagotonia induced by exercise training (Corrado et al., 2010). With individuals of Afrocaribbean descent, the presence of an elevated ST-segment with T-wave inversion in V2-V4 is often a benign finding.

#### 2.1.8 Sudden Cardiac Death and Exercise

Over the last few decades, participation in endurance and ultra-endurance events has risen considerably. SCD in endurance sports is very rare, with around 1 event for every 100,000 marathon participants (Lee, Pate, Lavie & Blair, 2012; O’Keefe et al., 2012). The 12-lead ECG has a greater clinical success for identifying asymptomatic athletes with potentially lethal heart disorders over and above history and physical examination (Corrado, Basso, Schiavon, Pelliccia & Thiene, 2008; Corrado & McKenna, 2007; Drezner, 2008; Myerburg & Vetter, 2007; Papadakis, Whyte & Sharma, 2008). Those athletes presenting training-unrelated abnormalities, representative of underlying heart disease, are at an increased risk of SCD and of developing life threatening conditions such as arrhythmias and fibrillations (Corrado et al., 2010). A national, state sponsored, pre-participation screening programme was introduced in Italy over 30 years ago as part of a legislation known as the “Medical Protection of Athletic Activities” law. The law states that all individuals engaged in sport must be routinely examined in regards to eligibility to participate in competitive sport which includes 12-lead ECG, as well as a history and physical examination (Pelliccia & Maron, 1995). Those with abnormalities are referred for further investigation including cardiac magnetic resonance imaging (cMRI) and echocardiography, and

those with potentially serious abnormalities are disqualified from competitive sport. The implementation of pre-participation screening resulted in a reduction in the incidence of SCD from 3.6 per 100,000 person years before screening to 0.4 per 100,000 person years 25 years after implementation in 2003-2004 (Corrado, Basso, Pavei, Michieli, Schiavon & Thiene, 2006). The Italian model was assessed in a study involving 4450 elite athletes who were judged eligible for participation after 12-lead ECG assessment. Subsequent echocardiography was conducted to assess previously undetected HCM (Pelliccia et al., 2006). Over an 8 year follow-up period only one individual was diagnosed with definitive HCM (0.02%) indicating a high negative predictive value of a normal ECG for HCM. Nonetheless, false positive detection of cardiomyopathies is a concern with 12-lead ECG interpretation. Even experienced cardiologists, with knowledge of the exercise-induced adaptations similar to the phenotypic manifestations of cardiomyopathies, have found an unacceptably high rate of false positive results (Pelliccia et al., 2000).

The causes of SCD resulting from physical exertion in individuals over the age of 30 commonly include coronary artery disease, coronary heart disease and myocardial infarction (Albano, Thompson & Kapur, 2012; Maron et al., 1996). Hypertrophic cardiomyopathy (HCM) is responsible for a large proportion of SCD in young athletes under the age of 30 (Albano, Thompson & Kapur, 2012; Maron & Pelliccia, 2006; Pelliccia et al., 2005). An ECG is unable to identify premature coronary artery disease and congenital coronary anomalies, therefore alternative measurement techniques are required to identify those at risk, such as arterial stiffness indices.

Emerging evidence suggests that exercise-induced cardiac remodelling may have a pathological component (La Gerche et al., 2010; O'Keefe et al., 2012; Wilson et al., 2011). Further study is warranted to determine if potentially pathological findings have any clinical impact and if any ECG findings may help identify the sub-population at risk of triggering this pathological remodelling (Weiner et al., 2011). Pre-participation screening with the use of ECG is an effective procedure in reducing SCD from cardiomyopathies, with a potential ethical concern regarding false positive results. This is a particularly effective tool for young athletes at a greater risk of SCD from cardiomyopathies. Coronary anomalies and premature coronary atherosclerosis are not detected by ECG and are responsible for a large proportion of SCD in athletes above 30 years of age. An appropriate pre-participation tool in the older population of athletes may involve greater use of measurements pertaining arterial stiffness.

## 2.2 Study 2

### 2.2.1 Background: Ultra-Endurance Exercise and Arterial Health

Substantial evidence supports the concept that moderate levels of exercise are effective for the prevention and treatment of many common illness' and disease states (Warburton, Nichol and Bredin, 2006). Nevertheless, acute exercise transiently elevates the risk of SCD and CV events (Corrado, Basso, Rizzoli, Schiavon & Thiene, 2003); yet the associated CV changes implicated in this transient period are not well understood. Furthermore, repeated exposure to prolonged exercise is linked with accumulative detrimental indices regarding CV health (O'Keefe et al., 2012; Schwartz et al., 2014). In light of recent increases in participation rates in ultra-endurance events (Hoffman, Ong, & Wang, 2010; Knechtle, Knechtle & Lepers, 2011), it is striking that we have a less than full understanding of the acute and long-term CV changes.

The arterial system is comprised of a complex organisation of elastic tubes, within which the cardiac pump generates pressure waves, which travel through the system. The pressure difference induced by the wave allows blood to flow through the system, albeit at a considerably reduced transfer rate to the wave itself (Caro et al., 1978). Early physicists recognised that no single arterial segment shares identical viscoelastic properties, therefore the properties of one segment cannot be generalised for the whole arterial tree (Edwards & Lang, 2005; Laurent et al., 2006). Extensive progress has been made in recent years to develop parameters of measuring arterial properties which includes the clinical application of such techniques. Current practice is now at a point that clinicians can predict outcome and assess arterial health in different populations (Laurent et al., 2006; Mattace-Raso et al., 2006). Furthermore, the degree of arterial stiffening is a direct risk factor for CV events and mortality, and has greater significance than central blood pressures (CBP) (Laurent et al., 2006).

Large elastic central arteries, such as the aorta, and medium sized muscular arteries, such as the femoral artery, have important roles as a conduit vessel and a buffer (or cushion) for blood flow pulsations (Nichols, O'Rourke & Vlachopoulos, 2011). There are two different concepts of these great vessels, that of compliance function, and that of conduit function. An artery with lower stiffness, and inversely greater compliance, allows for a higher pressure buffering capacity which creates a more efficient system during systole. Efficiency is increased due to compliant arteries attenuating energy loss and allowing the blood to flow more smoothly along the vessel (Davies, 2011). The most simplistic way to illustrate the cushioning function of arteries is to use the Windkessel model (figure 4). In this model, the large arteries are represented by a chamber (Windkessel) and peripheral arteries by the spout. The volume in the Windkessel is directly proportional to the excess pressure within it, and the flow rate is proportional to the pressure difference, with resistance in peripheral arteries at a constant (Caro et al., 1978). When the pressure in the large arteries rises during systole they expand,

subsequently when the pressure falls they contract to maintain a near constant flow-rate through peripheral vessels. This model is very simplistic since elastic properties are different throughout the arterial system and the model assumes that all arteries are distended simultaneously, which is not true. The Windkessel model has since been adapted to account for further characteristics of the arterial system (Westerhof, Lankhaar & Westerhof, 2009). Arterial compliance capacity may be augmented during moderate exercise to tolerate the increased blood flow (Kingswell et al., 1997; Maeda et al., 2008) or reduced after longer lasting endurance exercise (Burr et al., 2012; Vlachopoulos et al., 2010) and shorter eccentric exercise (Burr Boulter & Beck, in press). Reduced arterial compliance as a result of excessive exercise training is also not an uncommon finding (Burr et al., 2013).

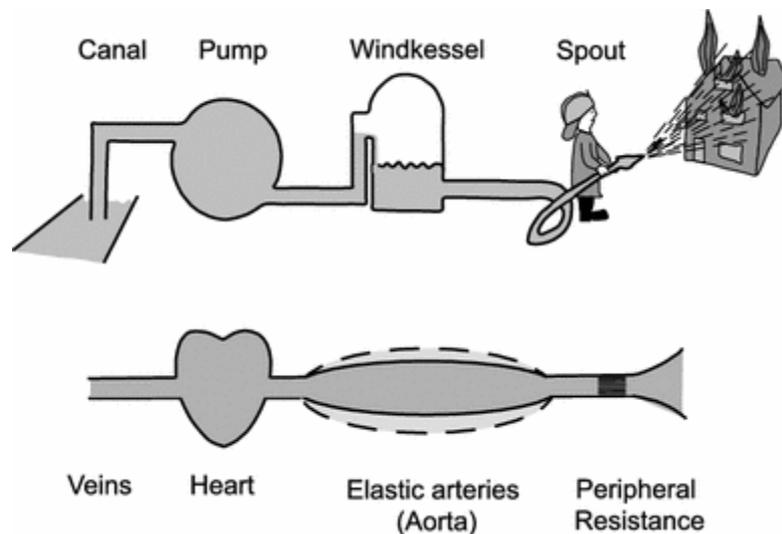


Figure 4. The Windkessel model of the arteries. Taken from Westerhof et al., 2008.

The large arteries act as the Windkessel. The combination of compliance, with aortic valve function and peripheral resistance results in a near constant peripheral flow.

### 2.2.1.1 Endurance Athlete's Artery

The Athlete's heart, as discussed previously (chapter 2.1.6.1), is now an accepted term to describe the characteristic morphology of the heart of some athletes. It is acknowledged that the heart of athletic individuals may develop different structural or functional characteristics as a result of exercise exposure (George et al., 2012). Likewise, the 'athlete's artery' has been suggested as a morphologic development, which like the athletes heart largely refers to endurance type exercise. Green et al. (2012) summarise findings of several studies with interest in the development of resistance and conduit vessels in response to training. Longer term studies assessed in this review support the hypothesis that athletes possess larger arteries with reduced wall thickness than non-athletic counterparts, as presented in figure 5 (Rowley et al., 2011; Schmidt-Truckäss et al., 2000; Thijssen, Cable & Green, 2012). Short-term studies such as Hambrecht et al. (2000) suggest adaptation of

resistance arteries precede that of conduit vessels. Interestingly, and rather paradoxically, evidence suggests athletes do not possess functionally enhanced arteries (DeSouza et al 2000; Green et al 1994; Green et al., 2013; Rowley, Dawson, Hopman et al., 2011). It may be that whilst moderate exercise exposure initially improves arterial function, the response is transient before returning to baseline (Tinken, et al., 2010; Tinken, Thijssen, Black, Cable & Green, 2008) and furthermore is superseded by structural remodelling. This remodelling including increased lumen size and reduced wall thickness is suggested to normalise shear stress; negating the need for functional enhancement. Excessive exercise may induce a different arterial stress response which is not fully understood.

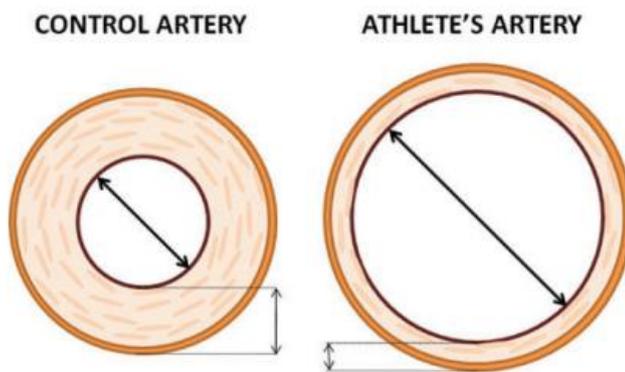


Figure 5. Typical representation of the structural characteristics of the endurance 'athlete's artery' adapted from Green et al. (2012).

Athlete's artery comprises of a larger lumen dimension and reduced wall thickness in comparison to a healthy non-athletic artery. It is proposed that the increase in lumen size and reduction in wall thickness helps to normalise shear stress and supersedes the functional responses to exercise.

### *2.2.1.2 The Possibility for Excessive Exercise Dose: Debate*

Recently, the health consequences of prolonged exercise on the CV system have been questioned. The potential of excessive exercise to elicit unfavourable CV consequences has been proposed in both epidemiological and intervention studies (Burr, et al., 2013; Lee, Pate, Lavie & Blair, 2012; O'Keefe et al., 2012; Paffenbarger et al., 1986; Quinn et al., 1990; Vlachopoulos et al., 2010). The U shaped dose-response regarding exercise-related benefits to health/mortality in these studies represents a disconcerting finding. For this reason, ultra-endurance athletes may represent a group of individuals at an elevated risk of CV events and death. The majority of recent intervention studies suggest a purely transient reduction in cardiac health in response to a single bout of excessive endurance exercise (La Gerche et al., 2008; Shave et al., 2010). However, epidemiological studies suggest an accumulative detrimental response to long-term involvement in excessive endurance exercise (Lee et al., 2012; O'Keefe et al., 2012).

Arterial stiffness is associated with increased CAD and CV events and can be assessed by validated and reproducible techniques and equipment such as the SphygmoCor apparatus (Laurent et al., 2006; Mancia et al., 2009). CAD accounts for a large portion of exercise-induced SCD fatalities, especially in individuals over the age of 35 (Albano, Thompson & Kapur, 2012; Maron & Pelliccia, 2006; Pelliccia et al., 2005). Despite these associations, arterial compliance changes in response to excessive training and exercise bouts has received very limited attention, especially when compared with literature pertaining to the cardiac consequences. Indirect arterial stiffness indices such as pulse wave velocity (PWV) and wave reflections including augmentation index (Aix) are powerful independent predictors of CV events and mortality (Laurent et al., 2006). In the chronic setting it has been indicated that ultra-endurance athletes may present with elevated arterial stiffness, and therefore CV risk, than recreationally active individuals (Burr et al., 2014; Vlachopoulos et al., 2010). Acute responses to prolonged endurance exercise indicate a possibility for differential peripheral and large arterial stiffening immediately after exercise. Stiffness in large arteries may be increased whereas peripheral arteries may have a decreased stiffening response, however the time frame is not clarified and is suspected to be transient, as with cardiac alterations. Short-term changes in arterial stiffness involve sympathetic tone, oxidative stress, cellular alterations and subsequent endothelial function. Longer term changes in arterial stiffness involve structural remodelling regarding arteriogenesis, angiogenesis, and collagen and elastin contributions to vessel walls. Oxidative stress likely has a major role in mediating the exercise-induced adaptations. Antioxidant supplementation therefore may alleviate oxidative stress and subsequent responses by maintaining a normal redox balance. Due to the growing number of participants in ultra-endurance exercise, there is an increased need to assess the health risks associated with such events. This is especially true for individuals preparing for their first ultra-endurance event; who may be unknowingly at risk of pathological CV remodelling.

The following review will discuss the findings of previous literature regarding the effects of differing volumes and intensities of exercise on markers of arterial stiffness. Additionally, mechanisms of action of the transient and long-term influences of exercise on arterial stiffness will be described. Furthermore, gold standard measuring techniques for arterial stiffness and central blood pressures are assessed with reference to reliability, validity and clinical applicability.

### **2.2.2 Habitual Moderate Exercise and Arterial Compliance**

Habitual moderate activity has widespread benefit to the physiological systems, preventing disease, improving health, and improving mortality (Corrado, Migliore, Basso & Thiene 2006; Warburton, Nichol and Bredin, 2006). Repetitive moderate cyclic stress to the physiological system with adequate recovery stimulates adaptation to various systems of the body, including arterial function and structure.

Moderate aerobic exercise has been shown to increase central arterial compliance, the inverse of arterial stiffness, after a brief period of 3 months in healthy middle-aged men irrespective of changes to body weight, blood pressure (BP), cholesterol, or rHR (Tanaka et al., 2000). Conversely, in this study peripheral muscular arterial compliance did not change with exercise training. Therefore it may be that some mechanical factors interact with structural or functional mechanisms to improve arterial compliance. Maeda et al. (2008) provided evidence of the beneficial influence of aerobic exercise training on arterial compliance. It was found that systemic arterial compliance was enhanced both at rest and following acute exercise after 6 months of aerobic training (30 min at 80% of ventilatory threshold, 5 days per week). Arterial compliance was not enhanced after acute exercise alone in this study indicating a training-induced functional sensitivity to exercise. The authors suggest the training-induced adaptation to arterial compliance was in response to the increased cardiac output experienced during exercise. The mechanism underlying the acute exercise-induced increase in arterial compliance in this instance was likely due to enhanced endothelial function. Acute stress responses are followed with accommodating, usually beneficial, adaptations to physiological systems chronic stress responses precede potentially detrimental remodelling and dysfunction.

### **2.2.3 Participation in Ultra-Endurance Events**

Ultra-endurance athletes represent a unique cohort of the athletic population taking part in long-term, highly demanding endurance training sessions. Ultra-endurance can be defined as exercise events lasting longer than 6 hours (Wortley & Islas, 2011; Zaryski & Smith, 2005); those participating in such events usually train between 20-40 hours each week, capable of expending over 70,000 kJ per week (O'Toole, Douglas & Hiller, 1989). Participation rates in ultra-endurance events has increased greatly within recent decades (Hoffman et al., 2010; Knechtle et al., 2011, Knez, Coombes & Jenkins, 2006). For example, since 1978 a greater than 10-fold rise in the number of finishers of 161 km ultra-marathon races has been observed in North American (Hoffman et al., 2010). Additionally, in 2007 over 1700 athletes competed in the annual Hawaii ironman, which is considerably greater than the very first ironman triathlon in 1978 which involved 12 finishers (Lepers, 2008).

### **2.2.4 Acute Effects of Endurance and Ultra-Endurance Exercise on Vasculature**

Ultra-endurance athletes may be at greater risk of CV abnormalities due to chronically increased physiological stress placed on the CV system (O'Keefe et al., 2012). Emerging data suggests that excessive endurance training may compromise vascular structure and function and may induce endothelial stiffening via accelerated atherosclerosis (Knez et al., 2008; Mastaloudis et al., 2001). Low to moderate exercise typically enhances flow mediated dilation (FMD) and endothelial function; however intense prolonged exercise is associated with increased oxidative stress and inflammation, capable of impairing NO-mediated dilation in blood vessels (Fisher-Wellman & Bloomer, 2009; Goto

et al., 2003; Green, Maiorana, O’Driscoll & Taylor, 2004). Nonetheless, the acute impact of prolonged endurance exercise on the vascular system has not been well studied. Oxidative stress and its effects on the vasculature is discussed further in chapter 2.2.7.2.7.

A single bout of prolonged endurance exercise is capable of eliciting at least transient differential changes in measurements of arterial stiffness depending on the regions influencing the measurement. Vlachopoulos et al. (2010b) present findings that a marathon race caused a significant fall in both Aix (12.2% vs. -5.8%,  $P < 0.001$ ) and Aix@HR75 (7% vs 0.0%,  $p < 0.01$ ), whereas PWV did not change significantly ( $6.66\text{m}\cdot\text{s}^{-1}$  vs.  $6.74\text{m}\cdot\text{s}^{-1}$ ,  $p = 0.690$ ). Burr et al. (2012) on the other hand found no significant difference in small artery compliance, however found a significant reduction in large artery compliance measured via applanation tonometry. The differential response in Aix and Aix@HR75 with PWV post-exercise may be indicative of enhanced peripheral artery function, but not aortic or large/medium artery function. This is because of the large reliance of wave reflections on peripheral arteries, discussed further in chapter 2.2.9.2. Whereas, reduced large artery compliance and indifferent small artery compliance observed by Burr et al. (2010) indicates the contrary. Both studies failed to elucidate the time course of the vascular response to ‘excessive’ endurance exercise therefore the long-term consequences of such events remain to be clarified.

This area remains a pertinent field of investigation since recently, only 40 minutes of downhill running was found to induce delayed and prolonged elevations to arterial stiffness and muscular soreness (Burr Boulter & Beck, in press). Measures of cfPWV and subjective muscle pain peaked 2 days post-exercise, and remained elevated 3 days post-exercise which marked the end of the study. The summation of these results imply that endurance exercise may reduce peripheral arterial stiffness, and increase large to medium arterial stiffness. In addition, previous investigations could have underestimated the maximal change in stiffness by performing measurements immediately post-exercise. Possible mechanisms regarding both transient and long-term changes in arterial stiffness in response to excessive exercise are discussed further in chapter 2.2.7

Table 2. Acute effects of exercise on measures of arterial stiffness post-exercise.

Author (Year)	Participants (Exercise)	Measurement timing	Arterial stiffness measure 1	Arterial stiffness measure 2	Arterial stiffness measure 3
Vlachopoulos, et al. (2010b)	n = 20 (foot race, 42.2km)	1 day pre-race vs. 10-15mins post-marathon	<b>No change</b> – PWV; $6.66\text{m}\cdot\text{s}^{-1}$ vs. $6.74\text{m}\cdot\text{s}^{-1}$ , $p = 0.69$	<b>Reduction</b> – Aix; 12.2% vs. -5.8%, $p < 0.001$	<b>Reduction</b> – Aix@HR75; 7.0% vs. 0.0%, $p < 0.01$
Burr et al. (2012)	n = 26 (foot race, 120-195km)	1 day pre-race vs. 20-60mins post-race	<b>Reduction</b> – large artery compliance ( $16.1 \pm 4.4$ ml/mmHg <sup>x10</sup> vs. $13.5 \pm 3.8$ ml/mmHg <sup>x10</sup> , $p = 0.003$ )	<b>No change</b> – small artery compliance ( $8.5 \pm 3.4$ mL/mmHg <sup>x100</sup> vs. $7.7 \pm 8.2$ mL/mmHg <sup>x100</sup> , $p = 0.65$ ).	Increased rHR, reduced SV, reduced SBP and DBP

Burr et al. (in press)	n = 12 (40min run at -12° gradient at 60% $\dot{V}O_{2max}$ )	Pre vs. 1) Post-run 2) 6hrs post 3) 24hrs post 4) 48hrs post 5) 72hrs post	<b>No change –</b> PWV; Pre: $5.1m \cdot s^{-1}$ vs. 1) $5.3m \cdot s^{-1}$ 2) $5.3m \cdot s^{-1}$ 3) $5.4m \cdot s^{-1}$ <b>Increase –</b> PWV; Pre: $5.1m \cdot s^{-1}$ vs. 4) $5.6m \cdot s^{-1}$ 5) $5.5 m \cdot s^{-1}$	<b>No change</b> Aix@HR75 Pre: -2% vs. 1) -0.7 2) -1.1 3) -1.5 4) -0.8 5) -1.8
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Aix = augmentation index, PWV = pulse wave velocity, rHR = resting heart rate, SV = stroke volume, SBP = systolic blood pressure, DBP = diastolic blood pressure.

Short duration exercise induces an initial, acute increase in arterial stiffness determined measurements. Sharman et al. (2005) showed that measures of arterial stiffness were increased at short durations of various cycling exercise intensities. Aix and PWV were significantly and progressively increased due to the initiation of exercise, as was HR and systolic blood pressure (SBP). Although markers of arterial stiffness were increased during exercise, so too were confounding factors HR and SBP (Lantelme et al., 2002). Therefore one should take caution when inferring changes to arterial stiffness irrespective of concomitant measurement error towards other biophysical properties. Furthermore the training status and endurance event experience may also influence the CV response to prolonged exercise. Those with limited previous experience to endurance exercise have been shown to elicit the greatest extent of cardiac damage biomarkers and ventricular dysfunction post-event (Neilan, Jannuzi et al., 2006; Sahlén et al., 2009). It would therefore be important to investigate the arterial health of experienced and less-experienced endurance athletes in response to prolonged exercise.

#### *2.2.4.1 Summary: Acute effects of Ultra-Endurance on Arterial Stiffness*

The area of prolonged endurance exercise bouts and arterial stiffness is limited by a lack of research. Recent evidence indicates that endurance exercise at least transiently influences arterial stiffness however studies have found conflicting results regarding the direction and location of this change. Nonetheless it is possible that prolonged exercise reduces peripheral arterial stiffness and increases large to middle arterial stiffness. The larger arteries are highly compliant, therefore would be expected to have different properties as a feature of their role in the circulatory Windkessel model (Westerhof, Lankhaar & Westerhof, 2009). Further investigations are required to determine the time frame of the arterial response to a single bout of excessive endurance exercise.

### 2.2.5 Long-term Effects of Habitual Prolonged Exercise on Vasculature

As mentioned previously, habitual ultra-endurance athletes may be at a greater risk of CV abnormalities due to the chronically increased physiological stress placed on the CV system (O'Keefe et al., 2012). Recent evidence suggests the large arteries may be at risk of adverse adaptation, which is possibly a result of elevated pro-atherogenic processes evidenced by plaque accumulation in ultra-endurance athletes (Burr et al., 2014; Schwartz et al., 2014). In consequence, long-term prolonged endurance training may place individuals at an increased risk of CV events (O'Keefe et al., 2012; Quinn et al., 1990).

Recent comparisons of habitual endurance athletes and recreationally active controls is presented in table 3. In a study by Vlachopoulos et al. (2010a) investigating marathoners and recreationally active controls, exercise volume ( $\text{min}\cdot\text{day}^{-1}$ ) was an independent determinant of increased arterial stiffness measured via carotid-femoral PWV ( $6.33\text{m}\cdot\text{s}^{-1}$  vs.  $6.89\text{m}\cdot\text{s}^{-1}$ ,  $P < 0.01$ ). No significant difference was observed in Aix between the groups. However, BP (both aortic and brachial) was also higher in the marathon group, which has a direct influence on PWV. Burr et al. (2014) observed a reduction in large artery compliance in ultra-marathoners compared to physically active age-matched controls, however no difference was observed between small artery compliance between the groups. This finding would indicate that the large conduit arteries, but not smaller arteries, are at greater risk of exercise-induced structural remodelling which would compromise vascular health.

In a study involving explicitly normotensive individuals, Radtke et al. (2013) observed no significant differences in Aix or brachial-ankle PWV between recreational, marathon, and ultra-endurance athletes. Hypertensive ( $>140/90$  mmHg,  $n = 5$ ) participants were excluded to avoid bias on arterial stiffness measures, however evidence suggests this be an expected adaptation in ultra-endurance athletes. Nonetheless, ultra-endurance athletes had significantly higher SBP values over 24 hours (8-9 mmHg) confirming that ultra-endurance exercise may predispose individuals to higher BP. Isawaki et al. (2003) demonstrated this adaptation in a study of 11 previously sedentary participants over a progressive 12 month training programme. In this study, diastolic BP, systolic BP, and total peripheral resistance (TPR) are reduced from month 3 to month 9. However no significant difference from baseline was observed at month 12, where training was most intense. Since BP is reported to be a powerful and independent predictor of aortic PWV (Cecelja & Chowienczyk, 2009; Radtke et al., 2013) excluding this common adaptation may result in potentially misguided conclusions. In the studies analysed in table 3, half found increases to large artery stiffness with endurance training, and the other half presenting no difference. Additionally 1 in 4 studies presented attenuated artery stiffness from wave reflections, with the other studies revealing no significant differences compared with recreationally active controls.

Table 3. Chronic effects of endurance exercise on measures of arterial stiffness.

Author (Year)	Participants	Duration of Involvement	Arterial stiffness measure 1	Arterial stiffness measure 2	Other related measurements
<b>Endurance Exercise</b>					
Edwards et al. (2005)	n = 16 competitive endurance vs. 16 recreationally active	Not-stated	<b>Not measured</b>	<b>Difference – Aix</b> -2.1 ± 2.1% vs. 4.5 ± 2.9%, p < 0.05	$\dot{V}O_{2max}$ 65 ±1.9 vs. 49 ±1.8
Vlachopoulos, et al. (2010a)	n = 49 marathoners vs. n = 46 recreationally active	11.6 years ± 9.1 years	<b>Difference – PWV</b> 6.89m·s <sup>-1</sup> vs. 6.33m·s <sup>-1</sup> , p < 0.01	<b>No difference – Aix</b> 13.8% vs. 13.9%, p = 0.34	Marathoners had higher SBP, DBP, pulse pressure and mean pressures.
<b>Ultra-Endurance Exercise</b>					
Knez et al. (2008)	n = 44 ultra-endurance vs. n = 44 recreationally active	6.2 ± 5.5 years of ultra-endurance training	<b>Not measured</b>	<b>No difference – Aix</b> 6 ± 12% vs. 6 ± 13%, p > 0.05	Exercise training volume related to central pulse pressure (r = -0.46, p = 0.002)
Radtke et al. (2013)	n = 16 recreationally active vs. n = 19 marathoners vs. n = 16 ultra-endurance	Lifetime training assessment	<b>No difference – between groups</b> baPWV, p = 0.95	<b>No difference – between groups</b> CAVI, p = 0.60	
Burr et al. (2014)	n = 18 ultra-marathon vs. 18 recreationally active	10 years ± 5 years	<b>Difference - lower large artery compliance in ultra-marathon</b> , p = 0.03	<b>No difference - In small artery compliance</b>	Decreased compliance related to longer running distance per training session r = -0.72, p = 0.03

Aix = aortic augmentation index, PWV = pulse wave velocity, baPWV = brachial to ankle pulse wave velocity, CAVI = cardio-ankle vascular index SBP = systolic blood pressure, DBP = diastolic blood pressure,  $\dot{V}O_{2max}$  = maximal volume of oxygen consumption per minute.

It is well acknowledged that the risk of CV events and mortality is associated with exercise volume (Burr, et al., 2014; O’Keefe et al., 2012; Paffenbarger et al., 1986; Quinn et al., 1990; Schnohr, O’Keefe, Marott, Lange & Jensen, in press; Schwartz et al., 2014). Increasing evidence suggests more may not always be better in terms of exercise load. For example, accelerated coronary plaque development was greater in marathon runners compared with sedentary control participants in a study conducted by Schwartz et al. (2014). Despite a lower BMI and lesser frequency of hypertensive participants, the marathon group had a paradoxically larger total coronary plaque volume, including

calcified plaque and non-calcified plaque (figure 6). Additionally, Möhlenkamp et al. (2008) found an association in healthy runners between the number of completed marathons and coronary artery calcification (CAC). Calcium deposition reduces vessel elasticity resulting in arterial stiffening and increased CV risk. The mechanisms underlying this paradoxical phenomenon, whereby the beneficial effects of exercise are reversed, are not fully understood. Nevertheless, several hypotheses have been identified including; an excessive release of circulating pro-inflammatory cytokines (Neubauer and König et al., 2008), elevated oxidative stress, (Knez, Jenkins & Coombes, 2007), and an increased production of vasoactive substances (Otsuki et al., 2007).

As discussed in chapter 2.1.8, exercise-induced SCD affects relatively few individuals, however its occurrence receives extensive publicity due to the seemingly healthy individuals at risk. Coronary artery disease (CAD) accounts for a large portion of SCD fatalities, especially in individuals over the age of 35 (Albano, Thompson & Kapur, 2012; Virmani, Burkem, Farb & Kark, 1997). Increased coronary plaque development and calcification increases the risk of CV events (Lafonte, 2003) and may represent an important underlying cause for correlations between CV events and exercise volume. Therefore potential elevations in arterial stiffness in habitual ultra-endurance athletes represents an increased risk of a CV event occurrence.

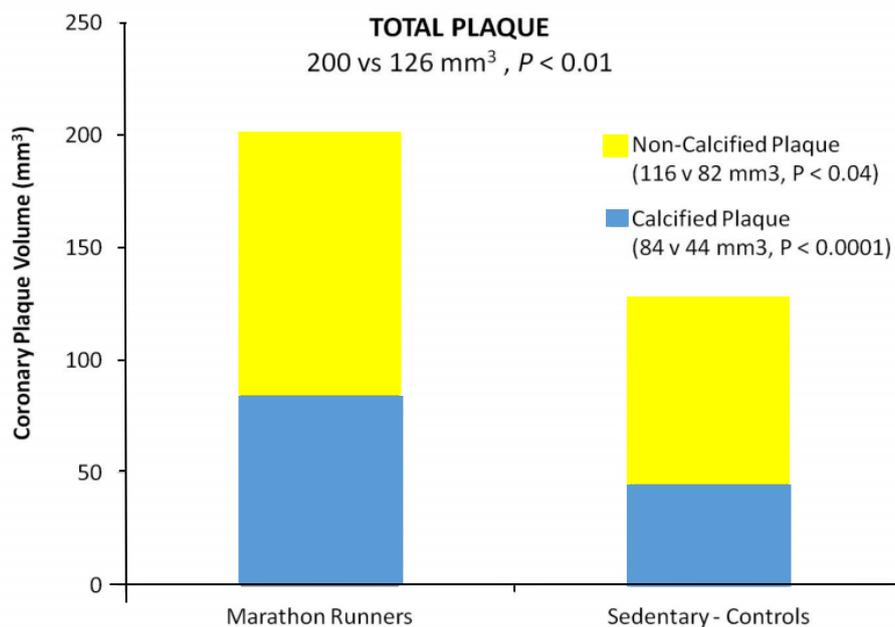


Figure 6. Marathon runners had significantly greater total coronary plaque volume, calcified plaque volume, and non-calcified plaque volume compared with sedentary control participants. Redrawn from Schwartz et al. (2014).

Coronary plaque volumes were assessed using high resolution coronary computed tomographic angiography (CCTA) in male marathon runners ( $n = 50$ ) and sedentary male control participants ( $n = 23$ ). Greater levels of non-calcified plaque accumulation leads to a greater risk of rupture leading to thrombotic occlusion.

### *2.2.5.1 Summary: Chronic Effects of Prolonged Exercise on Arterial Stiffness*

The aforementioned studies suggest habitual prolonged endurance exercise may promote arterial stiffening in large and medium size conduit arteries, whilst sparing or potentially enhancing peripheral arterial stiffness. Furthermore, accelerated coronary plaque development and calcification supports the hypothesis of large arterial stiffening in endurance athletes. Lastly, long-term prolonged endurance training may place individuals at an increased risk of CV events.

### **2.2.7 Proposed Mechanisms of Exercise-Induced Changes in Arterial Stiffness**

The underlying mechanisms responsible for the level of arterial stiffness in response to exercise involve different structural and functional processes. Usually, functional alterations such as changes in endothelial vasodilation occur first as a short-term adaptation, followed by subsequent structural remodelling. That being said the two adaptations are not mutually exclusive, for example structural changes such as vessel wall composition and relative contributions of smooth muscle cells would have an immediate effect on endothelial function as discussed in the following sections.

#### *2.2.7.1 Structural Adaptations to Blood Vessels*

Structural adaptations influencing arterial stiffness involves the physical remodelling of arteries and vessel walls including the enlargement of existing vessels and creation of new vessels. Stiffening of the arterial tree is not uniform throughout, but rather sporadic occurring predominantly in central, conduit vessels and sparing peripheral arteries (Burr et al., 2012; Burr et al., 2014; Vlachopoulos et al., 2010; Ziemann, Melenovsky & Kass, 2005). The compliance of the vascular wall is primarily dependent on the relative content of the two proteins collagen and elastin. An imbalance to the regulation of both proteins induced by an inflammatory environment leads to an increased production of collagen and reduced elastin, contributing to stiffness in the vessel walls (Intengan & Schiffrin, 2001). Within the extracellular matrix (ECM) of the vessel wall collagen and elastin are regulated by catabolic matrix metalloproteases (MMPs) (Ziemann, Melenovsky & Kass, 2005). Advanced glycation end products (AGEs), the result of nonenzymatic protein glycation, form irreversible cross-links between long-lived proteins such as collagen. AGE-linked collagen is stiffer and less disposed to hydrolytic turnover leading to an accumulation of structurally unsatisfactory collagen molecules. Elastin molecules are similarly susceptible to AGE modification affecting the elastic component of vessel walls (Kuzuya et al., 2001). AGEs are up regulated by conditions of increased blood glucose levels and oxidative stress caused by exercise (Goldin, Beckman, Schmidt & Creager, 2006; Henry et al., 2003). Frequent ingestion of high carbohydrate, high sugar foods, combined with excessive exercise characterised by ultra-endurance exercise may promote AGEs and subsequent damage to blood vessel structure.

Enlargement of vessels, termed arteriogenesis, and angiogenesis, the development of new blood vessels, occur in part as a response to increased cyclical blood flow leading to increased wall stress. Initially, wall mass increases to accommodate the higher pressure in the arteries caused by exercise which eventually leads to a larger mechanical stretch on the arterial wall (Prior, Yang & Terjung, 2004). Long-term training is therefore associated with lower arterial wall thickness likely due to normalisation of BP via increased lumen size (Rowley et al., 2011; Thijssen, Cable & Green, 2012). This process reflects a beneficial adaptation to arterial compliance, and concomitant arterial stiffness. However, endofibrosis and iliac artery kinking have been attributed to a small number of athletes, most prominent in cyclists (Feugier & Chevalier, 2004; Peach et al., 2012). Endofibrosis is characterised as the thickening of the iliac tunica intima, the innermost layer (Feugier & Chevalier, 2004). The physical position, muscular hypertrophy, arterial fixation, and endothelial dysfunction may contribute to the deformation of the iliac artery in high volume cyclists. These factors exacerbate endofibrosis and kinking due to: hyperflexion of the hip joint, mechanical vessel trauma, inability to move freely during exercise, and functional mechanisms discussed in chapter 2.2.7.2 (Peach et al., 2012).

#### *2.2.7.2 Exercise-Associated Functional Mechanisms Influencing Arterial Stiffness*

The functional properties of arteries can be influenced transiently or longer term in response to exercise and endurance training. Neural innervation, genotype expression, oxidative stress, inflammatory stress all influence the functional ability of arterial blood vessels which is largely mediated by shearing stress along the vessel walls.

##### *2.2.7.2.1 Neural Innervation*

Sympathetic tone refers to the degree of activation of the sympathetic nervous system (SNS), which together with the parasympathetic nervous system (PNS) and enteric nervous system form the 3 main divisions of the autonomic nervous system. Complex autonomic mediators have a major role during exercise for increasing HR, stroke volume (SV), and the relaxation of pre-capillary sphincters in active muscle capillary beds allowing a greater supply to working skeletal muscle (Sala-Mercado et al., 2006). Metabolic by-products are created by active skeletal and respiratory muscle when blood flow is providing inadequate oxygen and nutrients. The metaboreflex is characterised by the ability of the accumulation of these metabolites to stimulate group III and IV afferent nerve fibres which elicit selective elevations in sympathetic tone (Dempsey, Romer, Rodman, Miller & Smith, 2006; Sala-Mercado et al., 2006). Normal exercise does not elicit the metaboreflex as high levels of metabolites must be produced, as in high intensity or prolonged exercise. The metaboreflex has been shown to increase arterial stiffening and CBP (Davies et al., 2007). Sustained catecholamine exposure and sympathetic tone likely influences arterial stiffness by increasing HR, resulting in overall shortening of the cardiac cycle. Vessel recoil may then be shortened during diastole therefore restricting the degree

of compliance (Heffernan, et al., 2013). Indeed, markers of arterial stiffness including aortic Aix and PWV are influenced by HR (Burr et al., in press; Lantelme et al., 2002).

#### 2.2.7.2.2 Post-Exercise Hypotension in Blood Vessels

Prolonged endurance exercise is followed by exercise-induced hypotension and altered left ventricular function (Raine et al., 2001; Hart et al., 2010). Reductions in BP following exercise are suggested to normalise within minutes to hours of exercise (Rabowchuk, Stuckey, Millar, Gurr & MacDonald, 2009). One mechanism suspected to have a major role in this phenomenon is the arterial baroreflex which may be reset to operate at a lower point to maintain vasodilation after exercise (Raine et al., 2001). Subsequent BP reductions are concomitant with elevated HR to maintain adequate blood flow. Hart et al. (2010) found that after a 4 hour rowing protocol, carotid baroreflex control was reset to function at a lower arterial pressure and an elevated HR, and decreased LV ejection fraction. It was hypothesised that the observed LV dysfunction was at least in part caused by an attenuated LV filling during diastole, in agreement with previous research (George et al., 2005). These studies suggest the cardiac and arterial pressure baroreflex arcs do not respond in a mutually exclusive manner (Hart et al., 2010). The purpose and benefits of post-exercise vasodilation are not fully understood, although suggestions of greater angiogenesis, increased oxygen delivery, and enhanced glucose delivery may explain the primary responses (Halliwill, Buck, Laceywell & Romero, 2013).

#### 2.2.7.2.3 Endothelial Cell Phenotype

Low-to-moderate exercise loads may be associated with improvements in endothelial function, whereas greater exercise loads may attenuate such improvements via cellular and mechanical disruptions (Bergholm et al., 1999; Dawson et al., 2008; Goto et al., 2003). Chronic BP elevation results in epigenetic changes, specifically resulting in pro-atherogenic endothelial cell phenotypes (lower eNOS messenger ribonucleic acid (mRNA) expression, higher vascular cell adhesion protein-1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), endothelin-1 (ET-1) and ROS) (Laughlin, Newcomer & Bender, 2008). Overexpression of said phenotypes are associated with endothelial dysfunction and are suspected to differ when pressure stimulus involves transient cyclical elevation as opposed to chronic increases in BP (Laughlin, Newcomer & Bender, 2008; Widlansky Gokce, Keane & Vita, 2003). It is conceivable that ultra-endurance exercise presents a more chronic elevation in BP than the body is accustomed to, potentially allowing these mechanisms to be at least transiently promoted.

#### 2.2.7.2.4 Inflammatory Processes

As mentioned previously, ultra-endurance athletes may be at an elevated risk of increased AGEs formation and inflammatory cytokines. AGEs stimulate stress signalling and inflammatory processes, increasing NF- $\kappa$ B, ROS, proinflammatory cytokines, growth factors, and vascular adhesion molecules. Ultra-endurance exercise bouts can generate a significant inflammatory cytokine release into the

bloodstream, which can remain elevated for prolonged periods (Mastaloudis, Marrow, Hopkins, Devaraj & Traber, 2004; Marklund et al., 2013). Prolonged inflammatory stress leads to alternate, damaging structural remodelling of biological systems than would occur with acute inflammatory responses (Margonis et al., 2007). Abovementioned mediators can increase vascular stiffness via MMPs, contribute to endothelial dysfunction and oxidative stress, depress endothelial FMD and promote atherosclerotic plaque formation (Goldin et al., 2006; Kuzuya et al., 2001).

#### 2.2.7.2.5 Role of Shear Stress on Vascular Function

Shear stress refers to the frictional drag experienced by endothelial cells as blood flows along its surface. Shear stress stimulates the release of vasodilators which serve to relax and expand arteries. Moens et al. (2005) defines this endothelium dependent process facilitating the relaxation of an artery as FMD. Shear stress relates directly to blood flow, blood viscosity, and arterial radius and is a major stimulant for arterial remodelling (Silver & Vita, 2006). Transient inflammatory responses in vascular smooth muscle cells resulting from shear stress stimulates normal arterial remodelling. However, in the presence of atherosclerosis the remodelling becomes uncontrolled leading to excessive remodelling, and contributing to plaque vulnerability (Silver & Vita, 2006). Rupture of vulnerable plaque results in the contents being released into the bloodstream, causing coagulants to form on the blood vessel. This process leads to thrombotic occlusion of the associated artery resulting in obstructed blood flow and potentially SCD (Lafonte, 2003). Dawson et al. (2008) observed non-significant increases to shear stress of 20% in the femoral artery and decreases of 16% in brachial artery after marathon running. Furthermore a concomitant reduction in femoral, but not brachial, artery FMD was found post-marathon indicative of endothelial dysfunction only in the femoral artery. This localised endothelial dysfunction provides an explanation towards the differential response in arterial stiffening observed post-exercise as demonstrated in chapter 2.2.4.

#### 2.2.7.2.6 Nitric Oxide for Cellular Signalling

Nitric oxide, a potent blood vessel vasodilator, is upregulated by shear stress forces upon the endothelial cells releasing calcium, and in turn activating eNOS; an enzyme responsible for NO production. NO is produced from the amino acid L-arginine by nitric oxide synthase (NOS) with the use of several cofactors such as, oxygen, tetrahydrobiopterin (BH<sub>4</sub>), NADPH and flavin adenine nucleotides (Forstermann & Sessa, 2012). The two types of endothelial NOS (eNOS) are inducible NOS (iNOS) and constitutive NOS (cNOS). Expression of iNOS not calcium dependent, but is stimulated during inflammatory conditions by endotoxins, cytokines and other agents and once expressed is constantly active (Forstermann & Sessa, 2012). Shearing forces acting within the blood vessels induce a calcium release which activates cNOS. The enzyme cNOS converts L-arginine to NO inside the endothelial cell. NO has a very short half-life due to its high affinity for the free radical superoxide

anion ( $O_2^-$ ), forming peroxynitrate ( $ONOO^-$ ). Consequently, NO bioavailability is reduced by the  $O_2^-$ . NO predominantly takes one of two pathways; some NO diffuses into the blood, binding to haemoglobin in red blood cells (RBC's) where it is suggested to assist gaseous exchange by inducing vasodilation (Jensen, 2009). Interestingly RBC's have been shown to produce NO through the reduction of nitrite, which may escape during transport thereby encouraging vasodilation (Cosby et al., 2003). Another, more recognised pathway, is for NO to diffuse from the endothelium into the smooth muscle cells where it has a high affinity towards the enzyme guanylyl cyclase (figure 7). The consequential activation of the messenger cyclic guanosine monophosphate (cGMP) proactively influences vasodilation, inflammation and vascular wall thickness (Moncada & Higgs, 2006).

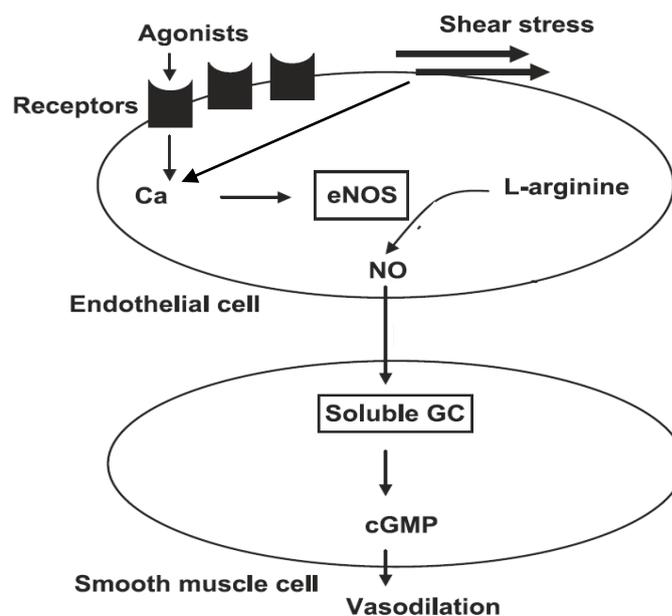


Figure 7. Nitric oxide (NO)- guanylate cyclase (GC)- cyclic guanosine monophosphate (cGMP) pathway resulting from shearing forces on the endothelial cells. Adapted from Higashi & Yoshizumi (2004). Blood flow shearing forces stimulates a calcium (Ca) release within the endothelial cell leading to activation of constitutive NO synthase (cNOS) and subsequent NO production from L-arginine. Inflammatory processes also instigate NO production via activation of inducible NOS (iNOS). iNOS and cNOS are types of endothelial NOS (eNOS). NO diffuses into the smooth muscle cell where it has a high affinity with the enzyme GC. GC converts guanosine triphosphate (GTP) to cGMP which influences several physiological responses such as vasodilation.

Greater NO synthesis and subsequent reactions together with metabolic energy production contributes to a greater production of reactive oxygen and nitrogen species (RONS). These reactive molecules present potential to disrupt cellular function and cause damage to cells particularly in the localised area, for example the endothelium. This damage contributes to stiffening of arteries, stimulating inflammatory processes described previously and promoting foam cell production (Liu et al., 1999).

#### 2.2.7.2.7 Exercise-Induced Oxidative Stress and Antioxidant Supplementation

Endurance athletes consume and use large volumes of oxygen during exercise, as well as experiencing an up regulation of NO production (Powers, Nelson & Hudson, 2011). These pathways encourage a greater production of reactive oxygen and nitrogen species (RONS) such as free radicals which can damage cells and lead to oxidative stress. The term oxidative stress refers to an imbalance of oxidants and antioxidants in favour of oxidant production (Powers & Jackson, 2008). A free radical is an atom that has one or more unpaired electrons, making them unstable and highly reactive. Since electrons usually exist in pairs, the unstable free radical donates or accepts an electron from another molecule, or alternatively joins onto a nonradical. In each process the nonradical molecule becomes a free radical in consequence. Unless the process is terminated, this sequence of events is repeated in a chain reaction of radical production, damaging the molecules and cells they reside in (Halliwell, 1991). Termination of this sequence is achieved with antioxidants. Many antioxidants become radicals themselves, forming part of a cooperative mechanism with other antioxidants that regenerate the original antioxidant (Powers, DeRuisseau, Quindry & Hamilton, 2004). The antioxidant defence system, which consists of endogenous and exogenous antioxidants, therefore is essential to the development of oxidative responses (Powers, Nelson & Hudson, 2011). The major cell bound non-enzymatic antioxidant in humans is glutathione, whilst superoxide dismutases provide a major source of enzymatic antioxidant protection (Powers & Jackson, 2008).

Low-to moderate levels of ROS have a major role in regulating gene expression, cell signalling pathways, glycogen synthesis, and therefore adaptation to exercise training (Powers & Jackson, 2008). Usually, a small release of ROS is met with adequate antioxidant defences (Halliwell, 2006). Excessive oxidant production can cause irreversible pathological damage, mutation, and negatively affect physiological function (Fisher-Wellman & Bloomer, 2009; Murphy, 2009); maintaining a redox balance with adequate antioxidant defence is essential for beneficial adaptation and prevention of oxidative damage. On the other hand, excessive doses of antioxidant supplementation may induce a reduced state, impairing skeletal muscle function and cellular signalling necessary for beneficial adaptations (Powers et al., 2004). Redox balance is an environment in which signalling and control in the body is not disrupted by excess oxidant production or reductive compounds (Powers & Jackson 2008). The

redox balance is demonstrated in figure 8, which is particularly useful in demonstrating the fine line between oxidative stress and reductive stress.

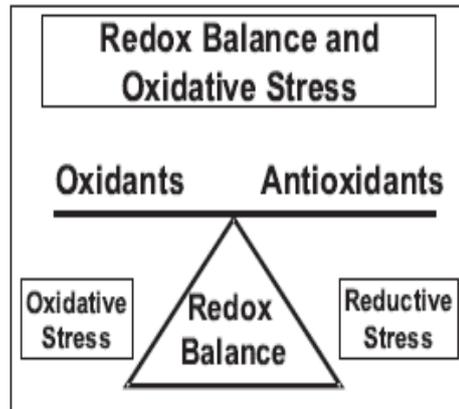


Figure 8. Illustration of the fine line between redox balance, oxidative stress, and reductive stress (Powers et al., 2004).

Greater oxidant production and inadequate antioxidant defences creates an environment of oxidative stress, leading to a chain reaction of oxidised molecules resulting in damage and cellular signalling disruptions. However, excessive antioxidants in the body results in a reductive stress environment, also resulting in signalling disruptions.

Davies et al. (1982) are frequently cited as the first evidence that free radicals are produced as a result of muscle contraction, and that exercise-induced ROS production has potential to damage body tissue. This study inspired extensive research into the area of ROS and tissue damage, including the recognition that free radicals may contribute to muscle function and fatigue (Barclay & Hansel, 1991). In addition, it has been established that low/basal levels of ROS are essential for normal force production and adaptive cellular signalling, yet excess oxidant production compromises force production as represented in figure 9 (Powers & Jackson, 2005; Reid, 2001; Seifried et al., 2007). Mitochondria have long been presumed the primary source of oxidants during exercise (Boveris & Chance, 1973) however, more current research carried out by St-Pierre, Buckingham, Roebuck & Brand (2002) considers the rate of mitochondrial ROS production to be significantly lower than previously accounted for. Powers & Jackson (2008) propose that the collective findings suggest a different predominant source of ROS production, indicating the heart, lungs and white blood cells may play a larger role than formerly understood.

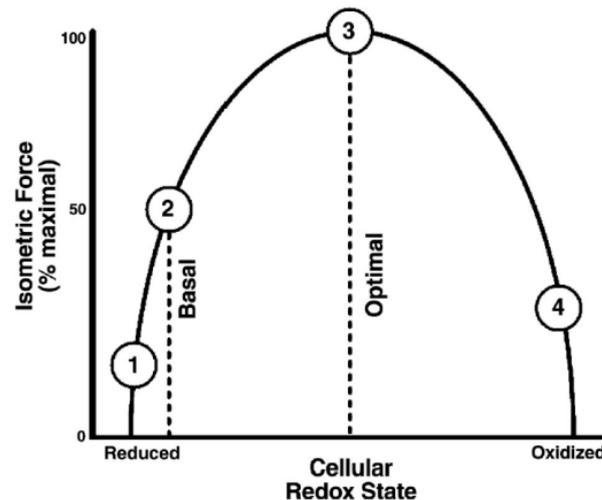


Figure 9. Biphasic effect of reactive oxygen species (ROS) on force production of the skeletal muscle redrawn from Reid, (2001) by Powers & Jackson (2005).

1) Unfatigued muscle exposed to reducing agent. 2) Muscle in basal state. 3) Unfatigued muscle exposed to low level of oxidants. 4) Detrimental effects of excessive oxidants on muscle force.

Several, but not all, studies have identified that excessive exercise increases oxidative stress (Kanter, Mastaloudis, Leonard & Traber, 2001; Margaritis, Tessier, Richard & Marconnet, 1997; Neubauer et al., 2006). Knez Coombes & Jenkins (2006) state that it is plausible that excessive exercise may advance atherogenesis due to oxidative stress and the subsequent LDL oxidation disrupting endothelial function. Additionally, the authors suggest the increased mortality rate and CVD risk seen in those participating in the most exercise, may be due to an elevated oxidative stress resulting from increased activity. This is despite the evidence that a greater antioxidant defence, and therefore enhanced protection against oxidative stress, is observed in those accustomed to endurance training (Bloomer et al., 2006; Jówko et al., 2011). The associated markers of oxidative stress observed in the aforementioned studies have been directly linked with an impairment to the CV system.

Gross et al. (2005) suggests the process of atherosclerosis may involve the oxidation of LDL particles, ROS production, and oxidative damage of cells. Formation of foam cells due to LDL oxidation may promote further oxidative damage and further disruption to the blood vessels. This hypothesis has previously been supported by Liu et al. (1999) finding an increased susceptibility of LDL oxidation in vitro after marathon running. An alternative or contributing mechanism involves the potential for ROS to reduce NO bioavailability. The interaction of ROS superoxide ( $O_2^-$ ) with NO to form peroxynitrite ( $ONOO^-$ ) elicits cellular damage to local areas such as the endothelium. Additionally,  $ONOO^-$  reduces  $BH_4$  to its inactive form, therefore reducing its bioavailability as a co-factor for NO production (Sindler, Delp, Reyes, Wu & Muller-Delp, 2009). In rodent-based studies, exhaustive exercise has impaired

endothelial function and increased arterial stiffness (Zhao et al., 2013). Interestingly the exercise-induced attenuation of eNOS expression was reversed by phytonutrient supplementation (*Lycium barbarum*) which had previously been shown to up-regulate NO level (Zhao et al., 2013). Accordingly, exogenous antioxidant supplementation may provide an effective strategy to attenuate exercise-induced oxidative stress and therein reduce the damaging effects on the CV system. Antioxidant supplementation can provide some protection, however optimal dosages remain to be elucidated. As of yet, there are limited scientific studies which investigate the effect of a multi-nutrient strategy to prevent oxidative stress resulting in excessive exercise and the related vascular compromise.

A large number of ROS can be produced in the body, and different antioxidants can scavenge types of free radicals at different biological sites (Hamid, Aiyelaagbe, Usman, Ameen & Lawal, 2010; Qin et al., 2008). Studies suggest that the use of several antioxidants has multiple potential target sites of action rather than overloading few sites with one substrate (Qin, He, Hai, Liang & Liu, 2008). With this reasoning, multiple antioxidants should be used to prevent situations of oxidative stress (Qin et al., 2008). Further supporting this postulation is the evidence of the adverse health effects of high dose isolated antioxidant supplementation (Gleeson et al., 2004 and Powers, Ji & Leeuwenburgh, 1999). Recent studies have identified an attenuating effect of antioxidant supplementation on exercise-induced cellular signals and adaptations in both vascular and skeletal muscle (Ji, Cabrera and Vina, 2006 and Meilhac, Ramachandran, Chiang, Santanam and Parthasarathy, 2001). Antioxidants such as vitamin C, E, and polyphenols, may exert pro-oxidant characteristics when intake far exceeds that required to obtain redox balance (Scalbert et al., 2005, Ristow et al., 2009, and Nieman et al., 2002). Additionally, Gliemann et al. (2013) has demonstrated that in aged men, resveratrol supplementation negated the beneficial effects of exercise training on CV health, contrary to prior investigation in animal studies (Murase et al., 2009; Dolinsky et al., 2012). With regard to arterial stiffness, it was suggested in a recent review of the literature that polyphenol-based interventions, which have effects further than redox properties and increase the bioavailability of NO, may be the most effective in reducing arterial stiffness (Pase, Grima & Sarris, 2011).

#### 2.2.7.2.8 Summary: Exercise and Vascular Health

The above literature demonstrate that moderate exercise is associated with cyclic, transient alterations in mechanical and cellular stress, resulting in subsequent long-term remodelling, baroreflex adjustments, and increased endothelial function (Hart et al., 2010; Laughlin, Newcomer & Bender, 2008). Long-term changes to arterial stiffness and vascular health have been found to be predominantly a result of structural adaptation. Excessive exercise training may promote an inflammatory environment resulting in reduced elastin and increased collagen in the vascular walls resulting in more rigid vessels, which may be further compromised by AGE modification in

hyperglycaemic and oxidative stress states. Additionally, short-term changes in arterial compliance are predominantly a result of sympathetic tone, cellular alterations and resultant endothelial dysfunction and vasoconstriction. Endurance exercise bouts promote oxidative stress via RONS production due to elevated oxygen consumption and NO production. Oxidative stress has potential to damage the vascular system by means of LDL modification causing endothelial cell damage and subsequent foam cell release leading to atherosclerotic plaque accumulation in the blood vessels. Alternatively, RONS may elicit damage to vascular cells directly as well as causing reduced bioavailability of NO, a potent vasodilator. Antioxidants, in particular phytonutrients and multiple antioxidant combinations, have potential to attenuate oxidative stress and subsequent vascular disruption and therefore be of value to prolonged endurance exercisers. Excessive amounts of one or more antioxidant however may induce a redox imbalance, resulting in pro-oxidant reactions and potentially damaging consequences.

### **2.2.8 Nutritional Factors Influencing Arterial Stiffness**

Nutrition intake prior to, during and post-exercise impacts both performance and recovery. Several nutritional factors affect measurements of arterial stiffness. Specifically caffeine, glucose, alcohol, and salt consumption have been associated with changes in arterial stiffness and wave reflections. (Draaijer et al., 1993; Papaionnou, Karatzi, Papamichael & Lekakis, 2005; Pase Grima & Sarris, 2010; van den Elzen et al., 2005; Zieman, Melenovsky & Kass, 2005).

#### *2.2.8.1 Caffeine*

Caffeine is a widely consumed substance in the world population, especially in athletes for its ergogenic effects (Graham et al., 2010). Current evidence provides support for the ability of caffeine to influence BP, wave reflections, and arterial stiffness. Indeed, Vlachopoulos, Hirata & O'Rourke (2003) showed a significant rise in Aix 30 minutes after caffeine consumption (~7%) which remained elevated 3 hours later; though this effect is not adjusted for concomitant HR elevations. Recently, caffeinated coffee (80mg caffeine) significantly increased PWV over decaffeinated coffee in a double blind crossover study (Karatzi et al., 2005). These data imply that caffeine acutely increases arterial stiffness. The effects of caffeine on arterial stiffness are likely due to the interaction with the central nervous system and HR (Papaioannou et al., 2005). Adenosine increases throughout wakefulness and has been shown to induce sleep and fatigue (Davis et al., 2002). Caffeine is able to cross the blood-brain barrier where it is suggested to influence CNS stimulation by blocking adenosine receptors (Davis et al., 2002). Additionally, the substantial influence of xanthine and theobromine, derivatives of caffeine, on the oxidant and antioxidant actions would affect arterial stiffness dependent on the conditions (Azam, Hadi, Khan & Hadi, 2003; Powers & Jackson, 2008; Powers, Nelson & Hudson, 2011).

### *2.2.8.2 Glucose*

Endurance athletes are known to and currently encouraged to ingest large amounts of carbohydrate and sugar during training periods (Peters 2003). As discussed previously, AGEs negatively influence the structure of arterial walls, stiffening collagen and elastin fibres, as well as compromising endothelial function. AGEs are up regulated by increased blood glucose levels, which may at least partially explain the accelerated stiffening observed with impaired glucose tolerance and diabetes (Goldin, Beckman, Schmidt & Creager, 2006; Henry et al., 2003; Stehouwer, Henry & Ferreira, 2008).

### *2.2.8.3 Alcohol*

Discrepancy exists as to the direction between the amount of alcohol consumption and the degree of arterial stiffness. Population studies have described a J-shaped relationship between alcohol consumption and mortality from CAD and stroke (Corrado, Bagnardi, Zambon & La Vecchia, 2004). This finding is supported by cross-sectional studies finding reduced arterial stiffness with light to moderate consumption but increased stiffness with heavy alcohol consumption (Sierksma et al., 2004; van den Elzen et al., 2005). Speculated beneficial effects refer to increased HDL, enhanced endothelial function, and antioxidant potential from partnered beverages such as wine. Detrimental mechanisms may involve MMP reduction of elastin, pro-oxidant activity, and pro-coagulant activity (Beilin, 2005).

### *2.2.8.4 Salt*

Greater dietary salt intake is associated with an augmented vascular stiffness the reverse is also true (Bagrov & Lakatta, 2004; Gates, Tanaka, Hiatt & Seals, 2004). Increase salt stimulates VSMC tone and vascular wall composition with abundant collagen production. Additionally, salt interacts with genetic polymorphisms which influence endothelial function as well as influencing endothelial function directly by diminishing NO bioavailability (Zieman, Melenovsky & Kass, 2005).

## **2.2.9 SphygmoCor Measurement Techniques for Arterial Stiffness**

### *2.2.9.1 Pulse Wave Velocity*

Arterial stiffness refers to a loss of elasticity within the arterial walls, which occurs naturally with age and arteriosclerosis. When the heart contracts, it generates a pulse wave which travels through the circulation; the speed of this pulse relates to the stiffness of the arteries (Laurent et al., 2006). Increases to pressure pulse wave velocity (PWV) represent increased arterial stiffening of the central arteries, since pulse wave propagation is faster along a stiffer tube (O'Rourke, Staessen, Vlachopoulos, Duprez & Plante, 2002). It is important to recognise that pulse wave transmission is different to blood flow. The pressure pulse generated by the LV reaches peripheral arteries almost immediately, yet the blood ejected by the LV may take several cardiac cycles to reach the same distance.

PWV is frequently considered as the 'gold standard' method of determining arterial stiffness (Laurent et al., 2006). The SphygmoCor apparatus is the most widely used non-invasive and commercially available device for determining arterial stiffness and wave reflection calculations (Boutouyrie, Briet, Collin, Vermeersch & Pannier, 2009)

PWV is related to the intrinsic properties of the arterial wall and its relationship can be calculated by the Moens-Korteweg equation:

$$PWV = \sqrt{\frac{Eh}{2r\rho}}$$

Where E = Young's elastic modulus, h = wall thickness of vessel, r = vessel diameter and  $\rho$  = blood density (Mackenzie, Wilkinson & Cockcroft, 2002; O'Rourke et al., 2002). The technique is relatively simple, non-invasive robust and reproducible. Carotid-femoral PWV, measured along the aortic and aortic-iliac pathway, is the most clinically relevant as it incorporates the arterial branches which are responsible for the majority of the pathophysiological effects of arterial stiffness. Carotid-femoral PWV is widely used to demonstrate the predictive value of aortic stiffness for CV events (Laurent et al., 2001; Shokawa et al., 2005).

### 2.2.9.2 Augmentation index

Pulse wave analysis readings allow an alternative estimation of aortic arterial stiffness and CBP. Pulse wave contours can be created from applanation tonometry measurements taken at the radial artery. The validated transfer function then estimates central pulse wave contours which can be used to estimate CBP and indices of arterial stiffness. The incident pressure pulse gets reflected back which gives the characteristic wave contour. Augmented SBP (AP) due to the naturally occurring wave reflections is divided by pulse pressure to give Aix as a percentage (Laurent et al., 2006).

Although both cfPWV and aortic Aix are markers of aortic arterial stiffness, the two are not mutually exclusive and may differ depending on the internal adaptations occurring (Edwards and Lang, 2005). PWV gives a regional arterial stiffness measure with influence by the aortic-femoral tract, whereas Aix gives an indication of wave reflections in the circulation which can be used to determine arterial stiffness. Peripheral, muscular arteries and arterioles serve as the major reflection sites therefore Aix is influenced by the function and structure of peripheral arteries.

### 2.2.9.3 Validation of SphygmoCor

#### 2.2.9.3.1 Pulse Wave Velocity

Carotid-femoral PWV (cfPWV) excludes the proximal segment of the aorta and includes a variable length of the iliac and femoral arteries. Wilkinson et al, (2010) suggest the intersecting tangent

algorithm should be used to determine transit time from the waveforms, as it has been shown to be the more accurate method subjected to less influence by wave contours. The path length should be calculated by subtracting the distance from the right common carotid artery and sternal notch from the right common femoral artery and sternal notch. Expert consensus has identified that this approach gives a truer estimate of the anatomical path length, therefore more physiological values of PWV (Laurent et al., 2006; Nichols & O'Rourke, 1998; Sugawara, Hayashi, Yokoi & Tanaka, 2008). More recent expert consensus advises using an alternative calculation of 80% of the direct carotid-femoral distance in order to provide a more accurate body surface distance estimate in a clinical population due to large bellies and breasts (Van Bortel, 2012). However the 80% distance method may be redundant in a well-trained endurance population due to the associated reduction in abdominal adipose tissue (Helge et al., 2003; Lee et al., 2005; Schütz et al., 2012). It should be ensured that there is no significant variation in HR or BP between cfPWV recordings (i.e. haemodynamic stability) which can be problematic in a highly trained population whereby sinus arrhythmia is a common occurrence. Furthermore, resting bradycardia observed in most athletes could affect wave reflections and pressure amplifications as well as impacting PWV assessments (Lantelme, Mestre, Lievre, Gressard & Milon, 2002). Therefore, interventions which elicit different HR's between sessions should be interpreted with caution.

A true aortic PWV (aPWV) can be assessed non-invasively via MRI or invasively by sequential or simultaneous recording of pressure/flow waveforms, just above the aortic valve and aortic bifurcation with high fidelity pressure sensors/indwelling catheters (Wilkinson et al., 2010). Recent studies have shown strong correlations between SphygmoCor cfPWV and invasive procedures;  $r = 0.59$ , and ultrasound techniques;  $r = 0.68$  (Ring et al., 2014; Vappou, Luo, Okajimi, Di Tullio & Konofagou, 2011). Correlations appear to become more scattered in the upper range of results greater than 7 or 8  $\text{m}\cdot\text{s}^{-1}$ , particularly in females.

#### 2.2.9.3.2 Central Blood Pressures (Generalised Transfer Function)

The generalised transfer function is applied to derive a central aortic waveform from a peripheral arterial waveform recording. Early studies demonstrate that clinically acceptable predictions of central arterial pressure and waveforms can be made with the use of a mathematical general transfer function from the radial artery pressure wave (Chen et al., 1997; Karamanoglou et al., 1993). The CBP estimation by the SphygmoCor system has been validated in various cohorts (Zuo et al., 2010) but the most comprehensive study approving the generalised transfer function was conducted by Pauca et al. (2001) in 62 patients. Differences in invasively measured, and estimated (from radial) waveforms agreed with the requirements of the Association for the Advancement of Medical Instrumentation (AAMI). The AAMI criteria for comparison of arterial pressures state that mean values are required to

correspond by  $5 \pm 8$  mm Hg or less in a sample of 25 comparisons; for invasive comparisons (White et al., 1993). Differences in the study by Pauca et al. (2001) were as follows: systolic ( $0.0 \pm 4.4$  mm Hg), diastolic ( $0.6 \pm 1.7$  mm Hg) pulse ( $0.7 \pm 4.2$  mm Hg) and mean arterial pressures ( $0.5 \pm 2.0$  mm Hg); suggesting a substantial equivalence between estimated and measured aortic waveforms. CBP derived from the SphygmoCor system is strongly correlated with invasive procedures (Ding et al., 2011; Sharman et al., 2006; Zuo et al., 2010), though recent studies suggest derivations may underestimate true values (Zuo et al., 2010). Since Aix is a relative quantity, underestimation of CBPs should not greatly influence its interpretation, so long as correlations with true CBP's remain high. Indeed studies have noted correlations between Aix ( $r = 0.97$ ;  $p < 0.001$ ) between the SphygmoCor and invasive procedures (Ring, Eriksson, Zierath & Caidahl, 2014).

### 2.2.9.3.3 Factors Influencing Arterial Stiffness Measurements

Various factors have been associated with altered indices of arterial stiffness such as age, HR, LVED and BP. Arterial compliance is diminished with advancing age with particular emphasis on central arteries, largely sparing peripheral arteries (Mitchell et al., 2004). It is of great interest that regular endurance activity can slow the natural ageing process associated with increasing arterial stiffness (DeVan & Seals., 2012; Tanaka et al., 2000).

Several studies have presented an association between HR and PWV independent of BP (Cunha et al., 1997; Lantelme et al., 2002). The underlying mechanisms regarding this association are largely unknown, however the reduced time available for vessel recoil probably contributes to a reduction in vessel compliance (Mangoni, Mircoli, Giannattasio, Ferrari & Mancia, 1996). It is suggested that HR be accounted for when interpreting changes in PWV, and gives rise to the need for larger studies to attempt correction factors as with Aix@HR75.

Corrections in Aix for a HR of 75 are based on 2 studies of small numbers of cardiac diseased participants (Wilkinson et al., 2000; Wilkinson et al., 2002). Aix was inversely associated with increased HR via cardiac pacing, however increasing HR was not associated with changes in arterial stiffness. High variations in data were apparent with linear declines in Aix of  $-0.39\%$  and  $5.6\%$  per  $10 \text{ beats} \cdot \text{min}^{-1}$  HR increase; respectively. Based on the average of the slopes of these studies an adjustment of  $4.8\%$  for each  $10 \text{ beats} \cdot \text{min}^{-1}$  increment was made by the SphygmoCor apparatus for Aix@HR75. The responsible mechanism is suggested to involve a shift in the reflected pressure wave from systole to diastole due to shortening of LVED (Wilkinson et al., 2002). Recently, Salvi et al. (2013) provided evidence to support this mechanism, finding that LVED is a stronger predictor of both PWV and Aix than HR. Therefore a more accurate adjustment may involve substituting HR for LVED. Two important points are brought up by these findings; 1) the adjustment of Aix@HR75 is based on few participants

with already compromised hearts therefore may not reflect the whole population, 2) LVED may represent a more accurate factor to base adjustments of Aix on.

Aix is directly influenced by changes in BP, in particular SBP and pulse pressure. In stiffer arteries, the pulse wave travels at a faster rate, therefore the reflected pulse wave occurs at an earlier point in the wave contour (O'Rourke, Staessen, Vlachopoulos, Duprez & Plante. 2002). As a result, the reflected wave causes an augmentation during systole rather than during diastole, leading to greater systolic BP and pulse pressures (Laurent et al., 2006).

As a result of these factors, changes in arterial stiffness measurements with concomitant changes in HR, LVED, or BP should be interpreted with caution and only large variations should be considered significant. Further studies are required to adjust measurement values for correction factors that relate more accurately with invasive procedures. Indices without correction factors have value regarding the mechanisms, structural or functional, involved with changes in measurements. Ultimately, this change results in an altered Aix, which is a ratio of augmented pressure and pulse pressure.

#### *2.2.9.4 Reliability of the SphygmoCor Device*

Reliability refers to the repeatability or consistency of measurements (Drost, 2011). It is essential to identify how reliable a measurement technique is in order to decide whether a particular measurement is of any value (Bruton, Conway & Holgate, 2000). There are two types of reliability, identified by Baumgartner (1989), relative and absolute reliability. Relative reliability refers to the degree to which individuals maintain their ranking over repeated measures, often assessed using Pearson's correlation coefficient. Correlation only identifies how two sets of data vary together, and can often be misleading if used as a sole indicator of reliability (Keating and Matyas, 1998). In clinical environments it is often necessary to determine whether values obtained are the same, not just proportional to each other. Absolute reliability is the degree to which repeated measurements vary for individuals, expressed as units of measurement.

Carotid-femoral PWV is the most widely used index of arterial stiffness and its predictive value has been demonstrated above that of classical risk factors in several studies based on different cohorts and different geographical locations (Laurent et al., 2006). PWV, independent of the measuring device, has been found inherently less reproducible than the measurement of Aix (McCrea, Skulas-Ray, Chow & West, 2012; Wilkinson et al., 1998). Wilkinson et al., (1998) found promising results regarding the use of SphygmoCor apparatus and pulse wave analysis (PWA) as a reproducible method for determining Aix in various patient groups. In this study two investigators measured Aix twice each in 33 participants and found a high intra and inter-investigator reproducibility determined by Bland-

Altman plots with small measurement differences of 0.49% and 0.23%; respectively. Additionally, the same authors used a similar protocol for cfPWV measurements in 24 participants. The intra and inter-observer measurement differences of PWV were  $0.07\text{m}\cdot\text{s}^{-1}$  and  $-0.30\text{m}\cdot\text{s}^{-1}$  with Bland-Altman plots indicating high reproducibility. For both Aix and PWV there was no significant difference between measurements made by different investigators or between first and second recorded measurements,  $p > 0.05$ . Furthermore neither indices of arterial stiffness showed trends to vary with the mean value.

#### *2.2.9.5 Clinical Applicability of PWV and PWA Measurements*

CV events can be predicted with great accuracy using PWV and PWA procedures. Blacher, Asmar, Djane, London & Safar (1999) were first to identify PWV as an independent risk factor for CV mortality and was strongly associated with atherosclerotic accumulation, albeit in a hypertensive population. In this study, a PWV greater than  $13\text{ m}\cdot\text{s}^{-1}$  was a strong predictor of CV mortality with high performance values;  $\geq 60\%$  sensitivity and specificity. Laurent et al. (2001) further support the clinical predictability of carotid-femoral PWV in a large study hypertensive populations over an average follow-up procedure of 112 months. An odds ratio (OR) of 1.51 was produced for an increase in  $5\text{ m}\cdot\text{s}^{-1}$  PWV for predicting CV mortality. Regression analysis revealed that in terms of mortality risk, an increase in PWV of  $5\text{ m}\cdot\text{s}^{-1}$  was equivalent to that of aging 10 years. More recently, increasing PWV has repeatedly been associated with CVD and mortality in various geographic, age and health-related populations (Cruickshank et al., 2002; Mattace-Raso et al., 2006; Shokawa et al., 2005). The European Society of Hypertension support findings from indicating a 50% increase in the risk of a CV event from a PWV of  $12\text{ m}\cdot\text{s}^{-1}$  or greater (Mancia et al., 2009).

Both aortic Aix and Aix@HR75 have been associated with a higher incidence of CAD in middle-aged and older individuals (Weber et al., 2004). Chorinos et al. (2005) and London et al. (2001) also support the clinical predictability of Aix in diseased individuals for all-cause mortality. Further work is necessary to establish the predictive value of Aix for younger individuals. Evidence does not appear to be unanimous regarding the clinical use of Aix over arterial pressures. In other clinical-based studies, Aix fell out of the regression analysis as an independent predictor of clinical outcomes (Pini et al., 2008; Williams et al., 2006). This may be due to the radial derivation of values in the radial-aorta transformation. The inherent inclusion of the degree of peripheral arterial stiffness may obscure the aortic transformation.

Indirect measurements of arterial stiffness provide clinical value irrespective of age; of which, PWV provides the greatest independent predictive value of CV events with values greater than  $12\text{ m}\cdot\text{s}^{-1}$  indicative of a high risk. Aix and Aix@HR75 may provide good predictive significance for future CV events, although further evidence is required since their robustness has been questioned in several

studies. Clinical applicability of the previously discussed arterial stiffness biomarkers in the athletic population requires further long-term follow-up investigation.

#### **2.2.10 Summary: Ultra-Endurance, Vascular Health and Protective Theories**

Arterial stiffness reflects the elasticity and function of arterial blood vessels in the body and has been adopted as a surrogate marker for CV risk. Moderate activity promotes beneficial vascular adaptations whereas habitual prolonged endurance exercise may be associated with detrimental adaptations to large and medium sized arteries. Likewise, prolonged endurance exercise may acutely and differentially affect arterial stiffness. Importantly, large to medium sized arterial stiffness have been shown to increase, whereas smaller peripheral arteries show reduced stiffness in the immediate period post-exercise.

Oxidative stress is a major mechanism responsible for exercise-induced alterations in the physiological system. Reducing oxidative stress may ameliorate structural and functional adaptations responsible for the transient and long-term impairment to CV health. Therefore antioxidant supplementation, particularly of different forms, may provide an appropriate protective strategy for individuals performing prolonged endurance exercise. However, excessive antioxidant supplementation may negatively influence the beneficial adaptations associated with exercise training. A delicate balance must be retained between oxidative stress and antioxidant defence.

The SphygmoCor apparatus provides a valid and reproducible method of detecting arterial stiffness non-invasively by means of regional PWV and wave reflections. Of which, PWV is the best and most reliable measure. As CV events are strongly predicted by such measurements they provide an appropriate means of assessing the vascular health of various heterogeneous populations. However, several nutritional factors should be controlled for prior to measurements being obtained which have been shown to transiently and chronically influence arterial stiffness.

## **Chapter Three: General Methods**

### 3.1 Research Design

This research project was split into 2 studies, study 1 and study 2. Study 1, “physiological profiling of the adaptations to endurance training”, adopted a longitudinal research design which studied the normal adaptations occurring along a 6 month incremental endurance training period. Study 2, “the influence of athletic status and effects of a long-distance triathlon on markers of arterial stiffness”, adopted a non-randomised longitudinal research design which explored the transient and longer term effect of an ultra-endurance, ‘ironman’ distance triathlon on markers of arterial stiffness.

Participants attended the laboratory prior to the study, whereby pre-screening assessments were made. Individuals then followed a standardised training programme over a period of 9 months. Multiple physiological parameters were measured in 2 month intervals over a 6 month period, i.e. months 0, 2, 4 and 6. A subgroup of individuals were recruited to take part in study 2, which began testing in month 9, whereby measurements of arterial stiffness were obtained one week prior to a long-distance triathlon. This ‘ironman’ distance triathlon consisted of 3.86km swim, 180.25km cycle, and 42.2km run. Measurements of arterial stiffness were repeated the day after the event, 7 days post-event, and 28 days post-event. A control sample was also collected with repeated measures and corresponding time intervals as the triathlon group.

### 3.2 Participant Recruitment

Study 1 participants were recruited via mass advertisement in magazines, social media, email and word of mouth. Prior to any pre-screening assessment, potential participants were sent information regarding the study design, inclusion/exclusion criteria, and what commitments were to be expected.

A cohort of participants from study 1 were recruited via email and word of mouth to take part in an additional study (study 2). These participants were already set to race in the Barcelona long-distance triathlon as part of study 1 criteria. Additionally, a control population were recruited via email and word of mouth. All individuals received information regarding participation expectations, study design, and inclusion/exclusion criteria. See Appendix 1 and 2 regarding study 2 participant information sheets.

### 3.3 Anthropometric Measurements

#### 3.3.1 Height Measurement

Individuals removed shoes and stood with their feet flat and heels together, with their back to the stadiometer (Seca model 214, Hamburg, Germany). The participants were asked to inhale and maintain a neutral position whilst looking directly ahead. The horizontal headboard was lowered to the top of the participants head and height was measured to the nearest 0.1 cm.

### 3.3.2 Body Mass Measurement

Participants were asked to arrive in appropriate minimal clothing with an empty bladder, and to remove excessive clothing and items from pockets prior to measurements. Individuals were then asked to step onto the electrical digital scales (Seca model 780, Hamburg, Germany), face forward, and remain still whilst the measurement was obtained. Body mass was measured to the nearest 0.1 kg.

### 3.4 Peripheral Blood Pressure

A British Hypertension Society (BHS) approved automated oscillometric device (Omron MX3 Plus, Hoofddorp, The Netherlands) was used to indirectly determine peripheral BP (Coleman, Freeman, Steel & Shennan, 2005). The cuff was centred over the brachial artery of the left arm after 5 minutes of rest and with the participant seated comfortably. The arm was supported at the level of the heart and the automated device was initiated. Participants remained still during the measurement and environmental stimulus was kept to a minimum. A duplicate measurement was obtained after a two minute interval and an average of the two measurements was recorded. If a difference of greater than 5 mmHg occurred a third measurement was obtained.

### 3.5 Blood Analysis

Participant's finger tips were sterilised using an alcohol wipe and allowed to air dry. A lancet was used to pierce the skin on the finger and the surface blood was wiped away. Finger capillary blood was collected into 10 µl glass capillary tube (EKF-diagnostic GmbH, Barleben, Germany) which were placed in 0.5 ml micro-centrifuge tubes with haemolysing solution (EKF-diagnostic GmbH, Barleben, Germany). The sample was gently inverted repeatedly to mix and was allowed to rest for 5 minutes before being taken to for analysis using the Biosen lactate analyser (Biosen C-line analyser, EKF-diagnostic GmbH, Barleben, Germany).

### 3.6 Respiratory Analysis

A mask was fitted to ensure an appropriate size was selected. Gas analysis was achieved through on-line methods using a breath-by-breath respiratory system (MetaLyzer 3B, Cortex Biophysik GmbH Leipzig, Germany) and presented through MetaSoft software (Cortex Biophysik GmbH, Leipzig, Germany). The volume transducer and gas sample line were connected to the mask which allowed the respiratory system to calculate respiratory parameters. The mask was tightened prior to the test and it was ensured that no expired gas was escaping. To do this the investigator placed a flat hand over the volume transducer and the participant was asked to breathe hard through the transducer. Last minute standardisation of the MetaLyzer device was achieved prior to each test by conducting a

clean ambient air measurement with the gas sample line stabilised away from interference such as breathing and air conditioning.

Respiratory equipment was calibrated, allowing for 45 minutes of warming up, on each morning of tests according to the manufacturer's guidelines. Firstly, the barometric pressure sensor was measured against a reference barometer and offset value was transferred to the device. Next, a 3 litre syringe (series 5570 3L syringe, Hans Rudolph, Kansas, U.S.A.) was used to calibrate the volume transducer sensor. Lastly, a two-point gas calibration was executed. Clean ambient air with reference concentrations of 20.93% O<sub>2</sub> and 0.03% CO<sub>2</sub>, was used for gas 1 measurement. The gas sample line was stabilised away from interference such as breathing and air conditioning during this measurement. A known reference concentration of oxygen and carbon dioxide was used as the gas 2 measurement, with expected reference values input to the device prior to measurement. Oxygen and carbon dioxide offsets/factors were assessed and the new values were transferred if they were acceptable.

### **3.7 Heart Rate during Exercise**

Collection of HR was conducted with the use of an electronic HR monitor via telemetry (Polar FT1, Polar Electro, Kempele, Finland). An elastic strap connected to a HR monitor was attached around the chest of the participant and was adjusted if until the accompanying watch detected values. The HR monitor was dampened with water if necessary to enhance conductivity.

### **3.8 Rating of Perceived Exertion**

The 15 point Borg (6-20) rating of perceived exertion (RPE) scale was used to measure the subjective intensity of exercise. The RPE scale was explained to participants prior to its use, stating that perceived exertion is how hard an individual feels their body is working combining physical stress, effort, and fatigue; with reference to all parts of the body. Participants were urged to be as honest as possible.

### **3.9 Ergometer Rig**

Participant's bicycles were supported by a stable rear-axle mount trainer stand (CompuTrainer Pro 8002, RacerMate, Seattle, Washington). The rear tyre was adjusted to the centre of the load generator friction roller which was connected to a laboratory computer and monitor allowing for real-time visual feedback for each performance test. Tyres were allowed to warm prior to standardising the equipment to  $3.73 \pm 0.07 \text{ kg}\cdot\text{m}\cdot\text{s}^{-2}$  and drag factor was set to 100%. A magnetic cadence sensor was attached to the chain stay and a magnet was attached to the crank arm to operate cadence. The rig was set up to automatically make adjustments in load as the protocol changed. For this reason the same cadence would produce different workloads at different points of the protocol.

### **3.10 Study 1 Laboratory Environment**

Testing was performed in the Human Performance Laboratory, College Lane Campus, at the University of Hertfordshire. The laboratory was maintained at controlled conditions throughout experimental procedures (temperature,  $22.4 \pm 0.9^{\circ}\text{C}$ ; barometric pressure – range, 97.3-102.3 kPa; humidity – range, 21-56%).

### **3.11 Data Analysis**

Statistical analysis was performed using the Statistical Package for the Social Sciences Version 21 (SPSS Inc., Illinois, United States of America). Graphs and box plots were drawn using Microsoft Excel 2013. Data were initially analysed to determine normality or non-normality. Where significant interactions were observed, Bonferroni post-hoc tests or Wilcoxon signed rank tests were employed to ascertain specific effects. Alpha levels were set at 0.05, and adjusted for multiple comparisons where necessary. Detailed descriptions of data analysis procedures are given within specific sections.

## **Chapter Four: Study 1 - The influence of ultra-endurance training on previously recreationally active individuals**

## 4.1 Abstract

**INTRODUCTION:** There are limited longitudinal data investigating the performance and health-related influence of beginning ultra-endurance training. Rather, several cross-sectional studies exist finding those performing most exercise may be at an increased risk of CV events. It is important to understand the expected physiological adaptations of ultra-endurance training for future participants. This understanding will allow individuals to track their progress and add to literature regarding the safety of such sports.

**PURPOSE:** To assess the development of several physiological factors associated with exercise training and to gain a greater insight regarding the changes in cardiac electrical conductance from endurance training.

**METHOD:** Seventy-six previously recreationally active participants (63 males and 13 females) underwent a tailored 6 month endurance training programme, in preparation for an iron-distance triathlon. Submaximal and exhaustive cycling tests were performed alongside BF assessments at months 0, 2, 4 and 6. Part 1 of this study assessed the data from these tests in a male cohort ( $n = 55$ ,  $34.9 \pm 7.4$  yr,  $178.3 \pm 5.6$  cm,  $79.4 \pm 10.2$  kg and  $46.6 \pm 6.1$  ml·kg<sup>-1</sup>·min<sup>-1</sup>). Participants were also split according to age 18-32 ( $n = 18$ ), 33-38 ( $n = 17$ ), and 39-50 ( $n = 20$ ). Part 2 of this study assessed 12-lead ECG findings recorded at months 0, 2, 4 and 6, in both male ( $n = 63$ ) and female ( $n = 13$ ) participants.

**RESULTS:** All effects are reported as significant at  $p < 0.05$ . Part 1: After 6 months,  $\dot{V}O_{2max}$  significantly increased by  $5.4$  ml·kg<sup>-1</sup>·min<sup>-1</sup>. Of this change, 65% and 79.6% had occurred after 2 and 4 months, respectively.  $HR_{max}$  minimally decreased by 2 beats·min<sup>-1</sup> 4 months into the programme. Maximal cycling power increased by 32 watts; 46.9% and 75% of this change was observed after 2 and 4 months, respectively. BF percentage decreased by 2.3% and lactate threshold increased by 20 watts. There were no significant interactions between time point and age group for any variables. Part 2: ECG data revealed that rHR decreased bimonthly by 3 beats·min<sup>-1</sup> progressively, up to 9 beats·min<sup>-1</sup> at month 6. Frontal plane vector loops revealed that QRS axis, P wave axis and T wave axis did not change significantly after 6 months of training. QT interval was significantly faster at month 2 than month 4 and 6 by 18 ms and 38 ms, respectively. Additionally, 13% of participants presented abnormal ECG findings on at least 1 occasion. Furthermore, there was a progressive increase in the percentage of individuals presenting training-related ECG changes from 68%, 78%, 85% and 87%, at month 0, 2, 4 and 6, respectively.

**CONCLUSION:** Training caused significant changes to related physiological factors. Most known training-related adaptations occurred in the initial months of training, with smaller changes as training

progressed. Age did not influence the training-related adaptations of variables. Abnormal ECG findings increased with the duration into the training programme, as did training-related ECG findings. Endurance training may induce bioelectrical patterns of abnormal criteria, reflecting a normal change to what was previously thought of as abnormal findings, or pathological manifestations in previously healthy individuals.

## 4.2 Introduction

The ironman distance triathlon is a relatively new event which requires participants to swim 3.86km, cycle 180.25km, and run 42.2km. In recent years, the number of individuals participating in ultra-endurance events has increased vastly (Hoffman, Ong, & Wang, 2010; Knechtle, Knechtle & Lepers, 2011). Therefore, a greater number of recreationally active participants are deciding to train for these prolonged endurance events. Few longitudinal studies have investigated the pattern of CV and performance-related physiological adaptations, which initially occur in recreationally active individuals deciding to train for ultra-endurance events. Furthermore, the volume of exercise training, and the extent of training-induced adaptations, which is necessary to develop individuals to a level whereby they are able to complete such events is not well recognised.

Multiple physiological adaptations occur with endurance training, resulting in an enhanced capacity to perform well. Such adaptations include increased type I muscle fibre recruitment, capillary supply to skeletal muscle, enzymatic activity of the electron transport chain, as well as a shift in substrate utilisation to fat oxidation over glucose, and a reduction in cellular acidosis (Hawley, 2002; Robergs et al., 2004; Venables & Jeukendrup, 2008). Further insight of the physiological adaptations occurring in preparation for a long-distance triathlon will help monitor the progress of recreational athletes in the early period of endurance training. Further insight would enable appropriate estimations of sample size requirements, and the sensitivity of associated parameters for future studies in the area.

Studies have identified an increased relative risk of CV disease (CVD) in those performing most aerobic exercise, highlighting the possible adverse effects of exercise (Burr, et al., 2014; O'Keefe et al., 2012; Paffenbarger et al., 1986; Quinn et al., 1990; Schnohr, Marott, Lange & Jensen, 2013). For this reason, ultra-endurance athletes may represent a group of individuals at an elevated risk of CV events. Hence, there is ethical concern that with increasing take-up of recreationally active individuals, more persons may be unknowingly putting themselves at risk of damaging consequences. The majority of recent intervention studies suggest a purely transient reduction in cardiac health in response to a single bout of excessive endurance exercise (La Gerche et al., 2008; Shave et al., 2010). However, epidemiological

studies suggest an accumulative detrimental response to long-term involvement in excessive endurance exercise (Lee, Pate, Lavie & Blair, 2012; O’Keefe et al., 2012). Those athletes presenting training-unrelated abnormalities, representative of underlying heart disease, are at an increased risk of SCD and of developing life threatening conditions such as arrhythmias and fibrillations (Corrado et al., 2010). Investigating the changes to the electrical activity of the heart over a long-term, high load training programme will provide an insight of the bioelectrical adaptations of the ‘athlete’s heart’.

### 4.3 Aims and Hypotheses

The aim of the current study was to provide a physiological profiling of the changes expected to occur in male participants during the initial 6 months of an endurance training programme. Additionally the influence of age on laboratory-based physiological adaptations was investigated. Furthermore, the study sought to gain an insight of the changes to the electrical activity of the heart over a long-term, high load training programme in male participants and assess the frequency of ECG criteria in male and female participants. It was hypothesised that training-related physiological parameters such as  $\dot{V}O_{2max}$ , lactate threshold, maximal power,  $HR_{max}$ , BF, would change throughout the study period. It was also hypothesised that endurance training would influence ECG changes such as rHR, vector loops and conduction intervals. It was hypothesised that the prevalence of training-related ECG findings would increase and there would be no change in training unrelated ECG findings. The full list of hypotheses can be found in chapter 1.4.

## 4.4 Method

### 4.4.1 Participants

One hundred and ten individuals were recruited to participate in this study. Participants were excluded if they: were aged < 18 / > 51 yr, participated in a long-distance event previously, not currently training 1-4 days per week, non-recreationally active with  $\dot{V}O_{2max}$  < 35 / > 55 ml·kg<sup>-1</sup>·min<sup>-1</sup>; male and < 25 / > 45 ml·kg<sup>-1</sup>·min<sup>-1</sup>; female, or had history of heart abnormalities/hypertension/coronary heart disease or diabetes. Fifty-five males were carried through to part 1 of this study for analysis, after exclusion criteria, withdrawal and missed appointments were accounted for. Age, height, body mass and  $\dot{V}O_{2max}$  in the selected participants at the beginning of training was 34.9 ± 7.4 yr, 178.3 ± 5.6 cm, 79.4 ± 10.2 kg and 46.6 ± 6.1 ml·kg<sup>-1</sup>·min<sup>-1</sup>, respectively. These selected participants were also split according to age 18-32 yr (n = 18), 33-38 yr (n = 17), and 39-50 yr (n = 20) for analysis purposes. In part 2 of this study, 50 males were initially assessed for training-induced changes in certain ECG

parameters, thought to be related to training (5 males from part 1 of the study were excluded due to missing ECG data). Seventy-six participants (male = 63, female = 13) were selected to assess the frequency of ECG criteria, regardless of missed appointments. It was deemed necessary to include all participants in this part of the study, regardless of whether there was missing data at certain time-points; this was for the reason that excluding data may have resulted in important findings going unnoticed. All participants provided written informed consent along with a letter from their respective general practitioners. See appendix 4 for a flow diagram of the study methodology and participant attrition.

#### 4.4.2 Study Design

This non-randomised longitudinal study involved a 9 month training protocol in preparation for a long-distance triathlon involving a 3.86 km swim, 180.25 km cycle, and 42.2 km run; of which, the initial 6 months involved bimonthly assessments. Individuals meeting the inclusion criteria were invited to attend the University of Hertfordshire for pre-screening consisting of anthropometric measurements, a 12-lead electrocardiogram (ECG) and a cycling  $\dot{V}O_{2max}$  test. ECG data was screened by a qualified cardiologist and volunteers were excluded if abnormality was found.

Participants were asked to complete a minimum of 80% of the training sessions of a standardised daily training programme, designed for this study by a supervisor (Dr Justin Roberts). This programme prescribed relative training intensities dependent on HR zones and/or RPE to elicit an equivalent physiological stress among participants (Mann, Lamgerts & Lambert, 2014). Participants completed a daily activity diary consisting of training type, session RPE (sRPE) and session volume (mins). Weekly training load was quantified in arbitrary units (AU) by the sum of each sessions RPE multiplied by the volume in minutes (Foster et al., 2001). Training load was assessed over a 3 week period every 2 months, incorporating a recovery week, a medium intensity week, and a hard week. The average weekly assigned training programme (100%) consisted 1789 AU, 2569 AU, and 2961 AU for month 0 to 2, month 2 to 4, and month 4 to 6, respectively. Participant weekly training load increased from  $1948 \pm 668$  AU (month 0 to 2) to  $2628 \pm 853$  AU (month 2 to 4) and  $2921 \pm 801$  AU (month 4 to 6), as shown in figure 10.

Physiological assessment included 4 testing appointments in a 6 month period of 2 month intervals, i.e. months 0, 2, 4 and 6. Participants were asked to arrive at least 2 hours fasted. Each testing appointment included: anthropometric measurements, a resting 12-lead electrocardiograph (ECG), cycling lactate threshold and  $\dot{V}O_{2max}$  tests sequentially. All participants were instructed to abstain from caffeine containing substances and alcohol ( $\geq 12$  h), and told to refrain from exercise training for 24 hours before the tests. Additionally, participants were asked to complete an approved health screen

prior to testing. This protocol was approved by the University of Hertfordshire Health and Human Sciences Ethics Committee with delegated ethics authority (protocol number: LS6/9/12SRA multi-disciplinary investigation in the effects of sustained aerobic training in healthy, recreational individuals preparing for a long-distance triathlon).

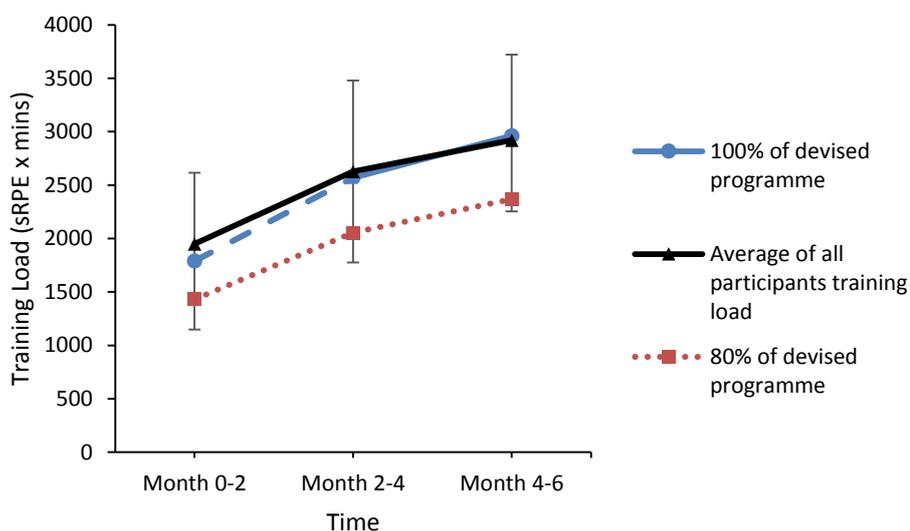


Figure 10. Training load over 6 month period (24 weeks). Training load in arbitrary units (AU) based on intensity (session rating of perceived exertion; sRPE) and volume (minutes) of assigned programme (mean  $\pm$  SD of participants weekly training load).

#### 4.4.3 Experimental Procedure

Participants were welcomed to the laboratory and undertook multiple physiological tests including; anthropometric measurements, resting 12-lead (ECG), cycling lactate threshold and cycling  $\dot{V}O_{2max}$  tests sequentially.

##### 4.4.3.1 Body Fat Composition

Sex, activity level (standard for all participants), height, and age were imputed into the bioelectrical impedance scales (Tanita BC418MA, Tokyo, Japan) to be used as part of the equation for body composition estimations. Estimated clothing weight was set to 0.3 kg for all measurements and participants were asked to step onto the scales and face forward whilst body mass was obtained. Participants then grasped the handles holding them with straight arms at an angle to their side. Electrical conductivity of segmental parts of the body and total body was detected using the 8 polar electrodes on the device. Total body fat (BF) percentage was given to the nearest 0.1% by using an in-built formula which has high reliability and high correlation with reference criteria measurements of body composition (Aandstad et al., 2014; Kelly & Metcalfe, 2012). The Tanita BC418MA model is

medically approved for use in clinical and university studies and hospitals (Medical Devices Directive (MDD) approved, non-automatic weighing instruments (NAWI) Class III).

#### *4.4.3.2 Electrocardiographic Measurements*

Participants were invited into a separated room and rested in the supine position for 5 minutes. Participants were asked to remove any excess chest hair prior to their appointments to ensure adequate contact with the skin. Electrode sites were cleansed with alcohol wipes and allowed to dry before 30 mm electrodes (Cardiacare Ltd. Essex, United Kingdom) were placed in the standard 12-lead ECG formation with cables attached to the electrocardiogram (Custo Med GmbH, Munich, Germany). Custo card m software (Custo Med GmbH, Munich, Germany) was interfaced with a laptop and used to visually display the measurements. Twelve-lead ECG's (10 mm = 1mV, 50 mm·s<sup>-1</sup>) were conducted by trained investigators on participants in the rested, supine condition.

Analysis included the frequency of occurrence of criteria for 20 uncommon, training-unrelated conditions as previously described in table 1. Five training-related conditions were assessed for frequency of occurrence including sinus bradycardia, first degree atrioventricular (AV) block, incomplete RBBB and isolated voltage for LVH as defined by Sokolow and Lyon (1949) criteria (table 1). ECG's of the entire population were analysed by computer-generated values with the exception of manual interpretation where values were not computed or obvious errors were apparent.

#### *4.4.3.3 Lactate Threshold Test*

A capillary blood sample was collected after resting ECG's had been obtained. Participants cycled for 8 minutes at a set intensity of 40% maximal work output (from pre-screening assessment), using the drag factor programme on the CompuTrainer. Participants then began a cycling lactate threshold protocol starting at an appropriate intensity for the individual's ability with drag factor set to 100%. Visual performance data was available to the participant during the test including: cadence, power, speed, and duration. Athletes were instructed to match real time power with expected load and to maintain a consistent cadence throughout, suggested at 90 revolutions per minute (RPM). The protocol consisted of 4 minute stages which included a 30 second 'ramp' of 15/20 watts, which marked the beginning of a new stage. A mask was attached for the full protocol and gas analysis was achieved by using the MetaLyzer equipment (chapter 3.6). Respiratory data was analysed from 2-3 minutes during each stage. RPE and HR were collected immediately prior to capillary blood samples at 3 minutes of each stage (chapter 3.7 and chapter 3.8). The investigator terminated the exercise test when lactate levels rose substantially above pre-test levels or RER > 1.0 and RPE ≥ 15. The point of lactate threshold was termed as the stage before which a direct identification of a 'break-point' was

observed in the blood lactate accumulation curve (Spurway & Jones, 2007). Power produced during this stage was used in the analysis.

#### 4.4.3.4 Maximal Exhaustion Test

The  $\dot{V}O_{2\max}$  protocol consisted of a 10 watt ramp increment every minute starting at one level below the lactate threshold power. Performance data was available to the participant during the test including: cadence, power, speed, and duration. Athletes were instructed to match real time power with expected load and to maintain a consistent cadence throughout, suggested at 90 RPM. Participants were instructed to continue with the test until maximal exhaustion. A mask was attached for the full protocol and gas analysis was achieved by using the MetaLyzer equipment (chapter 3.6). RPE and HR were collected in one minute intervals. Maximal HR and maximal load were observed as the participant reached exhaustion. Participants then underwent a 5 minute warm down at an undetermined power. Investigators were advised that a test was considered applicable for analysis if at least 2 of the following criteria were fulfilled: a levelling off of  $\dot{V}O_2$  (no greater than  $2.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  over a 60 second period), as derived from Taylor, Buskirk, and Henschel (1955); a HR within  $10 \text{ beats}\cdot\text{min}^{-1}$  of predicted maximum; peak RER values were greater than 1.10; in addition it was essential that Borg's RPE values were  $\geq 18$ . These values were preferred in accordance with the majority of previous recent research (Midgley et al., 2007).  $\dot{V}O_{2\max}$  was averaged over a 60 second period in accordance with previous research (Achten, Venebles & Jeukendrup, 2003; Jobson et al., 2008), and was manually adjusted to avoid automated placement errors and reflect an appropriate 60 second period that contains the  $\dot{V}O_{2\text{plateau}}$ .  $\dot{V}O_{2\max}$  relative to body mass was calculated by dividing the maximal volume of oxygen in millilitres by the body mass of the individual, in kilograms.

#### 4.4.4 Data Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences Version 21 (SPSS Inc., Illinois, United States of America). Study 1 of the thesis was split into 2 sections with different statistical tests conducted for each. The first part of this study focused on the CV and performance-related physiological measurements obtained in the male population, with secondary emphasis on age as a factor. Variables analysed in the first section included  $\dot{V}O_{2\max}$  relative to body mass,  $\dot{V}O_{2\max}$  absolute,  $HR_{\max}$ , maximal cycling power output, BF percentage, and cycling power output at lactate threshold. The second part of the results focused on resting 12-lead ECG data obtained over the same 6 month training period. The variables analysed in this section include resting rHR, QRS complex axis, P wave axis, T wave axis, QTc interval and QRS interval. Additionally, descriptive statistics are presented for the frequency of occurrence of criteria for non-training-related ECG abnormalities, as well as training-related ECG findings.

#### 4.4.4.1 Pre-Test Assumptions and Statistical Tests

##### 4.4.4.1.1 Part 1 – Laboratory-Based Variables

In order to ensure that parametric test could be run, pre-analysis checks were undertaken. Kolmogorov-Smirnov tests of normality were combined with visual analysis of box plots and P-P plots to assess the null hypothesis that data for within-subjects analysis was normally distributed ( $n = 55$ ). There was a significant violation to Kolmogorov-Smirnov tests of normality for the power produced at lactate threshold,  $p < 0.05$ . All other variables remained insignificantly non-normal according to Kolmogorov-Smirnov tests. Visual analysis of box plots and P-P plots were again utilised, in this instance to assess the distribution of data between age groups. Additionally Shapiro-Wilk tests of normality were considered due to the lesser number of participants between age groups (18-32,  $n = 18$ ; 33-38,  $n = 17$ ; and 39-50,  $n = 20$ ). Slight violations to the Shapiro-Wilk test are discussed in results section, however it should be noted that the ANOVA procedure is robust to slight violations of normality within a dataset (Field, 2009).

Levene's test of equality of error variances was performed on all dependent variables across time levels. Levene's test was found to be significant ( $p < 0.05$ ), representing a violation to the assumption of homogeneity of variance, and is further mentioned in the analysis. Depending on the extent of the violation, such situations resulted in a more conservative alpha level of 0.025 (Tabachnick & Fidell, 2013).

Mauchly's sphericity test was used to assess the assumption of sphericity within repeated-measures effects. Unless stated otherwise Mauchly's test was insignificant ( $p > 0.05$ ), therefore the assumption of sphericity was accepted. Greenhouse-Geisser correction for sphericity was used if the estimate of sphericity ( $\epsilon$ ) was  $< 0.75$  and Huynh-Feldt correction was used if the statistic was  $> 0.75$  (Girden, 1992). Due to the nature of this study it is unsurprising that Mauchly's test would be violated. The majority of differences in training-related dependent variables are likely to occur within the first periods of training for this sample population.

A two-way mixed measures analysis of variance (ANOVA) was conducted with time as the within-subjects factor (4 levels; month 0, month 2, month 4, and month 6) and with age as the between-subjects factor (3 levels; level 1: 18-32 [ $n = 18$ ], level 2: 33-38 [ $n = 17$ ], and level 3: 39-50 [ $n = 20$ ]). Age groups were designed to allow for sample sizes to be as equal as possible. Relative  $\dot{V}O_{2\max}$ , absolute  $\dot{V}O_{2\max}$ ,  $HR_{\max}$ , maximal cycling power output, and BF percentage were inserted as dependent variables. If a main time effect existed Bonferroni corrections for multiple comparisons were used to perform pairwise comparisons between all levels of time. Simple main effects were investigated by means of Bonferroni comparisons, adjusted for multiple comparisons, on the between-subjects factor

at each level of time. Parametric data were reported as mean  $\pm$  SD. Due to deviations from normality, the Friedman test was conducted with cycling power output at lactate threshold as the dependent variable. Post hoc comparisons were carried out using Wilcoxon signed-rank tests. Non-parametric data were reported as median (interquartile range).

#### 4.4.4.1.2 Part 2 – Resting Electrocardiograph Variables

Kolmogorov-Smirnov tests of normality were combined with visual analysis of box plots and P-P plots to assess whether data was normally distributed ( $n = 50$ ). The majority of data severely violated the Kolmogorov-Smirnov tests, suggesting a non-normal distribution. For this reason the Friedman test was conducted, which does not assume normality in the distribution of data.

Friedman's test has been reported as chi-square ( $\chi^2$ ), with degrees of freedom (df), and significance level;  $\chi^2(df) = \text{statistic}$ ,  $p = \text{significance}$ . This analysis reports whether there is an overall difference in the dependent variable being investigated. If significance was observed, post hoc analysis with Wilcoxon signed-rank test was conducted on the different combinations with a test statistic  $Z$ . The Bonferroni adjustment for multiple comparisons was undertaken to obtain a new significance level of 0.008 (0.05/6). In other words, if the  $p$  value was larger than 0.008 the result was not statistically significant. Median (IQR) values are reported with statistical test statistics.

## 4.5 Results

### 4.5.1 Iron-Distance Performance

Of the 68 participants who started the iron-distance triathlon in month 9, 56 (82%) crossed the finish line. Five participants were unable to start the race and 12 participants did not finish.

### 4.5.2 Part 1 – Laboratory-Based Variables

The results section contains a description of findings for each variable in sequential order. Violations within the variable dataset were outlined together with precautions made to control for the violations. Within-subject comparisons of time were first identified and, if significant, were followed by pairwise comparisons. The interaction effect was then described followed with simple main effects to ascertain the locations of significance. Lastly, the main effect of age group on the dependent variable was described. See appendix 5 for a full descriptive data of variables.

#### 4.5.2.1 Cardiorespiratory Fitness

##### 4.5.2.1.1 Maximal Volume of Oxygen Consumption Relative to Body Mass

Oxygen consumption was averaged over a 60 second period where values were largest. The volume of oxygen consumption, in millilitres, was divided by body mass, in kilograms, of the participant

( $\dot{V}O_{2max}$ ). Within-subjects tests found a significant main effect of time on relative  $\dot{V}O_{2max}$ ,  $F(3, 156) = 30.45$ ,  $p < 0.001$ ,  $\eta^2 = 0.37$ . Pairwise comparisons revealed significant differences between baseline ( $46.6 \pm 6.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) and all other months (month 2,  $50.1 \pm 6.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ; month 4,  $50.9 \pm 5.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ; month 6,  $52.0 \pm 5.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ),  $p < 0.001$ . Significant comparisons were not observed further than the inclusion of month 0. A significant increase in relative  $\dot{V}O_{2max}$  was found between month 0 and 2 as demonstrated in figure 11. Mean values remained larger for the duration of the training programme, but did not increase significantly more compared to other preceding months. As such, in 6 months  $\dot{V}O_{2max}$  increased by  $5.4 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ . Of this change, 65% and 79.6% had occurred after 2 and 4 months, respectively. No significant interaction effects were observed between the level of time into training and the age group of participants on relative  $\dot{V}O_{2max}$ . This indicates that the effect of exercise training on relative  $\dot{V}O_{2max}$  did not affect participants of different ages disproportionately. There was a significant main effect of age after between subject's analyses. Pairwise comparisons revealed that the youngest age group (18-32 yr) had significantly greater relative  $\dot{V}O_{2max}$  values compared to the eldest age group (39-50 yr) with a mean overall difference of  $5.15 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ,  $p < 0.05$ .

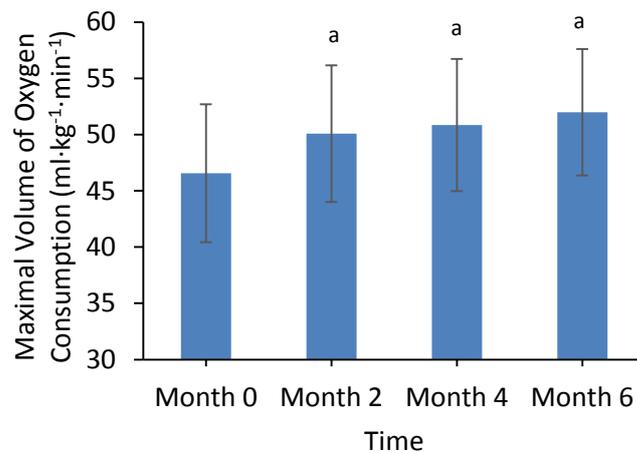


Figure 11. Comparison of maximal volume of oxygen consumption ( $\dot{V}O_{2max}$ ) relative to body weight between levels of time (mean  $\pm$  SD). Data represents all male participants who underwent a progressive training programme over the course of the study.

a. significantly different from month 0,  $p < 0.001$ .

#### 4.5.2.1.2 Absolute Maximal Volume of Oxygen Consumption

A significant main effect of time was observed for the dependent variable absolute  $\dot{V}O_{2max}$ ,  $F(3, 156) = 23.31$ ,  $p < 0.001$ ,  $\eta^2 = 0.31$ . Bonferroni pairwise comparisons revealed significant differences between baseline ( $3.675 \pm 0.454 \text{ L}\cdot\text{min}^{-1}$ ) and all other months (month 2,  $3.946 \pm 0.549 \text{ L}\cdot\text{min}^{-1}$ ; month 4,  $3.999$

$\pm 0.506 \text{ L}\cdot\text{min}^{-1}$ ; month 6,  $4.035 \pm 0.464 \text{ L}\cdot\text{min}^{-1}$ ),  $p < 0.001$ . No significantly different comparisons were observed past the inclusion of month 0. An interaction effect of time and age group was not significant,  $F(6, 156) = 1.00$ ,  $p = 0.44$ ,  $\eta^2 = 0.04$ . These results, shown in table 4, suggest that absolute  $\dot{V}O_{2\text{max}}$  increased significantly from baseline and took the same trend through the training programme irrespective of age. Between-subjects analysis showed a non-significant effect of age group,  $F(2, 52) = 1.19$ ,  $p = 0.31$ ,  $\eta^2 = 0.04$ . This result suggests absolute  $\dot{V}O_{2\text{max}}$  was statistically similar between age groups. The statistical inferences are similar to that observed for relative  $\dot{V}O_{2\text{max}}$ , described above, in that significant differences are only observed when comparing baseline values.

#### 4.5.2.1.3 Cycling Power produced at Lactate Threshold

Power at lactate threshold was not analysed as part of the mixed measures ANOVA or with a repeated measures only ANOVA due to the large deviation of normality. Kolmogorov-Smirnov tests of normality revealed a highly significant violation to the assumption of normality at month 0,  $D(55) = 0.20$ , month 2,  $D(55) = 0.21$ , month 4,  $D(55) = 0.17$ , and month 6,  $D(55) = 0.25$ ;  $p < 0.001$ . Additionally, power at lactate threshold violated the Shapiro-Wilk assumption of normality test for ages 18-32 at month 0;  $W(18) = 0.772$ ,  $p < 0.05$ , month 4;  $W(18) = 0.87$ ,  $p < 0.05$ , and month 6;  $W(18) = 0.80$ ,  $p < 0.05$ . Values for power at lactate threshold for ages 33-38 were significantly non-normal at month 6,  $W(17) = 0.89$ ,  $p < 0.05$ . The null hypotheses that data was non-normally distributed for ages 39-50 was rejected at month 0;  $W(20) = 0.887$ ,  $p < 0.05$ , and month 6,  $W(20) = 0.86$ ,  $p < 0.05$ .

Within-subjects analysis of cycling power produced at lactate threshold was conducted with the Friedman's test. There was a statistically significant difference in power production  $\chi^2(3) = 35.63$ ,  $p < 0.001$ , as shown in figure 12. Median (IQR) power was 160 watts (140 to 170 watts), 160 watts (140 to 180 watts), 180 watts (160 to 180 watts) and 160 watts (160 to 180 watts) at month 0, 2, 4 and 6, respectively. Wilcoxon signed-rank test were performed with Bonferroni multiple comparisons adjustment to obtain a new significance level of 0.008 (0.05/6). Wilcoxon signed-rank tests revealed significant differences between month 0 and month 4 ( $Z = -4.39$ ,  $p < 0.001$ ), and month 6 ( $Z = -4.58$ ,  $p < 0.001$ ), despite no differences in median values at month 0 and month 6. Additionally, significant differences were observed between month 2 and month 4 ( $Z = -3.95$ ,  $p < 0.001$ ).

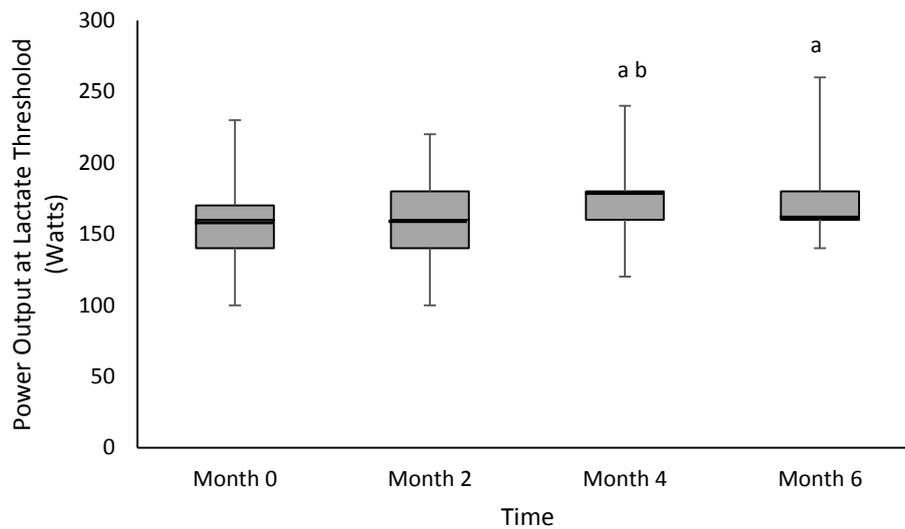


Figure 12. Comparison of cycling power output at lactate threshold during 6 months of a training programme in a male sample.

Upper box, upper 75% of values; lower box, lower 25% of values; middle/thick line, median; highest whisker, maximal value; lower whisker, minimum value.

a. significantly different from month 0,  $p < 0.001$ .

b. significantly different from month 2,  $p < 0.001$ .

#### 4.5.2.1.4 Maximal Power Output during Cycle Test

Maximal power was defined as the load, in watts, at which individuals could not cycle any longer during a continually increasing maximal test. A significant main effect of time on maximal power was found,  $F(3, 156) = 44.04$ ,  $p < 0.001$ ,  $\eta^2 = 0.46$ . Pairwise analysis revealed differences between all possible comparisons. Maximal power at month 0 was lowest compared with month 2, 4 and 6 ( $274 \pm 36$  watts vs.  $289 \pm 38$  watts,  $298 \pm 38$ , and  $306 \pm 37$  watts, respectively;  $p < 0.001$ ) as presented in figure 13. Maximal power at month 2 was significantly lower than month 4,  $p < 0.005$ , and month 6,  $p < 0.001$ . Likewise, power at month 4 was significantly lower than month 6,  $p < 0.05$ . Overall, maximal cycling power increased by 32 watts; 46.9 and 75% of this change was observed after 2 and 4 months, respectively.

No significant time and age group interaction was observed,  $F(6, 156) = 0.83$ ,  $p = 0.55$ ,  $\eta^2 = 0.03$ . Similarly, no significant between-subjects effect was found for age group on maximal power produced,  $F(2, 52) = 0.84$ ,  $p < 0.44$ ,  $\eta^2 = 0.03$ .

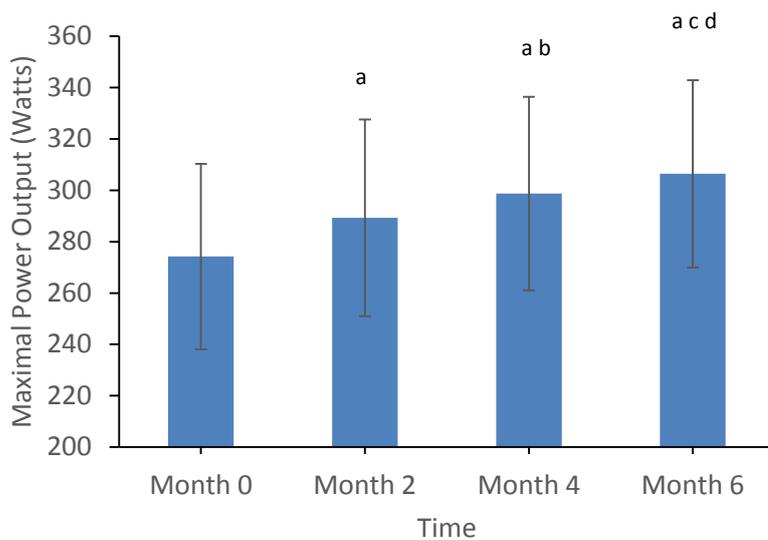


Figure 13. Comparison of maximal power output during a cycle test between different levels of time (mean  $\pm$  SD). Data represents all male participants who underwent a progressive training programme over the course of the study.

- a. Significantly different from month 0,  $p < 0.001$ .
- b. Significantly different from month 2,  $p < 0.005$ .
- c. Significantly different from month 2,  $p < 0.001$ .
- d. Significantly different from month 4,  $p < 0.05$ .

#### 4.5.2.1.5 Maximal Heart Rate

HR was continually observed during a progressively increasing cycle test to obtain a maximal value. Equal variance in  $HR_{max}$  between time-points was not accepted. Mauchly's test of sphericity was significant therefore the assumption of equal variances between time-points was rejected,  $\chi^2(5) = 16.00$ ,  $p < 0.05$ . To correct for this violation Huynh-Feldt adjustment for degrees of freedom was adopted  $\epsilon = 0.90$ . Analysis showed a significant main time effect of  $HR_{max}$ ,  $F(2.71, 141.06) = 4.40$ ,  $p < 0.05$ ,  $\eta^2 = 0.08$ . Bonferroni pairwise comparisons revealed a significant difference of 2  $\text{beats}\cdot\text{min}^{-1}$  between month 0 and month 4 only ( $184 \pm 9 \text{ beats}\cdot\text{min}^{-1}$  vs.  $182 \pm 9 \text{ beats}\cdot\text{min}^{-1}$ ,  $p < 0.05$ ). Comparisons of  $HR_{max}$  at month 0 with month 2 ( $183 \pm 9 \text{ beats}\cdot\text{min}^{-1}$ ) and month 6 ( $182 \pm 9 \text{ beats}\cdot\text{min}^{-1}$ ) remained statistically insignificant,  $p > 0.05$ . Means are presented in table 4. No significant interaction effect of time and age was found,  $F(5.43, 141.06) = 8.18$ ,  $p = 0.78$ ,  $\eta^2 = 0.02$ .

Between-subjects analysis revealed a significant main effect of age,  $F(2, 52) = 5.50$ ,  $p < 0.05$ ,  $\eta^2 = 0.18$ . Pairwise comparisons identified a statistically significant difference between the means of  $HR_{max}$  in the youngest age group (18-32 yr) and the eldest age group (39-50 yr), mean difference  $8 \pm 3 \text{ beats}\cdot\text{min}^{-1}$ ,  $p < 0.05$ .  $HR_{max}$  was greater overall in the younger group compared with the eldest group of participants.

#### 4.5.2.1.6 Body Fat

Average total BF percentage was hypothesised to decrease in the sample as the training programme progressed. Pre-test assumptions were violated for Mauchly's test of sphericity,  $\chi^2(5) = 35.25$ ,  $p < 0.001$ , indicating the differences between time-points were unequal. As a result of this violation, degrees of freedom were corrected using Greenhouse-Geisser method,  $\epsilon = 0.72$ . Within-subjects ANOVA tests found a significant effect of time on BF percentage,  $F(2.12, 112.94) = 20.00$ ,  $p < 0.001$ ,  $\eta^2 = 0.28$ . A significant difference was observed between baseline (month 0:  $18.6 \pm 5.7\%$ ) and month 2 ( $17.7 \pm 5.3\%$ ,  $p < 0.005$ ), month 4 ( $17.1 \pm 5.1\%$ ,  $p < 0.001$ ), and month 6 ( $16.3 \pm 5.4\%$ ,  $p < 0.001$ ) as presented in table 4. Similarly, significant differences were observed between month 2 and month 6 ( $p < 0.005$ ). BF percentage approached significance between month 4 and month 6 ( $p = 0.05$ ). In 6 months of endurance training, BF percentage decreased by 2.3%; 46.9% and 75% of this change was observed after 2 and 4 months, respectively.

There was no significant interaction effect of time and age,  $F(4.34, 112.94) = 0.57$ ,  $p = 0.70$ ,  $\eta^2 = 0.021$ . Likewise the main effect of between-subjects factor, age group, was insignificant,  $F(2, 52) = 2.38$ ,  $p = 0.10$ ,  $\eta^2 = 0.08$ . The results suggest BF percentage decreased significantly over time and that age did not significantly affect the decrease in BF percentage.

Table 4. Maximal oxygen consumption, maximal heart rate and body fat percentage

Measurement	Age Category	Month 0	Month 2	Month 4	Month 6
Absolute $\dot{V}O_{2max}$ (L·min <sup>-1</sup> )	18 to 32 yr	3.666 ± 0.399	3.867 ± 0.487	3.928 ± 0.467	3.981 ± 0.411
	33 to 38 yr	3.855 ± 0.523	4.103 ± 0.575	4.077 ± 0.512	4.173 ± 0.431
	39 to 50 yr	3.529 ± 0.403	3.885 ± 0.579	3.998 ± 0.550	3.968 ± 0.530
	Total	3.676 ± 0.454	<sup>a</sup> 3.946 ± 0.549	<sup>a</sup> 3.999 ± 0.506	<sup>a</sup> 4.035 ± 0.464
Maximal Heart Rate (beats·min <sup>-1</sup> )	18 to 32 yr	189 ± 9	187 ± 10	187 ± 9	187 ± 10
	33 to 38 yr	182 ± 6	182 ± 6	180 ± 7	181 ± 9
	39 to 50 yr	181 ± 9	180 ± 8	179 ± 9	178 ± 8
	Total	184 ± 9	183 ± 9	<sup>a</sup> 182 ± 9	182 ± 9
Body Fat (%)	18 to 32 yr	16.5 ± 4.9	15.9 ± 5.1	15.4 ± 4.5	14.9 ± 5.0
	33 to 38 yr	18.3 ± 5.9	17.3 ± 5.3	16.7 ± 5.4	15.8 ± 5.8
	39 to 50 yr	20.7 ± 5.6	19.6 ± 5.3	18.8 ± 5.1	17.8 ± 5.1
	Total	18.6 ± 5.7	<sup>a</sup> 17.7 ± 5.3	<sup>a</sup> 17.1 ± 5.1	<sup>a</sup> 16.3 ± 5.4

$\dot{V}O_{2max}$  = maximal volume of oxygen consumption; yr = years

Age category, 18 to 32 (n = 18); 33 to 38 (n = 17); 39 to 50 (n = 20). <sup>a</sup> significantly different from month 0; <sup>b</sup> significantly different from month 2.

### 4.5.3 Part 2 - Resting 12-lead Electrocardiographic Variables

The ECG data used in statistical analysis over the 6 month training period included resting rHR, QRS complex axis, P wave axis, T wave axis, QTc interval and QRS interval. This data reflects the male population of participants, from which full data was available at every appointment ( $n = 50$ ).

Additionally, the frequency of occurrence of criteria for non-training-related ECG abnormalities and training-related ECG findings were assessed in male and female participants ( $n = 76$ ;  $M = 63$ ,  $F = 13$ ). A greater number of participants were selected since all ECG appointments were considered to avoid the loss of important findings.

#### 4.5.3.1 Resting Heart Rate

It was predicted that rHR would decrease throughout the training programme. There was a statistically significant difference in rHR depending on the time-point at which measurements were obtained,  $\chi^2(3) = 23.42$ ,  $p < 0.001$ . Post hoc analysis with Wilcoxon signed-rank tests was conducted with Bonferroni correction applied. rHR values at month 0, 2, 4 and 6 were  $59 \text{ beats}\cdot\text{min}^{-1}$  (52 to 65  $\text{beats}\cdot\text{min}^{-1}$ ),  $56 \text{ beats}\cdot\text{min}^{-1}$  (48 to 61  $\text{beats}\cdot\text{min}^{-1}$ ),  $53 \text{ beats}\cdot\text{min}^{-1}$  (47 to 61  $\text{beats}\cdot\text{min}^{-1}$ ) and  $50 \text{ beats}\cdot\text{min}^{-1}$  (47 to 58  $\text{beats}\cdot\text{min}^{-1}$ ), respectively as shown in figure 14. There was a significant difference in rHR between month 0 and month 2 ( $Z = -3.07$ ,  $p < 0.008$ ), month 4 ( $Z = -3.68$ ,  $p < 0.001$ ), and month 6 ( $Z = -4.52$ ,  $p < 0.001$ ). Significance was not observed between other combinations of time-points.

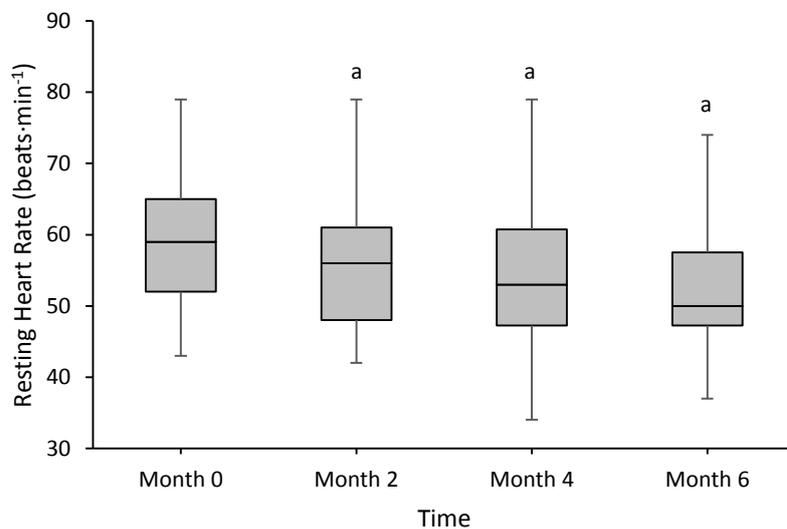


Figure 14. Comparison of resting heart rate (rHR) values obtained by a 12-lead electrocardiogram at several time-points during a training programme.

Upper box, upper 75% of values; lower box, lower 25% of values; middle line, median; highest whisker, maximal value; lower whisker, minimum value.

a. significantly different from month 0 (start of training programme),  $p < 0.001$ .

#### 4.5.3.2 Frontal Plane ECG Vector Loop

The summative direction of the electrical conductance from the QRS complex, P wave, and T wave of the frontal plane were obtained during 12-lead ECG analysis. There was no statistically significant difference in QRS complex axis any time-points of the training programme,  $\chi^2(3) = 4.95$ ,  $p = 0.18$ . Median QRS axis values at month 0, 2, 4 and 6 were  $52^\circ$  (44 to  $62^\circ$ ),  $52^\circ$  (35 to  $65^\circ$ ),  $53^\circ$  (40 to  $61^\circ$ ), and  $55^\circ$  (42 to  $62^\circ$ ), respectively. Similarly, no significant differences were observed between time-points for P wave axis direction ( $\chi^2(3) = 1.75$ ,  $p = 0.63$ ), and T wave axis direction ( $\chi^2(3) = 1.30$ ,  $p = 0.73$ ).

#### 4.5.3.3 Electrical Conduction Intervals

There was a statistically significant difference in QTc interval depending on the time into the training programme,  $\chi^2(3) = 25.13$ ,  $p < 0.001$ . QTc was 409 ms (393 to 424 ms) at month 0, 422 ms (404 to 444 ms) at month 2, 404 ms (388 to 420 ms) at month 4, and 384 ms (402 to 418 ms) at month 6. Wilcoxon signed-rank tests revealed a significant difference between month 2 and month 4 ( $Z = -3.42$ ,  $p < 0.008$ ), and month 6 ( $Z = -3.86$ ,  $p < 0.001$ ). This indicates that QTc was longer at month 2 in comparison to month 4 and month 6, as displayed in figure 15. Likewise, there was no significant difference in QRS interval between time-points as identified by the Friedman's test,  $\chi^2(3) = 4.17$ ,  $p = 0.24$ .

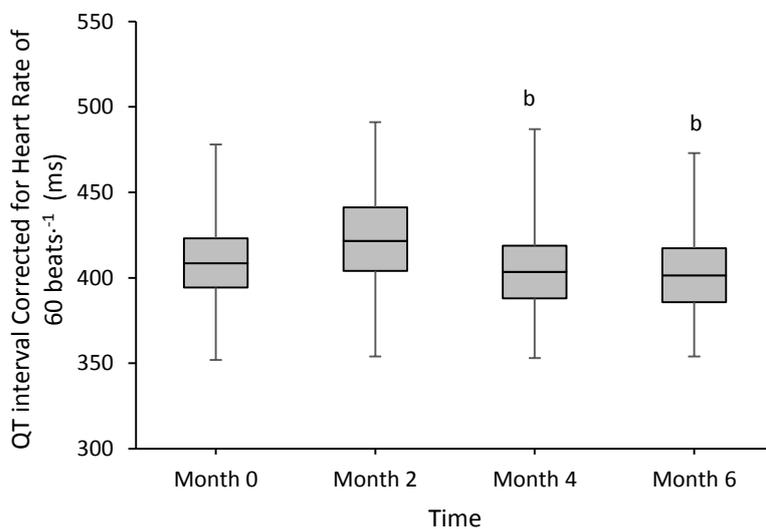


Figure 15. Comparison of QT interval corrected for a heart rate of  $60 \text{ beats}^{-1}$  by Bazzetts formula. Values were obtained using a 12-lead electrocardiogram at several time-points during a training programme.

Upper box, upper 75% of values; lower box, lower 25% of values; middle line, median; highest whisker, maximal value; lower whisker, minimum value.

b. significantly different from month 2,  $p < 0.001$ .

#### 4.5.3.4 Group 1, Training-Unrelated Electrocardiographic Abnormalities

Two-hundred and ninety three 12-lead ECG tracings were obtained from 77 individuals. A total of 34 abnormal ECG findings were identified in 10 participants. This suggests that 13% of individuals displayed abnormal training-unrelated ECG criteria on at least 1 time-point. The frequency and distribution of training-unrelated ECG findings are presented in table 5.

ECG criteria was not fulfilled at any stage for T wave inversion, ST segment depression, complete left or right bundle branch block (BBB), left atrial enlargement (LAE), (RVH), ventricular pre-excitation, short QT interval, profound bradycardia, premature ventricular arrhythmias and atrial tachyarrhythmias. Six participants (a, d, e, q, g, x) were found to have abnormal ECG criteria fulfilled only at one time-point for a condition, indicating a potential abnormality or lead placement error. Potential lead placement errors are noted when necessary.

Pathologically deep Q waves (> 3mm depth) were observed in participant "a" at month 2, which were not preceded at month 0 and did not persist at month 4 or 6. QRS complex axis was similar at month 0, 2, 4, and 6 (28°, 33°, 29° and 25° respectively) therefore the occurrence of a deep Q wave is unlikely to be due to an extreme rotation of the heart.

Participant "d" presented criteria for right atrial enlargement (RAE; high pointed P wave,  $\geq 0.25$  m, in leads II and II or  $V_1$ ) at month 4 which was not present at month 0 or month 2 and did not persist to month 6, although displayed borderline RAE. The criteria for RAE was also fulfilled in participant "c" at month 2 and month 6 and was borderline at both months 0 and 4. Long QT interval was also identified for participant "c" at month 4 and 6 but not at month 0 or 2, though almost fulfilled criteria at month 0 (QTc = 467 ms).

Participant "b" was recognised as having left axis deviation (LAD), intraventricular conduction delay, first degree atrioventricular block, and early repolarisation in several ECG tracings. LAD was a consistent finding throughout the bimonthly ECG analysis (figure 16). Criteria for intraventricular conduction delay was almost satisfied at month 0 (QRS duration = 118 ms) and was fulfilled from month 2 to month 6.

LAD was observed at month 0, 2 and 6 for participant "w". Criteria for LAD was not met at month 4, although a QRS axis of -28° was close to fulfilling the condition. Likewise, LAD criteria was seen in only 1 of the 4 ECG tracing's for participant "q". Criteria was not fulfilled at month 0, was almost met at month 2, and exceeded criteria at month 4 but not at month 6 (28°, -25°, -45°, and 2° respectively).

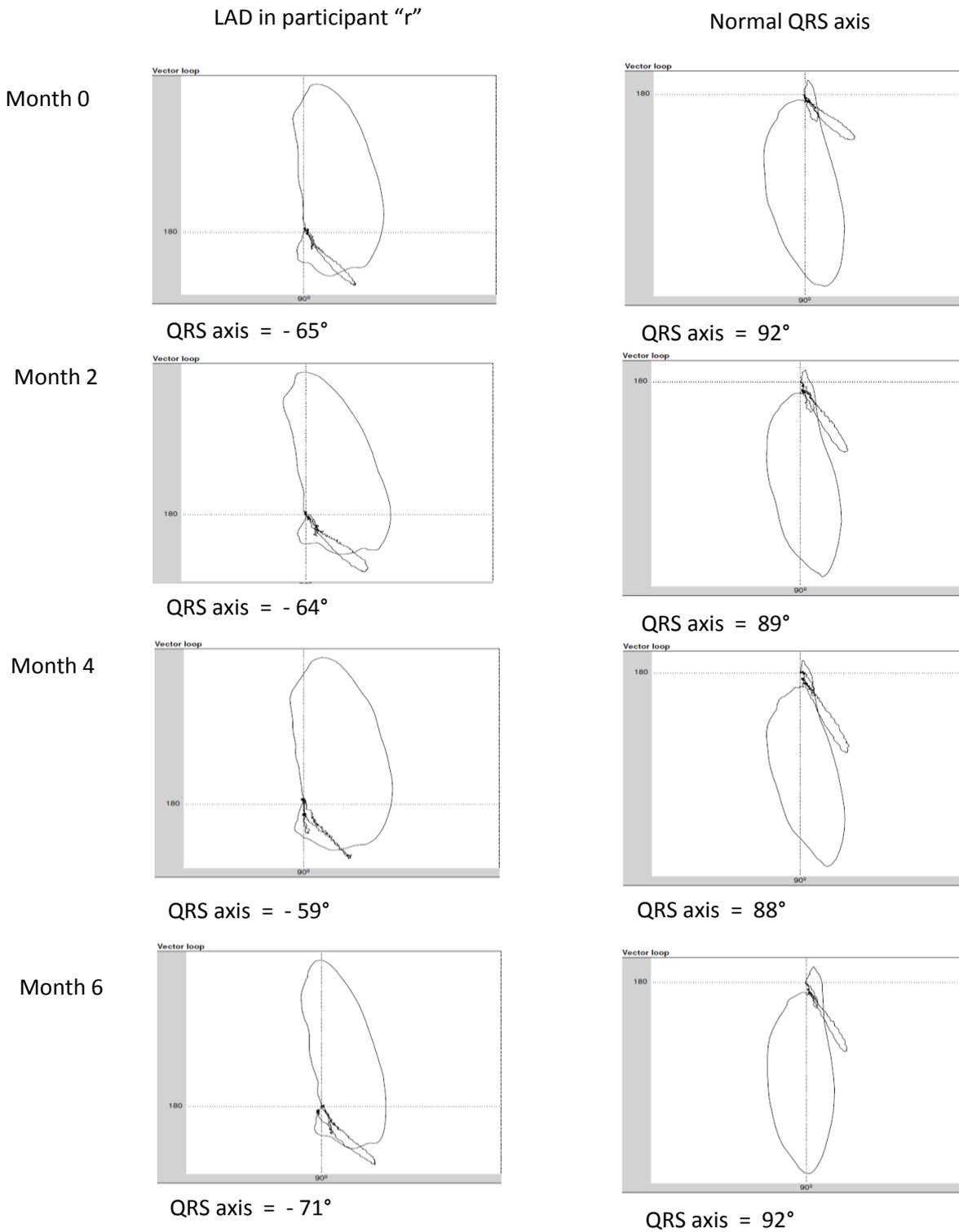


Figure 16. Frontal plane vector loop of left axis QRS deviation (LAD) in participant "r"; left side, and an example of a normal QRS axis of a healthy athletic individual.

QRS axis is considered to have a LAD if the mean electrical axis of ventricular contraction lies between  $-30^{\circ}$  and  $-90^{\circ}$ . A normal QRS axis for endurance trained individuals usually lies between  $-30^{\circ}$  and  $120^{\circ}$ . Past  $120^{\circ}$  a QRS axis is considered to have a right axis deviation (RAD).

*Note:* P and T wave axis are similar between individuals.

RAD was observed in one participant (g) at month 6 which was combined with extreme p wave and t wave axis deviation, none of which were found in previous months.

One participant (“e”) displayed criteria for intraventricular conduction delay at month 6 only, and for right atrial enlargement at month 4 only. QRS duration was borderline for intraventricular conduction delay in the months leading to month 6 for participant “e”.

Long QT interval was observed in participant “x” at month 0 but was not observed in subsequent ECG tracings for the same participant. rHR was 88 beats·min<sup>-1</sup> at month 0 for participant “x”, resulting in an exaggerated QTc. Indeed rHR reduced to 68, 67 and 67 beats·min<sup>-1</sup> (month 2, 4 and 6 respectively). It is supposed that the 1 case of right axis deviation (“g”) may have been erroneous as a result of incorrect lead placement. P, QRS, and T axis were all deviated at month 6 with this subject but were normal in previous ECG’s. An epsilon wave appeared at month 2 in participant “r” and was continually found in subsequent ECG’s for this individual (figure 17).

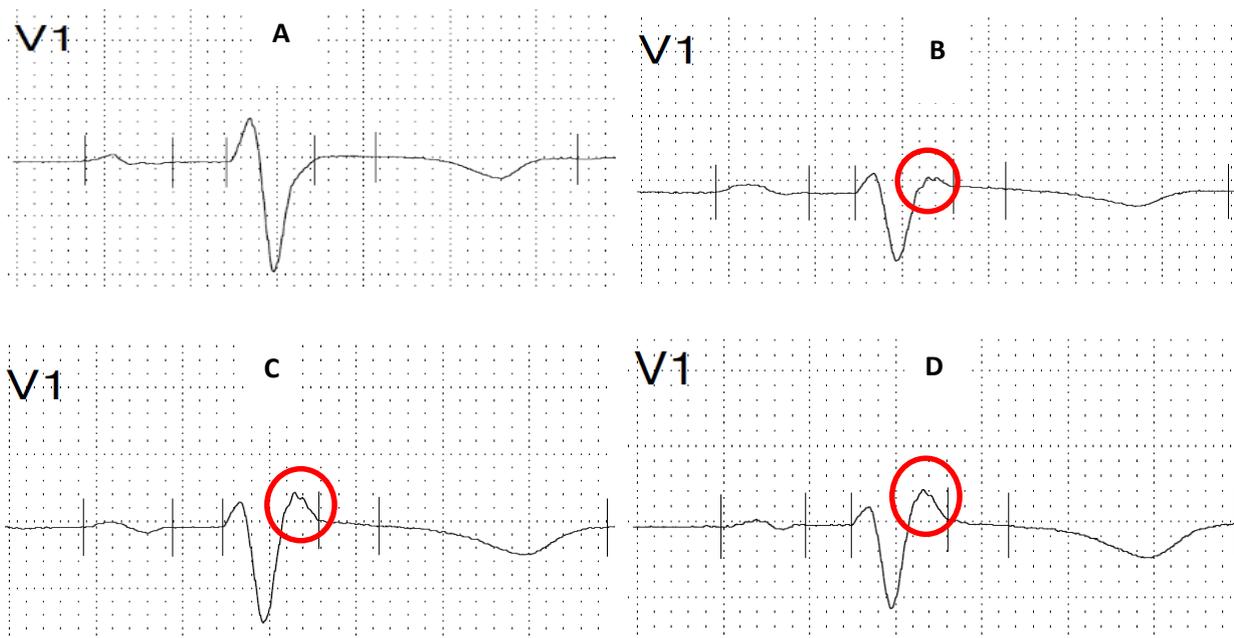


Figure 17. Epsilon wave in participant “r”, depicted as a small negative deflection just beyond the QRS complex in V1 highlighted by a red circle.

A, month 0; B, month 2; C, month 4; D, month 6.

Table 5. Frequency and distribution of group 1, abnormal ECG criteria.

	Month			
	0 (n = 72)	2 (n = 77)	4 (n = 74)	6 (n = 70)
<b>Abnormal ECG finding</b>				
T wave inversion				
ST segment depression				
Q wave abnormality		a		
CLBBB				
CRBBB				
Intraventricular conduction delay		b	b	b, e
LAE				
RAE		c	d, e	c
LAD	b, w	b, w	b, q	b, w
RAD				g
RVH				
Ventricular pre-excitation				
Long QT interval	x		c	c
Short QT interval				
Epsilon wave		r	r	r
Profound bradycardia				
Premature ventricular arrhythmias				
Atrial tachyarrhythmias				
<b>Frequency of abnormalities: TOTAL</b>	<b>3</b>	<b>6</b>	<b>7</b>	<b>8</b>
<b>% of participants showing ≥ 1 ECG abnormality</b>	4.2%	6.5%	8.1%	8.6%
<b>Frequency of abnormalities: Male</b>	1 (n = 59)	5 (n = 64)	7 (n = 61)	7 (n = 57)
<b>% of Males showing ≥ 1 ECG abnormality</b>	3.4%	6.3%	8.2%	8.8%
<b>Frequency of abnormalities: Female</b>	2 (n = 13)	1 (n = 13)	0 (n = 13)	1 (n = 13)
<b>% of Females showing ≥ 1 ECG abnormality</b>	7.7%	7.7%	0%	7.7%

Note: Alphabetised participant identification. Month 0 ECG data was not available for participant e and g. Those with long QT interval were below the cut-off value for unequivocal long QT.

CLBBB, Complete left bundle branch block; CRBBB, complete right bundle branch block; LAE, left atrial enlargement; LAE, right atrial enlargement; LAD, left axis deviation; RAD, right axis deviation; RVH, right ventricular hypertrophy; M, male; F, female.

“a”, “b”, “c”, “d”, “e”, “g”, “q” and “r” were male participants.

“w” and “x”, were female participants.

#### 4.5.3.5 Group 2, Common Training-Related Electrocardiographic Findings

Frequency and distributions of training-related ECG findings are presented in table 6. At the start of the training programme 68.1% of participants displayed a training-related ECG finding. The most common finding was sinus bradycardia, defined by a HR below 60 beats·min<sup>-1</sup>, among all time-points. The percentage of participants with training-related ECG findings increased at month 2, 4 and 6 (77.9%, 85.1%, and 87.1% respectively). The percentage of female participants presenting training-

related findings did not change throughout the 6 month period. The total frequency of training-related findings increased during the 6 month training period from 64 at month 0, to 88 at month 2, and to 98 at month 4 and 6. Seven female participants displayed a rHR below 60 beats·min<sup>-1</sup> (sinus bradycardia) at month 0 which remained throughout to month 6. Only 1 other training-related finding was observed in the female population which was first degree AV block. Criteria for first degree AV block was found at month 0 and month 6 for this participant.

Table 6. Frequency and distribution of group 2, training-related ECG findings.

Frequencies	ECG finding	Month			
		0 (n = 72)	2 (n = 77)	4 (n = 74)	6 (n = 70)
	Sinus bradycardia	38	47	51	52
	First degree AV block	5	8	8	8
	Early Repolarisation	12	17	20	21
	Incomplete RBBB	7	7	7	6
	Isolated voltage criteria for LVH	2	9	12	11
	<b>Frequency of training-related findings: TOTAL</b>	<b>64</b>	<b>88</b>	<b>98</b>	<b>98</b>
	<b>% of participants showing ≥ 1 training-related findings</b>	68.1%	77.9%	85.1%	87.1%
	<b>Frequency of training-related findings: Male</b>	56 (n = 59)	81 (n = 64)	91 (n = 61)	90 (n = 57)
	<b>% of Males showing ≥ 1 training-related findings</b>	71.2%	82.8%	91.8%	94.7%
	<b>Frequency of training-related findings: Female</b>	8 (n = 13)	7 (n = 13)	7 (n = 13)	8 (n = 13)
	<b>% of Females showing ≥ 1 training-related findings</b>	53.8%	53.8%	53.8%	53.8%

Note: AV, atrioventricular; RBBB, right bundle branch block; LVH, left ventricular hypertrophy.

## 4.6 Discussion

### 4.6.1 Part 1

#### 4.6.1.1 Maximal Volume of Oxygen Consumption

The present study confirms the hypothesis that endurance training over a 6 month period would increase  $\dot{V}O_{2max}$ . These results confirm the findings of multiple previous studies (Isawaki et al., 2003; Jones & Carter, 2000), showing that in this investigation, 6 months endurance training increased  $\dot{V}O_{2max}$  by 5.4 ml·kg<sup>-1</sup>·min<sup>-1</sup>. Of this change, 65% and 79.6% had occurred after 2 and 4 months,

respectively. This finding supports the suggestion that large adaptations occur at the beginning of a training programme, followed by smaller adaptations with enhanced endurance ability (Isawaki et al., 2003; Scharhag-Rosenberger, Meyer, Walitzek & Kindermann, 2009). The extent of increases to  $\dot{V}O_{2\max}$  from endurance training depend on various factors including the initial fitness of the study population, the duration, intensity and frequency of training sessions, as well as the duration and intensity of the training programme (Jones & Carter, 2000). Changes in relative  $\dot{V}O_{2\max}$  are understandably heavily influenced by body weight reductions, however the present study also revealed similar results regarding absolute  $\dot{V}O_{2\max}$ . Absolute  $\dot{V}O_{2\max}$  increased by  $0.36 \text{ L}\cdot\text{min}^{-1}$  after 6 months of training, and was also only significantly different from baseline throughout the study.

Differences in  $\dot{V}O_{2\max}$  of  $5.15 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}$  were observed between the youngest and eldest age groups, consistent with other investigations (Kasch et al., 1999; Tanaka et al., 2000). Previous data has observed losses of 5.8 to 6.8% per decade (Kasch et al., 1999). Multiple mechanisms may be responsible for the age associated decline in  $\dot{V}O_{2\max}$  including, a decrease in exercise training stimulus, changes in body weight, and physiological determinants such as  $\text{HR}_{\max}$  and stroke volume (Tanaka & Seals, 2003). Differences in body weight is likely the major factor of the age-related differences in this study since absolute  $\dot{V}O_{2\max}$  was not found to be different between groups.

#### *4.6.1.2 Lactate Threshold*

The hypothesis that lactate threshold would be augmented due to endurance training was accepted. Lactate threshold significantly increased by month 4 and month 6 compared to the start of the training period, although median values were only greater at month 4. The intensity at which lactate threshold occurs has been identified as a powerful predictor of endurance performance (Jones & Carter, 2000). A major influencing factor is due to the coinciding cellular acidosis with lactate production; reducing its accumulation delays active muscular fatigue (Robergs et al., 2004).

Due to the nature of the outcome goal of the training programme designed for the present study participants (success in an iron-distance triathlon), there was a greater emphasis on lower HR zones and greater durations of exercise. Therefore, aerobic energy pathways were specifically targeted, and hence greater increases to lactate threshold were expected. Contrary to this, maximal power output rose progressively throughout the programme discussed in chapter 4.6.1.3. The training programmed was designed relative to individual's HR zones and perceived exertions, rather than absolute to training speeds. Nonetheless, it is possible that the programme did not provide sufficient stimulus for adaptation for those of a greater athletic status at the start, compared to their more sedentary counterparts. This is reflected by the lessening of the interquartile range displayed for lactate threshold power in figure 12.

Changes noted in physiological parameters over the training period in the current study, represent a cohort of individuals in whom the response to training may differ. It has been previously found that the training response regarding one parameter, is not necessarily followed by the same training response of other parameters (Scharhag-Rosenberger et al., 2012; Vol्लाard et al., 2009). Due to the existence of responders and non-responders to endurance training, caution should be applied when transferring the data from the current study to other individuals starting endurance training. Future studies should provide percentages or frequencies of the extents of physiological adaptations in order to categorise responders and non-responders.

#### *4.6.1.3 Maximal Power Output*

Maximal cycling power output progressively increased throughout the training period in the present study. Six months of endurance training increased maximal power output by 32 watts. Unlike other factors, muscle power was the only variable to increase significantly over each bimonthly test. Enhancing an athlete's anaerobic work capacity is suggested to benefit the individual's ability to respond to sections of a race that are above the average work output (Fukuba & Whipp, 1999; Jones & Carter, 2000). With increasing intensities, there is an increased reliance on oxygen-independent energy pathways (Bassett & Howley, 2000). For this reason, exercise training with a focus on improving endurance performance, should aim to increase the rate of energy production from aerobic as well as oxygen-independent pathways, match ATP production with hydrolysis, minimise cellular disturbances, increase efficiency, and improve fatigue resistance (Hawley, 2002). Maximal power represents an outcome variable which is impacted by these components, and therefore should be considered during endurance training. A more specific analysis of the anaerobic processes may have been to investigate the actual power increase past  $\dot{V}O_{2max}$  attainment (Bassett & Howley, 2000).

#### *4.6.1.4 Maximal Heart Rate*

Endurance training reduced  $HR_{max}$  by 2 beats·min<sup>-1</sup> at month 4, however results were insignificant by month 6. This is a lesser response to that seen in previous studies; Isawaki et al. (2003) for example, observed reductions of 9 beats·min<sup>-1</sup> within 3 and 6 months of endurance training compared to baseline. Discrepancies between training-induced responses may be due to methodological differences such as variations in training stimuli. Additionally, the population used in the present study were already recreationally active prior to participation. Many previous studies have observed differences in sedentary, untrained individuals undergoing a training period (Isawaki et al., 2003; Zavorsky, 2000). Whyte et al. (2008) and Boyette et al. (2013) suggest the decrease in  $HR_{max}$  is not due to high vagal tone, but can be explained by sinoatrial node remodelling. The results of the present study agree with previous investigations, finding a reduction in  $HR_{max}$  of 8 beats·min<sup>-1</sup> in those of the eldest age group compared with the youngest individuals. Multiple studies have shown advancing age

to be associated with a decrease in  $HR_{max}$  at just under a rate of  $1 \text{ beat}\cdot\text{min}^{-1}$  per year (Gellish et al., 2007; Hawkins et al., 2001).

#### *4.6.1.5 Body Fat*

Average BF decreased as a result of endurance training by 2.3%, again with the majority of change occurring within the first 2 months of training. This finding supports the hypothesis that BF is reduced with endurance training. The rise in training load undertaken by participants likely caused an increased metabolic demand with associated shifts in substrate utilisation from glucose to fat stores. In turn, calorific output is elevated and greater fatty acid oxidation occurs (Bruce et al., 2006, Venables & Jeukendrup, 2008). Although adipose tissue may be reduced as a result of endurance training, intramuscular triglycerides have been shown to be augmented in endurance trained individuals (Goodpaster, He, Watkins & Kelley, 2001; Kiens, 2006; Tarnopolsky et al., 2007). This finding is considered an endurance specific training response, allowing greater accessibility to highly dense fuel stores in active muscle fibres (Tarnopolsky et al., 2007). Therefore, global BF composition values as used in the current study do not represent specific changes in the body. In this context, BF percentage may be a flawed measurement. Separate measurements of intramuscular triglyceride and adipose triglycerides would give a greater representation of the physiological adaptations occurring in response to training.

Regardless of age-related differences in relative  $\dot{V}O_{2max}$  and  $HR_{max}$ , training-related adaptations were not influenced by age. This implies that endurance training stimulates similar adaptations in older aged individuals and younger individuals. With further analysis of the time response, perhaps differences would be observed reflecting variations in the upper limit of adaptations. For example, it has been speculated that maximal cardiac training responses, and subsequent full expression of the athlete's heart, occur when training is undertaken during growth and development (Zadeh et al., 2014).

## **4.6.2 Part 2**

### *4.6.2.1 Resting Heart Rate*

The current study found that rHR was slower by  $9.0 \text{ beats}\cdot\text{min}^{-1}$  after 6 months of endurance training. Therefore, the hypothesis that endurance training would reduce rHR can be accepted. This finding is a common change found with multiple previous studies. For example, 6 months of endurance training has been followed with reductions of 2.7 to  $9.0 \text{ beats}\cdot\text{min}^{-1}$  in rHR (Scharhag-Rosenberg et al., 2009; Wilmore et al., 1996). The mechanisms underlying this adaptation are multifactorial including the autonomic nervous system, sinoatrial remodelling, cardiac hypertrophy, baroreflex resetting and

individual's genetic predispositions (Corrado et al., 2010; Matelot, Schnell, Kervio, Du Boullay & Carré, 2013).

#### *4.6.2.2 Frontal Plane Vector Loop*

The hypothesis that QRS axis would significantly change in response to endurance training was rejected. Likewise no significant changes were observed with P wave axis and T wave axis. Previous studies have shown that endurance training results in increased LV wall thickness and LV dilation (Ellison et al., 2011; Muhl, Dassen & Kuipers, 2008). Recently, it was shown that the left and right ventricle develop differently in response to endurance training. Zadeh et al. (2014) showed that in the first 6-9 months of endurance training, the LV responds with concentric remodelling, followed thereafter with eccentric remodelling, restoring the mass to volume ratio. Conversely, the RV responds to endurance training with eccentric remodelling throughout. Measurements of average electrical directions are likely influenced by changes in weight, as a consequence of lead placement variations. Therefore potential axis deviations may be masked or obscured by weight reductions. It is also possible that the lack of change in QRS axis may reflect the increased reliance on the right ventricle with prolonged endurance exercise (La Gerche et al., 2008; La Gerche et al., 2012). These findings provide evidence of similar remodelling of the left and right chambers of the heart.

#### *4.6.2.3 Electrical Conduction Intervals*

QTc increased, albeit insignificantly from month 0 to month 2, where it was significantly longer than month 4 and month 6. rHR on the other hand significantly decreased after 2 months of the training, and remained lower 6 months into the training programme. Furthermore, QRS interval remained unchanged throughout the study. It is recognised that HR is directly correlated with QT interval, hence the use of Bazett's formula correcting QT for HR (Corrado et al., 2010). Together, this information indicates that the initial adaptation of a reduced rHR does not confer similar responses to the QTc or QRS interval. The reduction in QTc, observed from month 2 to month 4, may be a result of a prolonged adjustment of the electrical conduction of the heart to a reduced rate of contraction.

#### *4.6.2.4 Group 1 and Group 2 ECG Findings*

The frequency of group 1 training-unrelated ECG findings increased over 2 fold throughout the 6 month period. 8.6% of participants presented at least one training-unrelated finding at month 6, compared to 4.2% at month 0 of the study. Recent investigation has found an increased prevalence of both training-related and training-unrelated ECG findings in elite endurance trained athletes compared to non-endurance athletes (Brosnan et al., 2014). Likewise, both groups of changes were more prevalent in elite endurance athletes than the frequencies observed previously in competitive mixed discipline athletes (Papadakis et al., 2011). In elite endurance athletes, the frequency of at least

one training-unrelated finding was as high as 29.9%, compared with findings of less than 10% in competitive athletes, which is more comparable to the current study. The increased frequency of training-unrelated ECG findings throughout the current study, with progressive training load elevations, supports the suggestion that some changes are not exclusively training-unrelated as previously thought. Brosnan et al. (2014) have suggested that group 1 abnormal changes may actually be non-pathological and related to endurance exercise have been those of right ventricle association, such as RVH and right precordial T wave inversion (Brosnan et al., 2014). It could not be implied from the current study which group 1 findings were related to endurance exercise due to the lack of incidences, likely because of insufficient participants. Although suggestive of pathological adaptations, without further clinical investigation with cardiac imaging or echocardiographic measurements, underlying CV disease cannot be assumed. The occurrence and subsequent disappearance of certain ECG criteria for abnormal findings may represent a normal morphology of the heart at a particular stage of exercise-induced cardiac adaptation. Alternatively pathological findings may indicate an increased risk of a cardiac event during the period whereby the electrical activity of the heart is compromised.

In regards to normal training-related ECG changes, sinus bradycardia was the most prevalent, followed by early repolarisation patterns. Incidences of isolated voltage criteria for LVH increased substantially from 2 participants at month 0 to 11 participants at month 6. The overall percentage of individuals presenting training-related findings increased from 68.1% to 87.1% from month 0 to month 6. Interestingly, the percentage of females presenting training-related changes did not change over the course of the study, therefore the increased prevalence of findings was solely contributed by male participants. Additionally, only one female presented findings of group 1 abnormal ECG findings throughout, indicating the increased prevalence of training-unrelated findings was due to the male population. However, male participants far outweighed the participation of female participants in this study, therefore results cannot be generalised. Nonetheless, pre-menopausal females also have a lower incidence of CV disease than males (Deschamps & Murphy, 2009). Oestrogen has been suggested the responsible factor, and has been shown to alter gene expression and non-genomic pathways which activate signalling pathways for cardioprotection (Murphy & Steenbergen, 2007). Such pathways include eNOS bioavailability and mitochondrial biogenesis. Literature is scarce regarding the cardiac specific influence of oestrogen, although the data from the current study provide evidence that females are less likely to undergo changes to the electrical conductance of the heart. Lack of adaptation to the electrical conductance may not be advantageous to sporting performance, but it may decrease the likelihood of undergoing pathological remodelling.

### 4.6.3 Methodological Issues

#### 4.6.3.1 Part 1 Limitations

The training programme used was progressive over time, with the specific goal of attaining a level of fitness that would allow individuals to complete an iron-distance triathlon. It is true that the training programme designed contained components of high intensity training and functional, strength training therefore is not entirely specific to endurance training. Variability in training programmes between studies means caution should be made when comparing physiological adaptations. Furthermore, participants were included for analysis if they met the total training load requirements, assessed by volume and intensity. As such, participants not complying with the set training programme, performing sessions of low volume and high intensity, may have been deemed as performing the same unit of training load as those performing typical high volumes with lower intensities. This would stimulate alternative physiological processes, contributing to individual variation in the adaptive responses incurred over the course of the study.

Participants recruited for this study were required to train for 9 months prior to an iron-distance triathlon. However due to a change in study design, CV and associated measurements relevant to this study were only collected until 6 months of training. For this reason, the influence of tapering prior to the race, an integral technique for competitive athletes, was not considered. Nevertheless, previous long-term investigations have found most improvements to endurance capacity over the first 3 to 6 months of training (Scharhag-Rosenberg, 2009). As with the present study, most adaptations occurred at the beginning of the training, with diminishing responses as training progressed. Therefore, it is unlikely that substantial changes occurred in the final 3 months of training. The study design was changed to assess other parameters, such as time-trial performance, which subsequent studies will use to identify ultra-endurance performance predictor variables.

The continuous step protocol used to obtain lactate threshold had large incremental steps in power output for each stage. Therefore, the actual power produced at lactate threshold may be largely obscured. Additionally, at the first testing procedure, approximations were made as to which power load was suitable for each participant. As a result, lactate threshold curves often contained very few or numerous stages, resulting in offset lactate thresholds due to lactate kinetics. The maximal lactate steady state (MLSS) may provide a better assessment of endurance performance due to the steady state nature, however would not reflect lactate kinetics (Faude et al., 2009). On the other hand, using such a protocol as the MLSS would have required a far greater time commitment from participants and investigators.

The percentage  $\dot{V}O_{2max}$  at lactate threshold may have given a more indicative parameter for the assessment of endurance ability.  $\dot{V}O_{2max}$  alone has previously been shown to increase and

subsequently level off over the beginning months of training, whereas  $\dot{V}O_{2\max}$  at lactate threshold continues to change over time (Bassett & Howley, 2000). Though this finding may provide a greater mechanistic insight to the specific physiological adaptations which occur, the use of power production at lactate threshold as used in the current study, provides an outcome variable integrating other important physiological factors.

Though bioelectrical impedance assessments of body composition show high correlation with reference methods such as dual-energy X-ray absorptiometry (DEXA) scanning, measurement errors are not eradicated (Völgi et al., 2008). Impedance varies as a result of water distribution and quantity within the body. The distribution of water in the body is vulnerable to intra-day variability as a result of food and drink intake as well as exercise. Therefore, although it was urged that participants maintained uniform conditions prior to repeat measurements, inter-day fluctuations are unavoidable to an extent. However, the reliability of the bioelectrical impedance method has been repeatedly approved in test-retest methods in various population samples (Aandstad, Holtberget, Hageber, Holme & Anderssen, 2014; Kelly & Metcalfe, 2012). Additionally, body composition measurements should not be compared directly with values from other studies using different techniques. Knechtle et al. (2011) demonstrated significantly larger values of fat and skeletal muscle mass compared with anthropometric equations in an ultra-endurance athletic sample.

#### *4.6.3.2 Part 2 Limitations*

QTc has been criticised for its applicability to the athletic population. Since QT is corrected for R-R interval to quantify QTc there is a heavy reliance on HR at the time of the ECG. In normal, healthy individuals 450ms in males and 460ms in females, is likely to distinguish a long QT interval (Viskin, 2009). This value is increased in the criteria used in this study allowing for elevated values in the athletic population and to increase specificity (Drezner, 2012). The majority of QTc values were similar and realistic throughout the bimonthly ECG tracings with the exception of some spurious values which were frequently deviated due to a different HR at the time of the ECG.

Family and personal history are important considerations when investigating 12 lead ECG findings. As such, this information was considered as part of the pre-screening procedure. Additionally, symptoms such as chest pain and syncope provide important insight together with ECG findings regarding the development of CVD. Throughout the study period, participants were not asked if they were experiencing related symptoms. Providing this information would have enabled further understanding of the CV adaptation to prolonged endurance training. Another limitation to this study is that although an attempt was made to display information in relative terms, such as percentages, the low number of female participants highly obscures the information. The low number of female participants means that a potentially small frequency of observed findings will unnaturally exaggerate the relative

percentage of occurrences. Additionally, this limited cohort of female participants can not reflect the female population appropriately.

It would have been appropriate to analyse 12-lead ECG's immediately or shortly after measurements were obtained in order to assess other factors which might have been influencing results, such as hyperkalaemia, hypercalcaemia and acidosis. Additionally, assessing ECG's earlier would have allowed the identification of individuals potentially at risk from SCD prior to the ultra-endurance exercise. Referring such individuals and undergoing further clinical investigations, such as the use of echocardiography and imaging techniques, would have given a greater insight into developments and associated conditions. Furthermore, the identification of criteria reflecting pathological conditions may have been exaggerated in the current study. Despite the simplicity of use regarding electrode placement of the 12-lead ECG, it is possible that the contribution of relatively inexperienced investigators jeopardised accurate results. Although pre-participation screening was conducted by a cardiologist who was aware of the similar manifestations of the athletic and pathologic heart, false positive detection is still a concern (Corrado et al., 2010). This same cardiologist was not available to interpret ECG data used in the data analysis. Moreover, the investigator responsible for identification of abnormal ECG patterns did not have certification in the field of cardiac physiology. All learning was conducted through the use of books, research articles and online courses. For this reason, human error should be considered as a limitation and interpretation should be made with caution.

## 4.7 Conclusion

This study demonstrates that 9 months endurance training is suitable to produce an acceptable rate of completion for an iron-distance triathlon event (82% success in those who competed). Additionally, this study provides data on physiological adaptations expected to occur in response to an endurance type training programme in previously recreationally active male individuals. Over 6 months  $\dot{V}O_{2\max}$  increased by  $5.4 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}/0.36 \text{ L}\cdot\text{min}^{-1}$ , maximal power increased by 32 watts, rHR decreased by 9  $\text{beats}\cdot\text{min}^{-1}$ , HR<sub>max</sub> decreased by 2  $\text{beats}\cdot\text{min}^{-1}$  and BF decreased by 2.3%. Median lactate threshold values increased by 20 watts at month 4 but not at 6 months; nonetheless, interquartile ranges were greater at month 6. Regardless of age-related differences in  $\dot{V}O_{2\max}$  and HR<sub>max</sub>, age did not influence the training-related adaptations. The majority of training-related adaptations were greatest in the initial periods of endurance training from month 0 to month 2 or 4. Likewise, the prevalence of both training-related and unrelated ECG findings increased most from month 0 to month 2 and 4. Additionally, males were more likely to undergo bioelectrical cardiac changes than female participants. This adds further weight to the debate regarding the potentially damaging effects of endurance training. Prospective evaluations should conduct further clinical examination on participants displaying abnormal ECG criteria to confirm or exclude pathological origin.

### Key points:

- Nine months of endurance training is sufficient training period to allow recreationally active individuals complete an ironman-distance triathlon.
- In previously recreationally active individuals, most known training-related adaptations occur within the initial months of training. Subsequent changes are smaller as the time in to training progresses.
- Age differences only existed for relative  $\dot{V}O_{2\max}$  ( $5.15 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) and HR<sub>max</sub> ( $8 \text{ beats}\cdot\text{min}^{-1}$ ). Nevertheless, training-related adaptations were not influenced by age.
- QRS axis is not altered with endurance training, suggestive of similar left and right ventricular adaptation.
- Group 1 training-unrelated findings were more frequent as the training period progressed.
- Training-related and training-unrelated bioelectrical cardiac changes were more prevalent in males than females.
- The increased prevalence of abnormal ECG criteria implies either i) certain abnormal findings are not necessarily training-unrelated as previously thought and may still reflect normal bioelectrical patterns, or ii) endurance training induces pathological manifestations of the heart in individuals previously thought to be healthy.

## **Chapter Five: Pilot Testing: Intra-observer reliability of SphygmoCor Equipment**

## 5.1 Introduction

It is important in research to determine the reliability of a procedure being used. Perfect reliability, no random variation and no systematic variation, is very rare in most clinical research (Bruton, Conway & Holgate, 2000). Usually, reliability testing is conducted to assess the instrumental reliability, observer reliability, and variable reliability (Bruton et al., 2000). The most common form of reliability testing is retest reliability, which refers to the reproducibility of values when you conduct the measurement twice on the same individual in the same condition (Hopkins, 2000).

The reliability and validity of the SphygmoCor device have been previously discussed in chapter 2.2.9. Nonetheless, reliability of equipment may be jeopardised by several factors including, observer experience and equipment damage. SphygmoCor measures of arterial stiffness were assessed for test-retest reliability prior to the main investigation. PWV and Aix and Aix@HR75 were measured on two separate occasions.

## 5.2 Pilot Study 1: Reliability of Pulse Wave Velocity Measurements

### 5.2.1 Method

Eight normotensive participants were recruited for this pilot study (female, n = 2 and male, n = 6). Descriptive participant characteristics are presented in table 7. Resting peripheral BP measurements were obtained from the left arm after 10 minutes of seated rest as in chapter 3.4. The participant was asked to rest for a further 10 minutes in the supine position while the equipment was set up as outlined below. PWV was obtained and the procedure was duplicated immediately after the first measurement was completed. All measurements were conducted by the same investigator.

Table 7. Descriptive characteristics of pilot study 1 participants (n = 8).

Participant Characteristic	Mean $\pm$ SD
Age (yr)	35.8 $\pm$ 16.5
Height (cm)	175.4 $\pm$ 8.8
Body Mass (kg)	71.8 $\pm$ 14.2
Resting Heart Rate (beats·min <sup>-1</sup> )	60 $\pm$ 7
Peripheral Systolic Blood Pressure (mmHg)	122 $\pm$ 13
Peripheral Diastolic Blood Pressure (mmHg)	67 $\pm$ 7

### 5.2.2 SphygmoCor CVMS Measurements

Arterial Stiffness was measured non-invasively via applanation tonometry using the SphygmoCor equipment (SphygmoCor, AtCor Medical, Sydney, Australia). The device was calibrated for brachial BP prior to measurements along with anthropometric data including height and body mass. A high fidelity tonometer was applied with gentle pressure over the artery so that it was only partially occluded. The

tonometer was adjusted until a satisfactory and repeatable waveform was being captured, and was held stable throughout data capture. The data acquisition box was interfaced with a laptop installed with SphygmoCor CVMS software (AtCor Medical, Sydney, Australia) to display visual information.

All participants were asked to abstain from caffeine and alcoholic substances (>12 hrs), food (3 hrs) and exercise (12 hrs) prior to their appointments. Testing was conducted in laboratory controlled conditions at temperature,  $21 \pm 1^\circ\text{C}$ ; barometric pressure – range, 97.3-102.3 kPa; humidity – range, 21-56%.

#### *5.2.2.1 Pulse Wave Velocity Measurements*

Participants were instructed to lie in the supine position and rest for 5-10 minutes prior to data acquisition. During this time the sites for carotid and femoral artery pulse on the right side of the body were palpated and suitable sites were identified and marked. Distance was measured from the suprasternal notch to the distal site, femoral artery, and from the suprasternal notch to the proximal site, carotid artery of the right side of the body (Seca, 201 ergonomic circumference measuring tape, Hamburg). Electrode sites were cleansed with alcohol wipes, allowed to dry, and three 30 mm electrodes (Cardiacare Ltd. Essex, United Kingdom) were placed according to the SphygmoCor manual recommendations, see figure 18. Cables were attached to the electrodes and the SphygmoCor device. Carotid and femoral artery sites were cleansed with an alcohol wipe and allowed to dry. Using the tonometer, pulse waves were obtained transcutaneously at the carotid and femoral artery of the right side sequentially for a 12 second acceptable period. Data was obtained for at least 2 minutes at each site before processing to allow familiarisation of the conditions.

#### *5.2.2.2 Carotid and Femoral Artery Measurements*

For carotid artery assessment, the patient lay on the bed in the supine position with the absence of a pillow. The patients head was tilted slightly backwards and to their left. The operator felt for the position of the strongest pulse at the right carotid artery and placed the tonometer directly over the skin of this point. The operator was sitting behind the patient with a solid platform to rest the forearm during the measurement. For the femoral assessment, the patient remained supine on the bed with their right knee slightly flexed and thigh rotated away from the body. Right sided femoral pulse was felt midway between the anterior superior iliac spine and the front of the pubic bone. The tonometer was placed directly over the point of the skin where the pulse was strongest until an adequate reading was obtained. Latex gloves were worn for the duration of the femoral artery assessment.

PWV was measured using the intersecting tangent algorithm using the foot-to-foot velocity method from a minimum of 3 waveforms, as presented in figure 19 (Chiu et al., 1991). An ECG trace was obtained concurrent to the pulse wave recording and the time delay ( $\Delta t$  or transit time) was measured

between the feet of the two waveforms. PWV was calculated as  $PWV = D \text{ (meters)} / \Delta t \text{ (seconds)}$ , where  $D$  equals the proximal distance (suprasternal-carotid) subtracted from the distal distance (suprasternal-femoral). Measurements were repeated if the standard deviation of the time differences was greater than 10% or if rHR varied by greater than 5 beats·min<sup>-1</sup>.

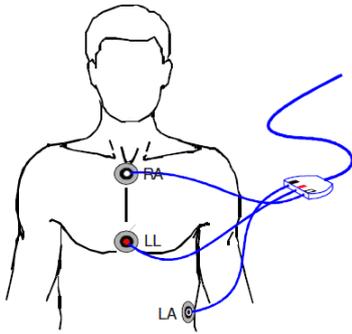


Figure 18. Three-cable electrocardiograph (ECG) configuration on SphygmoCor equipment.

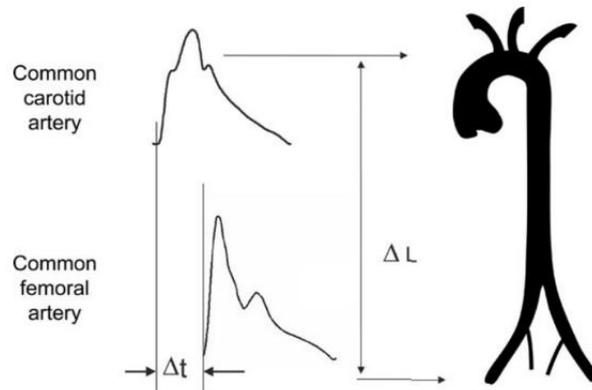


Figure 19. Measurement of carotid-femoral pulse wave velocity (PWV) with the foot to foot method.

### 5.2.2.3 Data Analysis

Paired samples t-tests were conducted to examine differences between measurement 1 and measurement 2. Significance was determined by an alpha level of  $p < 0.05$ . Repeatability coefficient (CR) was used to present measurement error over the two tests using the following formula as recommended by the British Standards Institution (1979):

$$CR = 1.96 \times \sqrt{\frac{\sum (d_2 - d_1)^2}{n}}$$

Where 'd' is the difference between two measurements, and 'n' is sample size (Bland and Altman, 1986).

Linear regression was conducted on the difference between measurement 1 and 2 and the mean values of measurement 1 and 2. The hypotheses was tested that the mean difference coefficient was 0.

### 5.2.3 Results

On average, PWV was not significantly different in measure 1 ( $5.6 \pm 1.0$ ) than to measure 2 ( $5.8 \pm 0.9$ ),  $t(7) = -1.618$ ,  $p > 0.05$ . Pearson's correlation was 0.96 ( $p < 0.001$ ). The test-retest coefficient of variation was 18% for measurement 1 and 16% for measurement 2. The estimated repeatability

coefficient was  $0.7 \text{ m}\cdot\text{s}^{-1}$ . Linear regression revealed no significance for the test that the coefficient for the mean difference was not 0,  $t = -0.94$ ,  $p = 0.38$ , indicating no proportional bias. In addition, Bland-Altman plots indicated good reproducibility of PWV, 100% within 2 standard deviations (figure 20).

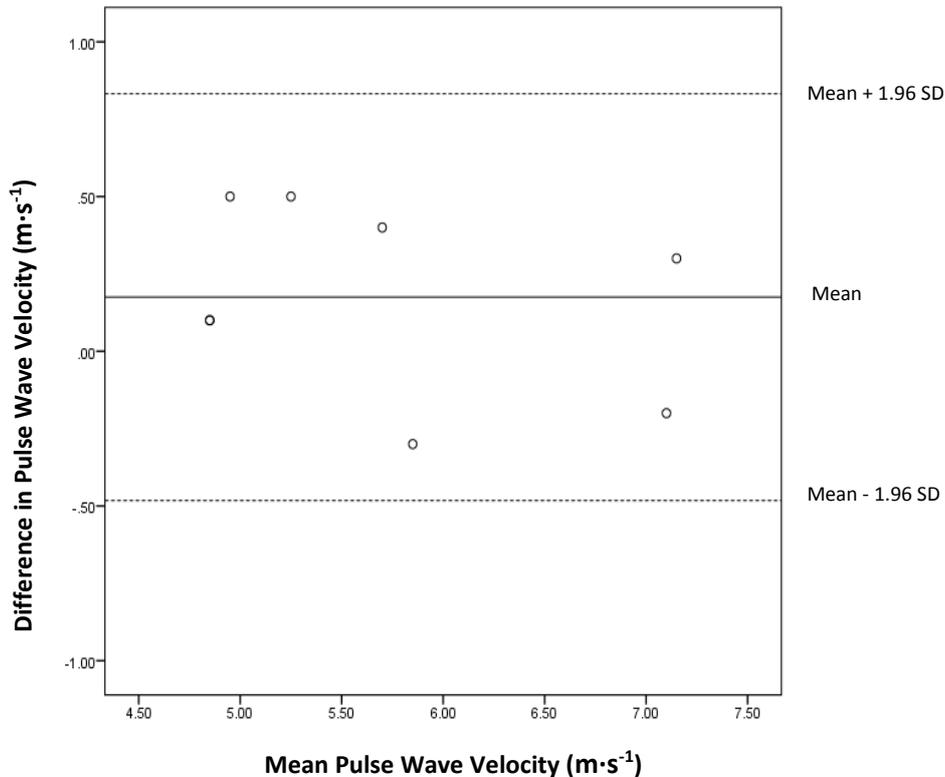


Figure 20. Bland-Altman plot from repeated measures of pulse wave velocity (PWV) showing the extent of intra-observer variability.

Mean difference =  $0.18 \text{ m}\cdot\text{s}^{-1}$ ; lower 95% =  $-0.48 \text{ m}\cdot\text{s}^{-1}$ ; upper 95% =  $0.83 \text{ m}\cdot\text{s}^{-1}$ .

#### 5.2.4 Discussion

PWV measurements were obtained one after the other in 8 participants with the same investigator. The reliability of these intra-investigator measurements were examined in preparation for their use within a prospective main study. Bland-Altman plots indicated a high reproducibility for PWV. Repeatability coefficient represents the value below which the difference between 2 measurements will lie with 95% probability. In this study, an estimated repeatability coefficient of  $0.7 \text{ m}\cdot\text{s}^{-1}$  means we can expect the absolute difference between two future measurements made by one investigator on one participant to be no greater than  $0.7 \text{ m}\cdot\text{s}^{-1}$  on 95% of occasions. The acceptance of the null hypothesis that the coefficient of the mean difference between measurements was 0, indicates there was no proportional bias. In other words, no trend was observed where there are more points above

or below the mean difference line. This suggests there is a high level of agreement for PWV measurements. The Pearson's correlation value of 0.96 represents a high correlation between the test-retest measurements.

### 5.3 Pilot Study 2 - Reliability of Pulse Wave Analysis Measurements

#### 5.3.1 Method

Thirteen normotensive participants were recruited for this pilot study (female,  $n = 1$  and male,  $n = 12$ ). Descriptive statistics of this sample are presented in table 8. Resting peripheral BP was obtained from the left arm after 10 minutes of seated rest. The SphygmoCor equipment was set up to record PWA as outlined below. The procedure for PWA was duplicated immediately after the first measurement was completed. All measurements were conducted by the same investigator.

Table 8. Descriptive characteristics of pilot study 2 participants ( $n = 13$ ).

Participant Characteristics	Mean $\pm$ SD
Age (yr)	30.9 $\pm$ 13.3
Height (cm)	177.1 $\pm$ 5.7
Body Mass (kg)	76 $\pm$ 11.5
Resting Heart Rate (beats·min <sup>-1</sup> )	59 $\pm$ 11
Peripheral Systolic Blood Pressure (mmHg)	113 $\pm$ 6
Peripheral Diastolic Blood Pressure (mmHg)	65 $\pm$ 6

#### 5.3.2 SphygmoCor CVMS Measurements

Arterial Stiffness was measured non-invasively via applanation tonometry using the SphygmoCor equipment (SphygmoCor, AtCor Medical, Sydney, Australia). The device was calibrated for brachial BP prior to measurements along with anthropometric data including height and body mass. A high fidelity tonometer was applied with gentle pressure over the artery so that it was only partially occluded. The tonometer was adjusted until a satisfactory and repeatable waveform was being captured, and was held stable throughout data capture. The data acquisition box was interfaced with a laptop installed with SphygmoCor CVMS software (AtCor Medical, Sydney, Australia) to display visual information.

All participants were asked to abstain from caffeine and alcoholic substances (>12 hrs), food (3 hrs) and exercise (12 hrs) prior to their appointments. Testing was conducted in laboratory controlled conditions at temperature,  $21 \pm 1^\circ\text{C}$ ; barometric pressure – range, 97.3-102.3 kPa; humidity – range, 21-56%.

##### 5.3.2.1 Pulse Wave Analysis Measurements

The participant remained in the seated position with the left arm relaxed at heart level. The skin site was cleansed with an alcoholic wipe and left to dry before the tonometer was placed perpendicular

to the strongest pulse at the radial artery of the left arm. After at least 2 minutes of data acquisition a 12 second recording was processed and the corresponding aortic pressure waveform was generated from the in-built transfer function. Aix was defined as a percentage of augmentation pressure (AP) divided by aortic pulse pressure (aPP). Aix was corrected for a HR of 75 beats·min<sup>-1</sup> (Aix@HR75). SphygmoCor software adjusts for this manipulating Aix at an inverse rate of 4.8% for each 10 beats·min<sup>-1</sup> increase in HR (Stoner, Young & Fryer, 2012; Wilkinson et al., 2000; Wilkinson et al., 2002). In compliance with the SphygmoCor operator manual, parameters determined from a left ventricular ejection duration (LVED) value outside the range of 200-450 ms were disregarded. Additionally, results were only accepted if operator index was greater than 75%, as suggested in the manufacturer's recommendations. Operator index refers to the number calculated from the quality control indices including average pulse height, pulse height variation, diastolic variation, and shape variation.

### 5.3.2.2 Data Analysis

As in part 1 of the pilot study, paired samples t-tests were conducted to examine differences between measurement 1 and measurement 2, of Aix and Aix@HR75. Significance was determined by an alpha level of  $p < 0.05$ . Repeatability coefficient (CR) was used to present measurement error over the two tests using the following formula as recommended by the British Standards Institution (1979):

$$CR = 1.96 \times \sqrt{\frac{\sum (d_2 - d_1)^2}{n}}$$

Where 'd' is the difference between two measurements, and 'n' is sample size (Bland and Altman, 1986).

Linear regression was conducted on the difference between measurement 1 and 2 and the mean values of measurement 1 and 2. The null hypotheses was tested that the mean difference coefficient was significantly different to 0.

### 5.3.3 Results

On average, Aix was not significantly different in measure 1 ( $15.2 \pm 12.1\%$ ) than to measure 2 ( $15.8 \pm 10.6\%$ ),  $t(12) = -0.50$ ,  $p = 0.63$ . Likewise, Aix@HR75 was not significantly different between measurements ( $7.9 \pm 14.2\%$  vs.  $7.7 \pm 12.8\%$ ),  $t(12) = 0.14$ ,  $p = 0.89$ . Pearson's correlation was 0.93 for Aix and 0.96 for Aix@HR75;  $p < 0.001$ . The test-retest coefficient of variation was not calculated since the type of data was interval. The estimated repeatability coefficient was 8.5% for Aix and 7.4% for Aix@HR75. The average quality control operator index score was  $95 \pm 6\%$ . Linear regression revealed no significance for the test that the coefficient for the mean difference was not 0,  $t = -1.23$ ,  $p = 0.24$  for Aix and  $t = -1.29$ ,  $p = 0.23$  for Aix@HR75. In addition, Bland-Altman plots indicated good

reproducibility of Aix, 100% within 2 standard deviations (figure 21), as well as Aix@HR75, 92% within 2 standard deviations (figure 22).

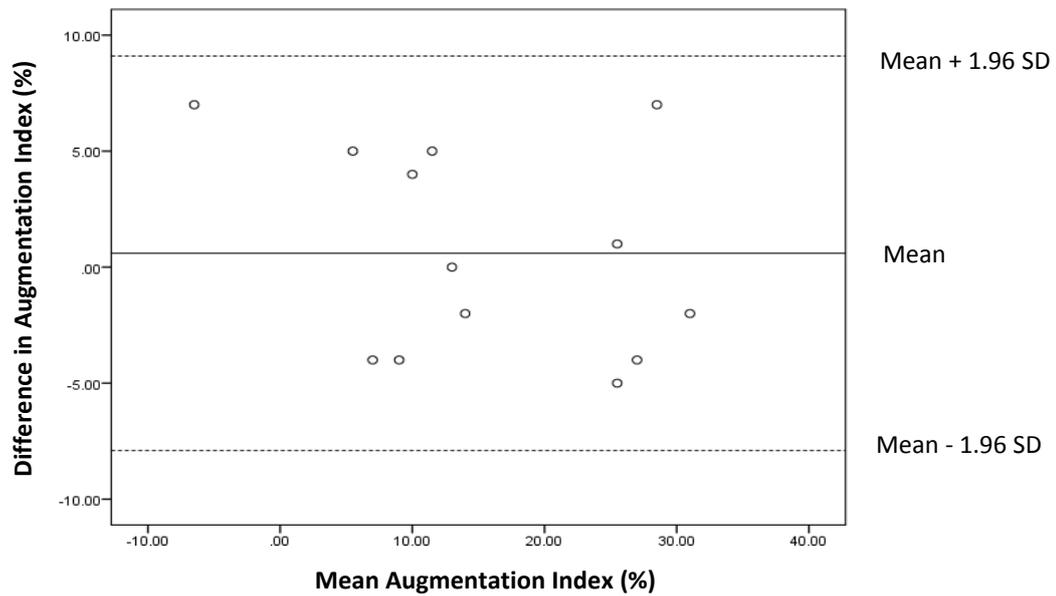


Figure 21. Bland-Altman plot from repeated measures of augmentation index (Aix) showing the extent of intra-observer variability.

Mean difference = 0.6%; lower 95% = - 7.9%; upper 95% = 9.1%.

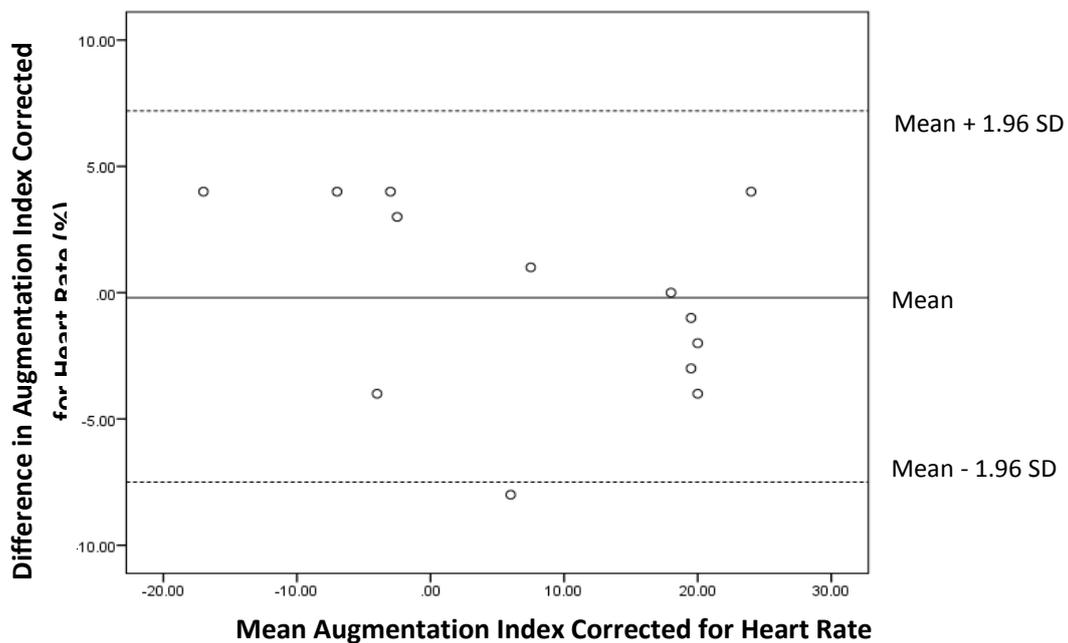


Figure 22. Bland-Altman plot from repeated measures of augmentation index corrected for a heart rate of 75 beats·min<sup>-1</sup> (Aix@HR75) showing the extent of intra-observer variability.

Mean difference = 0.2%; lower 95% = - 7.5%; upper 95% = 7.2%.

#### 5.3.4 Discussion

PWA procedures were conducted twice consecutively in 13 participants. Aix and Aix@HR75 were obtained and the reliability of intra-investigator measurements were examined. In line with previous research, Bland-Altman plots indicated a high reproducibility for Aix and Aix@HR75 (Papaionnou et al., 2007). An estimated repeatability coefficient of 8.5% for Aix means we can expect the absolute difference between two future measurements made by one investigator on one participant to be no greater than 8.5% on 95% of occasions. An estimated repeatability coefficient of 7.4% for Aix@HR75 confers similar assumptions. These values are considerable and may reflect inexperienced use with equipment. One measure to account for the large repeatability coefficient may be to increase the operator index acceptance level from 75% in this study, to 90%. This adjustment would allow for a tighter regulation of data provided by the SphygmoCor equipment.

The acceptance of the null hypothesis that the coefficient of the mean difference between measurements was 0 indicates there was no proportional bias. In other words, no trend was observed where there are more points above or below the mean difference line. This suggests there is a high level of agreement for PWV measurements.

#### 5.4 Overall Pilot Testing Conclusion

All of the measurements of arterial stiffness obtained by the SphygmoCor equipment showed adequate reliability. The hypothesis for proportional bias was rejected for all techniques. PWV appears a more reliable method of arterial stiffness measurement than Aix or Aix@HR75. These methods were considered suitable for use in the next phase of study with the knowledge that within-subject repeatability is adequate. However, the aforementioned repeatability coefficient values should be considered in the main section of the study when making conclusions regarding arterial stiffness. One method to adjust for this would be to consider a greater minimum operator index for pulse wave analysis measures, for example 90%.

## **Chapter Six: Study 2 - The effects of a long-distance triathlon on markers of arterial stiffness**

## 6.1 Abstract

**INTRODUCTION:** Repetitive episodes of arterial shear stress provide a stimulus that promotes vascular adaptations through endothelium-dependent remodelling. Longer exposure to stimuli, such as during endurance exercise, may compromise the vascular system. The mechanisms are likely due to shear stress, oxidative stress and inflammatory processes having an influence on endothelial function and aortic elasticity.

**PURPOSE:** To provide an insight to the vascular response to ultra-endurance training and an ultra-endurance event, and to determine whether antioxidant supplementation influences the arterial stiffening response to prolonged exercise.

**METHODS:** Eleven athletes (TRI; age:  $34.3 \pm 8.7$ yr; weight:  $73.9 \pm 10.3$  kg) and 10 recreational control participants (NOTRI; age:  $26.5 \pm 7.1$  yr, weight:  $72.7 \pm 5.6$  kg) were assessed on 4 occasions with identical time intervals. Additionally,  $n = 5$  TRI participants received antioxidant & anti-inflammatory supplementation for 12 weeks (S) whilst  $n = 6$  received no supplementation (NS). Carotid-femoral pulse wave velocity (PWV), and pulse wave analysis (PWA) procedures were measured 7 days prior (T1) to an iron-distance triathlon. This consisted of a 3.86km swim, 180.25km cycle, and 42.2km run. Repeated measurements were obtained 12 to 18 hours post-triathlon (T2), 7 days post-triathlon (T3), and 28 days post-triathlon (T4). The NOTRI group did not participate in the triathlon. Data presented as mean  $\pm$  standard deviation.

**RESULTS:** All effects are reported as significant at  $p < 0.05$ . Average triathlon race time was  $786 \pm 63$ mins. PWV was significantly greater in TRI than NOTRI at T2 and T3 (T2;  $6.1 \pm 0.4\text{m}\cdot\text{s}^{-1}$  vs.  $5.5 \pm 0.6\text{m}\cdot\text{s}^{-1}$  and T3;  $6.3 \pm 0.7\text{m}\cdot\text{s}^{-1}$  vs.  $5.6 \pm 0.6\text{m}\cdot\text{s}^{-1}$ ), but not at T1 or T4 (T1;  $5.9 \pm 0.6\text{m}\cdot\text{s}^{-1}$  vs  $5.6 \pm 0.8\text{m}\cdot\text{s}^{-1}$  and T4;  $6.0 \pm 0.6\text{m}\cdot\text{s}^{-1}$  vs  $5.8 \pm 0.8\text{m}\cdot\text{s}^{-1}$ ). No significant differences were observed for Aix or Aix@HR75. No significant differences in PWV, Aix or Aix@HR75 were observed in TRI alone between time-points. No significant time or group interactions were revealed for PWV, Aix or Aix@HR75 in S and NS between time-points.

**CONCLUSION:** The elevation of PWV but not Aix or Aix@75 in the TRI group is likely due to the different response to shear stress and acute systemic inflammation of large and small arterial vascular beds. Importantly, all measurements were statistically similar levels at the final time-point; suggesting only a short-term elevation of arterial stiffness. This response was not substantial enough to elicit a change in arterial stiffness markers in the triathletes compared against themselves over time-points. Prior antioxidant supplementation did not provoke a differential response in arterial stiffening from ultra-endurance exercise.

## 6.2 Introduction

Substantial evidence supports the concept that habitual exercise has CV health benefits contributing to an overall reduction in risk of SCD and cardiac events (Corrado, Migliore, Basso & Thiene 2006; Warburton, Nichol and Bredin, 2006). Prolonged endurance training on the other hand has been associated with unfavourable CV risk factors (Schwartz et al., 2014, O’Keefe et al., 2012; Vlachopoulos et al., 2010). Indirect arterial stiffness indices such as PWV and wave reflections including Aix are powerful independent predictors of CV events and mortality (Laurent et al., 2006). Therefore, elevated arterial stiffness measurements represent an increased CV risk. In the chronic setting, it has been indicated that ultra-endurance athletes may present an elevated arterial stiffness than recreationally active individuals (Burr et al., 2014; Vlachopoulos et al., 2010).

Growth of ultra-endurance events and participants competing in prolonged endurance exercise has increased in recent years (Hoffman, Ong, & Wang, 2010; Knechtle, Knechtle & Lepers, 2011), therefore the need to assess the health consequences of such events has become more important. Acute exercise transiently elevates the risk of SCD (Corrado, Basso, Rizzoli, Schiavon & Thiene, 2003). Despite rising participation and evidence of transiently compromised CV health, the acute and long-term responses to prolonged excessive exercise that are implicated in the transient period are not fully understood. Studies investigating the acute effect have indicated an increased large artery stiffness and reduced wave reflections, suggestive of enhanced peripheral artery compliance. The time response of arterial distensibility to an ultra-endurance bout have not yet been investigated, however as with the heart, it is not inconceivable to expect prolonged changes (La Gerche et al., 2008; Neilan, Yoerger et al., 2006). Research examining the area of ultra-endurance has typically focused on elite or long-term performers, however the recent increases in participation and thus sub-elite athletes allow the investigation of the responses in previously unexperienced athletes. It is probable that the associated stressors and outcomes of participation manifest differently in experienced and unexperienced individuals.

One of the suspected primary mechanisms regarding arterial stiffening with particular reference to prolonged exercise involves an up-regulation of oxidative stress (Gross et al., 2005; Sindler et al., 2009). Oxidative stress influences long-term, structural mediators of arterial stiffening as well as short-term, functional mechanisms. Antioxidant strategies may attenuate this oxidative stress and subsequent arterial stiffening (Qin et al., 2008; Zhao et al., 2013).

### 6.3 Aims and Hypotheses

The purpose of this study was to provide an insight to the CV response to ultra-endurance training and an ultra-endurance event, and to determine whether antioxidant supplementation influences the arterial stiffening response to prolonged exercise. The primary null hypothesis was that PWV, Aix, Aix@HR75, rHR, SBP, DBP and LVED would not differ between ultra-endurance trained and recreationally active controls at baseline. The secondary null hypothesis predicted that PWV, Aix, Aix@HR75, rHR, SBP, DBP and LVED would not change in the short or longer period post long-distance triathlon event. Thirdly, the null hypothesis predicted no differences in PWV, Aix, Aix@HR75, rHR, SBP, DBP and LVED between antioxidant supplemented and non-supplemented individuals at any time-point pre and post-event.

### 6.4 Method

#### 6.4.1 Participants

A total of 33 healthy individuals were recruited to take part in this study with 21 participants completing the protocol. Twenty athletes were originally recruited from study 1 of an adjunct larger scale study, and were previously signed up to compete in a long-distance triathlon in Barcelona consisting of a 3.86 km swim, 180.25 km cycle, and 42.2 km run. Only 11 athletes were able to complete the study largely due to failure to complete the long-distance event. Of these, 5 participants were receiving supplementation (S), and 6 were receiving no supplementation (NS). Twelve week supplementation consisted of a multi-nutrient formula, an essential fatty acid formula, an antioxidant formula, and a probiotic formula (Biocare Ltd., Birmingham, United Kingdom). See appendix 6 for full supplementation information. Thirteen participants were recruited to act as a control population who did not receive supplementation and did not complete the triathlon. Ten control participants were taken forward to analysis due to withdrawal. Control volunteers were considered 'recreationally active', completing no more than 4 exercise sessions per week. Participant information can be found in table 9. Inclusion criteria required that participants be aged between 18 and 51 years, have no history of heart abnormalities, hypertension, coronary heart disease or diabetes, have not previously completed a long-distance event, and are not taking any medication. Written informed consent was obtained prior to the study and a health screen was provided before each appointment. Upon initial arrival, participants were given a verbal brief regarding the study design, what was to be expected from them, and to ensure they understood the commitment and testing procedure sufficiently.

Participants were excluded if they: were aged <18/>51 years, participated in a long-distance event previously, not currently training 1-4 days per week, or had history of heart

abnormalities/hypertension/coronary heart disease or diabetes. All participants provided written informed consent along with a letter from respective general practitioners.

#### **6.4.2 Study Design**

This longitudinal study employed a non-randomised repeated measures experimental design to examine the short and longer term effects of prolonged exercise on human vasculature. Additionally it was sought to identify if nutritional supplementation influenced the vascular response to prolonged exercise. This protocol was approved by the University of Hertfordshire health and human sciences ethics committee with delegated ethics authority (protocol number: aLMS/PG/UH/00139 the influence of ultra-endurance exercise on the CV and related physiological systems).

#### **6.4.3 Experimental Procedure**

Arterial stiffness measures were obtained 7 days prior to the previously mentioned long-distance triathlon in the athletic population (T1). These measurements were repeated 12-18 hours post-event (T2), 7 days post-event (T3), and 28 days post-event (T4). Measures were also repeated with the same time intervals on a different group of 'recreational' individuals. Height, body mass and peripheral BP were obtained as in chapter 3.3 and 3.4. Appendix 7 shows the flow diagram of the method used in this study.

#### **6.4.4 SphygmoCor CVMS Measurements**

Arterial Stiffness was measured non-invasively via applanation tonometry using the SphygmoCor equipment (SphygmoCor, AtCor Medical, Sydney, Australia). A high fidelity tonometer was applied with gentle pressure over the artery so that it was only partially occluded. The tonometer was adjusted until a satisfactory and repeatable waveform was being captured, and was held stable throughout data capture. The data acquisition box was interfaced with a laptop installed with SphygmoCor CVMS software (AtCor Medical, Sydney, Australia) to display visual information.

Where possible, participants were booked in at the same time of the day to control for natural diurnal variance. Additionally, participants were asked to eat a similar meal prior to each testing appointment. All participants were asked to abstain from caffeine and alcoholic substances (>12 hr), food (3 hr) and exercise (12 hr) prior to their appointments. Testing points 7 days pre (T1), 7 days post (T3), and 28 days post-event (T4) were conducted in laboratory controlled conditions at temperature,  $21 \pm 1^\circ\text{C}$ ; barometric pressure – range, 97.3-102.3 kPa; humidity – range, 21-56%. Measurements for the triathlon group at 12-18 hr (T2) post-event were completed in a different environment due to logistical reasons, at temperature  $21 \pm 1^\circ\text{C}$ . To avoid inter-observer error all the measurements were conducted by the same investigator. Systematic errors and bias were minimised by using the same equipment on each participant.

Firstly, PWA measurements were conducted, providing values for: Aix, Aix@HR75, aortic systolic and diastolic BP, and LVED, as in chapter 5.3.2. Waveforms were only accepted if the operator index reached 90% or greater, for reasons observed in pilot study 2. Next, PWV and rHR were obtained in the same arrangement as chapter 5.2.2.

#### 6.4.5 Genova Diagnostics

Participants were instructed to follow the standardised instructions for the collection of bodily samples required for the 'optimal nutrition evaluation' (ONE) (Genova Diagnostics, Surrey, United Kingdom). Participants were required to overnight fast (8 hrs) and collect their first morning urine sample into the sterile cup provided. Participants were responsible for transferring the urine into the 3 collection tubes provided via pipette and were asked to freeze the samples for a minimum of 2 hours before shipping. Instructions were available to participants both in hardcopy and online format. Samples were sent to Genova Diagnostics for processing within 24 hours of collection. Further information can be found in the appendix 8. Lipid peroxide, 8-hydroxydeoxyguanosine (8-OHdG), and glutathione requirement were assessed in the TRI groups (NS and S). The ONE feedback document consisted of urinary lipid peroxide values given as microliters per gram of creatinine ( $\mu\text{mol}\cdot\text{g creatinine}^{-1}$ ). 8-OHdG were measured in micrograms per g of creatinine ( $\mu\text{g}\cdot\text{g creatinine}^{-1}$ ). Glutathione requirement was given in scale format (figure 23). Therefore, each major tick represented an incremental score of 1 (0 to 10) for analysis purposes.



Figure 23. An example of the visual feedback regarding glutathione requirement values from the optimal nutrition evaluation (ONE).

Urinary samples were collected and dispatched for analysis by participants. Glutathione requirement was determined via urinary analysis in an external laboratory by Genova Diagnostics. The green area reflects a normal score, yellow area indicates borderline requirement, and the red area indicates a high need. Major tick marks represent an incremental score of 1 for analytical purposes, with 0 representing the lowest score and 10 representing a maximal score. X indicates the score for the individual.

#### 6.4.6 Data Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences Version 21 (SPSS Inc., Illinois, United States of America). Pre-test checks were conducted prior to statistical analysis to assess for violations of assumptions. These include the Shapiro-Wilk tests of normality, with visual analysis of histograms, box plots and P-P plots, Levene's test of equality of variances, and Mauchly's sphericity test. Two mixed measures analysis of variance (ANOVA) models were conducted and are

described separately in part 1 and part 2 of the results section. Independent samples t-tests were performed on baseline variables to demonstrate whether the groups were matched at baseline or not.

The first two-way mixed measures ANOVA (part 1) used time as within-subjects factor (4 levels; T1: 7 days pre-event, T2: 12 to 18 hours post-event, T3: 7 days post-event, and T4: 28 days post-event) and with condition as between-subjects factor (2 levels; TRI: athletes participating in iron-distance triathlon, and NOTRI: recreationally active participations, tested with equal variance between time-points as TRI group). In the second two-way mixed measures ANOVA (part 2) time was again used for within-subjects comparisons. In this section only the TRI participants were used for data analysis with group (2 levels; NS: non-supplementation group, and S: supplementation group) as the between-subjects factor.

Within-subjects analysis investigated the main time effect on the dependent variables as well as the interaction effect of the within-subjects factor (time) and the between-subjects factor (condition or group). If a main time effect existed Bonferroni comparisons were used to perform pairwise comparisons between all levels of time. Significant interactions were followed with simple main effects using Bonferroni comparisons on the between-subjects factor at each level of time. Main effects were also conducted for the between-subjects factor. Adjustments for multiple comparisons were made for pairwise analysis, accounting for the number of potential hypotheses within each test. Correcting for the total amount of hypotheses within the study would have created an alpha level extremely conservative. This procedure would significantly reduce the chance of a type I error, but also increase the risk of a false negative significance occurring.

## 6.5 Results

### 6.5.1 Pre-Test Assumptions

Shapiro-Wilk tests of normality were conducted for assessment of the distribution of data along with visual analysis of histograms and P-P plots. No large deviations from normality were observed, and since most comparison procedures perform well under small deviations from normality it was deemed appropriate to use parametric tests (Field, 2009). The ANOVA is particularly robust to slight violations of normality within a dataset (Field, 2009).

Levene's test of equality of error variances was performed on all dependent variables across time levels. The assumption of homogeneity of variance was met if Levene's test accepted the null hypothesis that error variance was equal across groups. Significance ( $p < 0.05$ ) in Levene's test, representing a violation to the assumption of homogeneity of variance, is mentioned in text.

Depending on the extent of the violation, such situations resulted in a more conservative alpha level of 0.025 (Tabachnick & Fidell, 2013).

Mauchly's sphericity test was used to assess the assumption of sphericity within repeated-measures effects. Unless stated otherwise Mauchly's test was insignificant ( $p > 0.05$ ), therefore the assumption of sphericity was accepted. Greenhouse-Geisser correction for sphericity was used if the estimate of sphericity ( $\epsilon$ ) was  $< 0.75$  and Huynh-Feldt correction was used if the statistic was  $> 0.75$  (Girden, 1992).

### 6.5.2 Part 1: Triathletes vs. Recreationally Active

Part 1 of this study used a 4 by 2 mixed measures ANOVA with time as the within-subjects factor (4 levels) with between-subjects comparisons made between TRI (athletes participating in iron-distance triathlon) and NOTRI (recreationally active participants not participating in triathlon) conditions. Arterial stiffness biomarkers, rHR, aortic BP, and LVED were inserted into the ANOVA as dependent variables. Full descriptive data can be found in appendix 9.

#### 6.5.2.1 Baseline Comparisons

Table 9 presents the baseline participant characteristics between conditions. An independent sample T-test was conducted for age, height and body mass between TRI and NOTRI conditions at T1. A significant difference in age was found at baseline between TRI and NOTRI conditions,  $t(19) = 2.23$ ,  $p = 0.04$ . Significant differences were not observed for height,  $t(19) = -0.91$ , or body mass,  $t(19) = 0.33$ ;  $p > 0.05$ .

Table 9. Baseline study 2 participant characteristics between conditions.

	TRI (n = 11)	NOTRI (n = 10)	
Age (years)	34.4 ± 8.7	26.5 ± 7.1	$p < 0.05$
Height (cm)	174.5 ± 7.4	177.0 ± 5.3	$p = 0.25$
Body mass (kg)	73.9 ± 10.3	72.7 ± 5.6	$P = 0.29$

TRI = triathlete participants; NOTRI = recreationally active participant; yr = years; cm = centimetre; kg = kilogram.

#### 6.5.2.2 Arterial Stiffness Biomarkers – Pulse Wave Velocity

Within-subject analysis found no significant main time effect on PWV,  $F(3, 57) = 0.697$ ,  $p = 0.56$ ,  $\eta^2 = 0.04$ . Likewise no significant interaction was observed for time and condition for PWV,  $F(3, 57) = 1.610$ ,  $p = 0.20$ ,  $\eta^2 = 0.08$ . This finding indicates that TRI and NOTRI participants were not affected differently over time.

Between-subjects analysis found a significant effect in PWV between TRI and NOTRI conditions,  $F(1, 19) = 4.46$ ,  $p < 0.05$ ,  $\eta^2 = 0.19$ . Simple effects analysis was conducted with Bonferroni adjustment for

multiple comparisons. TRI and NOTRI conditions were compared across each level of time. A significant difference in PWV was found at T2 ( $F(1, 19) = 9.24, p < 0.05, \eta^2 = 0.33$ ) and T3 ( $F(1, 19) = 6.228, p < 0.05, \eta^2 = 0.25$ ) (figure 24). These results indicate that PWV was significantly greater in those who competed in the triathlon than participants who did not, at 12 to 18 hours post-event (T2:  $6.1 \pm 0.4\text{m}\cdot\text{s}^{-1}$  vs.  $5.5 \pm 0.6\text{m}\cdot\text{s}^{-1}$ , respectively) and 7 days post-event (T3:  $6.3 \pm 0.7\text{m}\cdot\text{s}^{-1}$  vs.  $5.6 \pm 0.6\text{m}\cdot\text{s}^{-1}$  respectively). There were no significant differences between TRI and NOTRI 7 days pre-event or 28 days post-event (T1:  $5.9 \pm 0.6\text{m}\cdot\text{s}^{-1}$  vs  $5.6 \pm 0.8\text{m}\cdot\text{s}^{-1}$  and T4:  $6.0 \pm 0.6\text{m}\cdot\text{s}^{-1}$  vs  $5.8 \pm 0.8\text{m}\cdot\text{s}^{-1}$ ). Average PWV remained stable between time-points in NOTRI (maximal change  $0.2\text{m}\cdot\text{s}^{-1}$ ) and TRI (maximal change  $0.4\text{m}\cdot\text{s}^{-1}$ ).

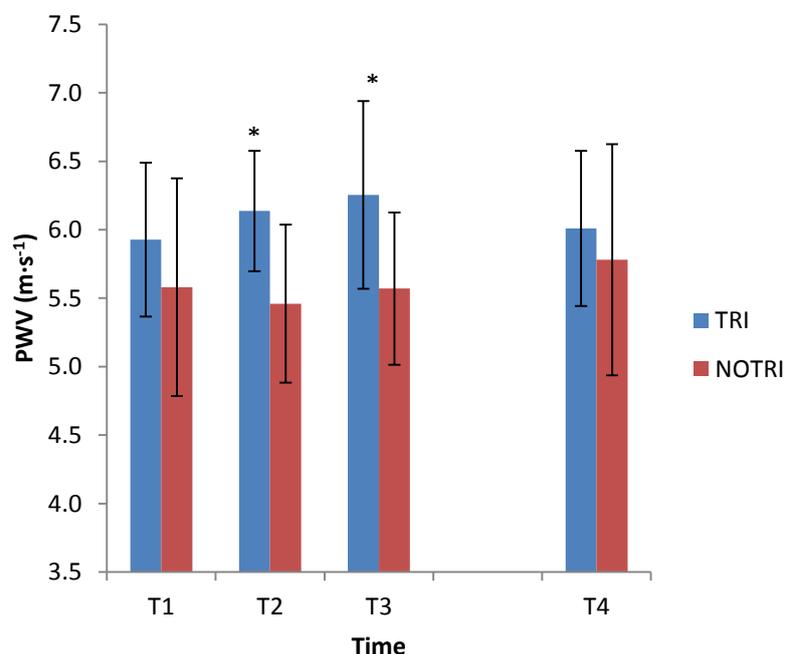


Figure 24. Comparison of pulse wave velocity (PWV) values between both conditions TRI (n = 11) and NOTRI (n = 10) across 4 time-points (mean ± SD).

TRI = triathlete participants; NOTRI = recreationally active participants; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event (in respect to TRI group). Time-points reflect equal variance for NOTRI group conducted at a later period. \* significant difference between conditions,  $p < 0.05$ .

### 6.5.2.3 Arterial Stiffness Biomarkers – Augmentation Index

Levene's test of equality of error variances was violated at T3,  $F(1, 19) = 5.44, p = 0.03$ . Owing to this violation, an alpha level of 0.025 was accepted. There was not a significant main effect of time on Aix,  $F(3, 57) = 2.74, p = 0.05, \eta^2 = 0.13$ . There was no significant interaction effect of time and condition for Aix,  $F(3, 57) = 1.493, p = 0.226, \eta^2 = 0.07$ . This suggests that TRI and NOTRI participants were not affected differently across any level of time as presented in figure 25 (T1:  $14.6 \pm 12.1\%$  vs.  $10.0 \pm$

10.4%; T2:  $9.2 \pm 9.5\%$  vs.  $12.7 \pm 7.6\%$ ; T3:  $6.1 \pm 12.7\%$  vs.  $7.9 \pm 7.9\%$ ; T4:  $11.2 \pm 10.0\%$  vs.  $12.2 \pm 8.2\%$ ; respectively,  $p < 0.05$ ). Aix decreased from T1 to T3 by 8.5% in the TRI group, almost a 3 fold reduction, however was non-significant as previously mentioned. Between-subjects analysis found no significant main effect of condition on Aix,  $F(1, 19) = 0.01$ ,  $p = 0.91$ ,  $\eta^2 = 0.00$ .

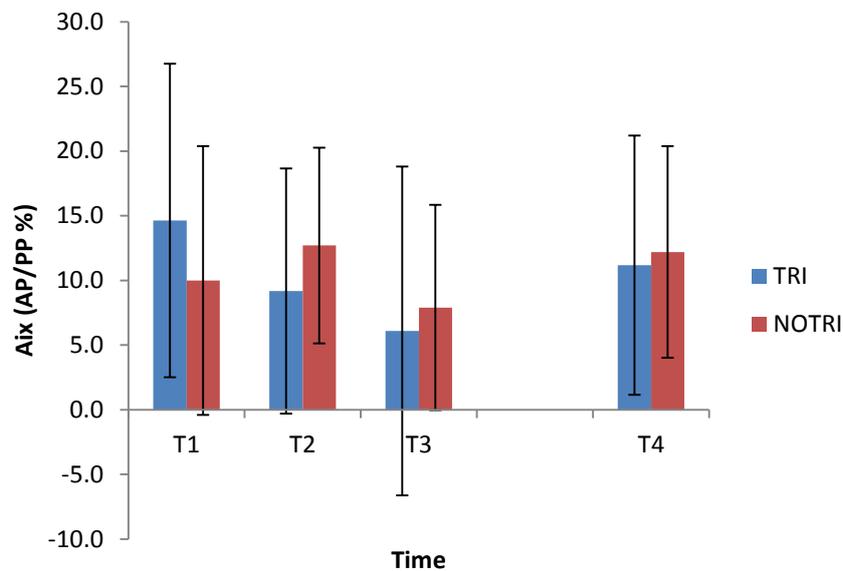


Figure 25. Comparison of augmentation index values (Aix; AP/PP) between TRI ( $n = 11$ ) and NOTRI ( $n = 10$ ) across 4 time-points (mean  $\pm$  SD,  $p > 0.05$ ).

TRI = triathlete participants; NOTRI = recreationally active participants; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event (in respect to TRI group). Time-points reflect equal variance for NOTRI group conducted at a later period.

#### 6.5.2.4 Arterial Stiffness Biomarkers – Augmentation Index Corrected for a Heart Rate of 75 $\text{beats} \cdot \text{min}^{-1}$

Analysis of within-subjects effects found no significant main time effect,  $F(3, 57) = 0.90$ ,  $p = 0.14$ ,  $\eta^2 = 0.09$ , and no significant interaction of condition and time,  $F(3, 57) = 0.168$ ,  $p = 0.917$ ,  $\eta^2 = 0.01$ . The results displayed in figure 26 suggest Aix@HR75 did not significantly differ between conditions across any time-point (T1:  $5.5 \pm 10.6\%$  vs.  $5.5 \pm 9.8\%$ ; T2:  $6.3 \pm 6.9\%$  vs.  $5.8 \pm 7.6\%$ ; T3:  $1.6 \pm 10.3\%$  vs.  $2.9 \pm 8.1\%$ ; T4:  $3.8 \pm 9.8\%$  vs.  $5.4 \pm 6.9\%$ ; respectively,  $p > 0.05$ ). A very large variation in findings is represented by the SD's in figure 26 ranging into negative values, a normal finding for low wave reflections. Similarly, between-subjects analysis found no significant differences in TRI or NOTRI conditions for Aix@HR75,  $F(1, 19) = 0.03$ ,  $p = 0.86$ ,  $\eta^2 = 0.00$ .

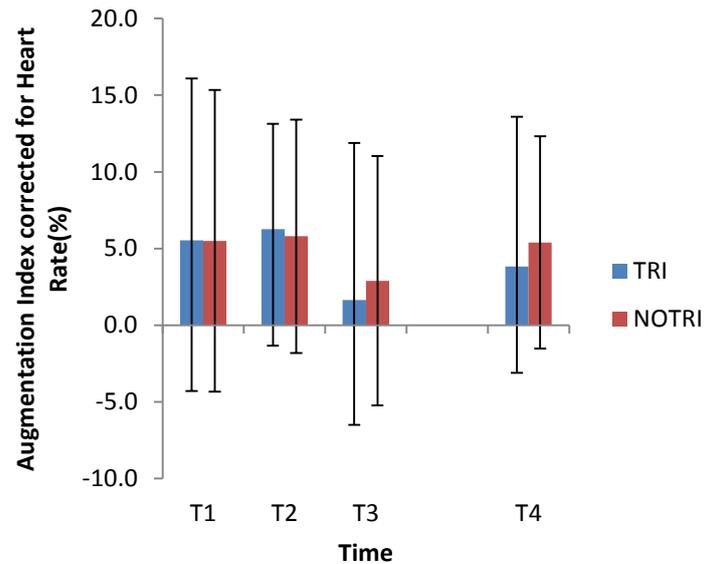


Figure 26. Comparison of augmentation index corrected for a heart rate of 75 beats·min<sup>-1</sup> (Aix@HR75) between TRI (n = 11) and NOTRI (n = 10) across 4 time-points (mean ± SD, p > 0.05).

TRI = triathlete participants; NOTRI = recreationally active participants; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event (in respect to TRI group). Time-points reflect equal variance for NOTRI group conducted at a later period.

#### 6.5.2.5 Other Cardiovascular Measurements - Resting Heart Rate

Within-subjects analysis displayed no significant main time effect, which suggests the values for rHR were in general similar across levels of time,  $F(3, 57) = 1.85$ ,  $p = 0.15$ ,  $\eta^2 = 0.09$ . An interaction effect of condition and time on HR was found to be significant,  $F(3, 57) = 10.28$ ,  $p < 0.001$ ,  $\eta^2 = 0.35$ . Bonferroni pairwise comparisons displayed a significant difference in rHR between TRI and NOTRI at T2,  $69 \pm 9$  beats·min<sup>-1</sup> vs.  $56 \pm 11$  beats·min<sup>-1</sup>,  $F(1, 19) = 6.57$ ,  $p < 0.05$ ,  $\eta^2 = 0.29$  but not at T1 ( $56 \pm 7$  vs.  $63 \pm 13$  beats·min<sup>-1</sup>,  $p = 0.13$ ), T3 ( $65 \pm 8$  vs.  $60 \pm 8$  beats·min<sup>-1</sup>,  $p = 0.2$ ) or T4 ( $58 \pm 9$  vs.  $61 \pm 10$  beats·min<sup>-1</sup>,  $p = 0.60$ ). Figure 27 presents values for rHR for TRI and NOTRI participants over the 4 time levels.

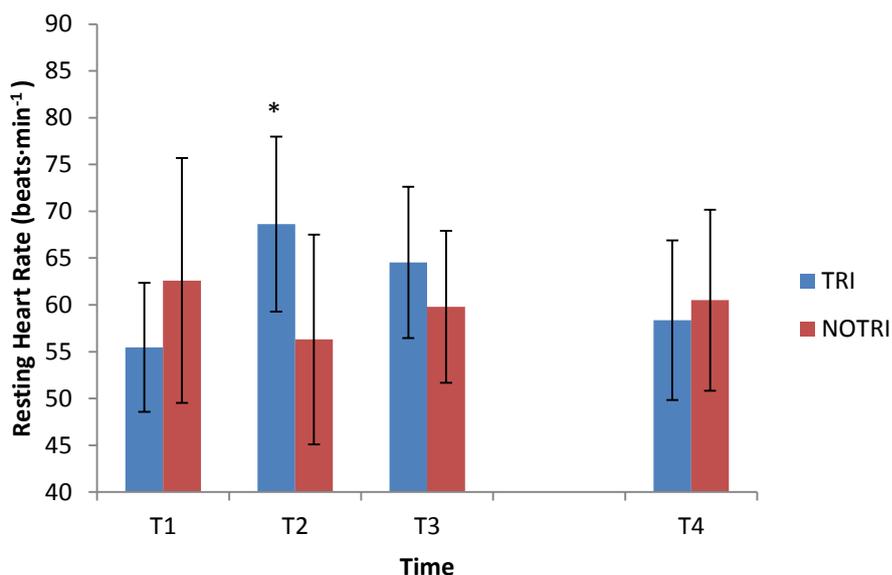


Figure 27. Comparison of resting heart rate (rHR) between TRI (n = 11) and NOTRI (n = 10) conditions across 4 time-points (mean  $\pm$  SD).

TRI = triathlete participants; NOTRI = recreationally active participants; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event (in respect to TRI group). Time-points reflect equal variance for NOTRI group conducted at a later period.

\* significance between TRI and NOTRI,  $p < 0.05$ .

#### 6.5.2.6 Other Cardiovascular Measurements - Aortic Systolic Blood Pressure

Mauchly's test indicated that the assumption of sphericity had been violated,  $\chi^2(5) = 11.43$ ,  $p = 0.04$ .

Owing to this violation, degrees of freedom were corrected using Greenhouse-Geisser correction,  $\epsilon = 0.69$ . Within-subjects analysis found no significant effect of time,  $F(2.06, 38.19) = 1.35$ ,  $p = 0.27$ ,  $\eta^2 = 0.07$ . Similarly, an interaction effect of time and condition remained statistically insignificant,  $F(2.06, 38.19) = 1.04$ ,  $p = 0.38$ ,  $\eta^2 = 0.05$ . There was no significant main effect of condition, indicating aSBP values for TRI and NOTRI participants were in general the same,  $F(1, 19) = 1.25$ ,  $p < 0.05$ ,  $\eta^2 = 0.06$  (table 10).

#### 6.5.2.7 Other Cardiovascular Measurements - Aortic Diastolic Blood Pressure

Levene's test of equality of error variances was violated at T1,  $F(1, 19) = 7.54$ ,  $p < 0.05$ , and at T2,  $F(1, 19) = 8.69$ ,  $p < 0.05$ . As a result a more conservative alpha level of 0.025 was accepted. There was no significant main effect of time on aDBP,  $F(3, 57) = 1.91$ ,  $p = 0.14$ ,  $\eta^2 = 0.09$  or interaction effect between time and condition,  $F(3, 57) = 1.22$ ,  $p = 0.31$ ,  $\eta^2 = 0.06$ . This indicates that aDBP of different levels of time did not differ according to whether participants were in the TRI or NOTRI condition (table 10). Between-subjects effects found no overall significant difference between TRI and NOTRI conditions,  $F(1, 19) = 1.33$ ,  $p > 0.05$ ,  $\eta^2 = 0.07$ , indicating aDBP was similar between athletes participating in the triathlon and recreationally active individuals.

### 6.5.2.8 Other Cardiovascular Measurements - Left Ventricular Ejection Duration

A significant main time effect was found for LVED,  $F(3, 57) = 2.82$ ,  $p < 0.05$ ,  $\eta^2 = 0.13$ , with a linear contrast,  $F(1, 19) = 10.40$ ,  $p < 0.005$ ,  $\eta^2 = 0.35$ . Additionally, within-subject analysis found a significant time and group interaction,  $F(3, 57) = 6.45$ ,  $p < 0.005$ ,  $\eta^2 = 0.25$ , indicating that LVED was affected by the different conditions across different levels of time (table 10). Simple main effects using Bonferroni adjustment for multiple comparisons was used to clarify the effect of condition. Analysis found a significant difference between TRI and NOTRI conditions at T3,  $F(1, 19) = 4.60$ ,  $p < 0.05$ ,  $\eta^2 = 0.20$ . This suggests that LVED was similar between TRI and NOTRI conditions pre-event (T1:  $316 \pm 20$  ms vs.  $301 \pm 21$  ms) and 12 to 18 hours post TRI only event (T2:  $298 \pm 21$  ms vs.  $311 \pm 21$  ms). LVED was significantly shorter for TRI compared with NOTRI at 7 days post-event (T3:  $290 \pm 17$  ms vs.  $305 \pm 17$  ms) but not at 28 days post TRI only event (T4:  $302 \pm 20$  ms vs.  $296 \pm 21$  ms). Between-subjects effects of condition was insignificant,  $F(1, 19) = 0.09$ ,  $p = 0.77$ ,  $\eta^2 = 0.01$  indicating LVED was similar between athletes participating in the triathlon and recreationally active individuals.

Table 10. Descriptive statistics of additional cardiovascular measurements between conditions.

Measurement	Time-Point	TRI (n = 11)	NOTRI (n = 10)
aSBP (mmHg)	T1 – 7d Pre	109 ± 12	103 ± 11
	T2 – 12-18h Post	103 ± 8	103 ± 11
	T3 – 7d Post	108 ± 10	102 ± 8
	T4 – 28d Post	103 ± 9	101 ± 7
aDBP (mmHg)	T1 – 7d Pre	73 ± 6	70 ± 11
	T2 – 12-18h Post	70 ± 7	67 ± 11
	T3 – 7d Post	74 ± 6	67 ± 9
	T4 – 28d Post	70 ± 7	67 ± 8
LVED (ms)	T1 – 7d Pre	302 ± 20	296 ± 21
	T2 – 12-18h Post	290 ± 17	305 ± 17
	T3 – 7d Post	*298 ± 21	311 ± 21
	T4 – 28d Post	316 ± 20	301 ± 21

TRI = triathlete participants; NOTRI = recreationally active participant; aSBP = aortic systolic blood pressure; aDBP = aortic diastolic blood pressure; LVED = left ventricular ejection duration.

\* significant difference compared with NOTRI at level of time ( $p < 0.05$ ).

### 6.5.3 Part 2: Supplementation vs. Non-supplementation

Part 2 of this study used a 4 by 2 mixed measures ANOVA with time as the within-subjects factor (4 levels) and with comparisons made between and NS and S groups (non-supplementation group and supplementation group, respectively) using TRI participants only (athletes participating in iron-distance triathlon). Arterial stiffness biomarkers, rHR, aortic BP, and LVED were inserted into the ANOVA as dependent variables.

#### 6.5.3.1 Baseline Comparisons

Table 11. shows the baseline participant characteristics between NS and S groups (NS: no supplementation and S: supplementation). Independent samples t-tests were conducted for baseline (T1) age, height and body mass between groups as reflected in table 11. Age was statistically non-significant between groups  $t(9) = -1.35$ ,  $p = 0.21$ . Likewise, no significant differences between groups were observed for height,  $t(9) = -1.07$ ,  $p = 0.31$ , or body mass,  $t(9) = 0.33$ ,  $p = 0.75$ . Additionally,  $\dot{V}O_{2max}$  values for NS and S participants were not significantly different,  $t(9) = 0.19$ ,  $p = 0.85$ , along with race performance,  $t(9) = 1.07$ ,  $p = 0.31$ .

Table 11. Baseline participant characteristics between groups.

	TRI (n = 11)		p
	NS (n = 6)	S (n = 5)	
Age (years)	31.2 ± 7.9	38.0 ± 8.9	p = 0.21
Height (cm)	172.3 ± 8.5	177.0 ± 5.1	p = 0.31
Body mass (kg)	70.8 ± 10.6	77.6 ± 9.6	p = 0.75
Relative $\dot{V}O_{2max}$ (ml·kg <sup>-1</sup> ·mins <sup>-1</sup> )	51.0 ± 7.1	50.2 ± 6.5	p = 0.85
Race Performance (dec mins)	804.7 ± 65.3	764.2 ± 59.1	p = 0.31

NS = non-supplementation group; S = supplementation group; yr = years; cm = centimetre; kg = kilogram; relative  $\dot{V}O_{2max}$  = maximal volume of oxygen uptake per kilogram of body mass per minute; dec mins = decimal minutes.

There were no significant differences observed between NS and S for urinary markers of oxidative stress and antioxidant reserve capacity pre-event (table 12). Independent samples t-tests were conducted for pre-event oxidative stress biomarkers including: urinary lipid peroxides  $t(8) = 0.02$ ,  $p = 0.98$  and 8-hydroxydeoxyguanosine (8-OHdG)  $t(8) = -0.14$ ,  $p = 0.90$ . Additionally, glutathione requirement scores were not significantly different between groups  $t(9) = -0.72$ ,  $p = 0.49$ .

Table 12. Comparison of urinary oxidative stress biomarkers and antioxidant reserve capacity obtained pre-race.

	TRI (n = 11)		p
	NS (n = 6)	S (n = 5)	
Lipid peroxides ( $\mu\text{g}\cdot\text{g creatinine}^{-1}$ ) <sup>1)</sup>	5.75 $\pm$ 1.61	5.75 $\pm$ 1.87	0.98
8-OHdG ( $\mu\text{g}\cdot\text{g creatinine}^{-1}$ )	10.83 $\pm$ 2.48	11.00 $\pm$ 1.41	0.90
Glutathione Requirement Score	2.51 $\pm$ 2.95	3.60 $\pm$ 2.07	0.49

NS = non-supplementation group; S = supplementation group; 8OHdG = 8-hydroxydeoxyguanosine. Glutathione requirement was scored from 0 to 10 for each participant.

### 6.5.3.2 Arterial Stiffness Biomarkers - Pulse Wave Velocity

The null hypothesis was tested that there would be no difference in PWV between time-points, and there would be no difference between those receiving antioxidant supplementation and those not receiving supplementation. Analysis showed no significant main time effect for PWV,  $F(3, 27) = 1.20$ ,  $p = 0.33$ ,  $\eta^2 = 0.12$ , indicating the results were in general the same across time-points (T1:  $5.9 \pm 0.6$   $\text{m}\cdot\text{s}^{-1}$ , T2:  $6.1 \pm 0.4$   $\text{m}\cdot\text{s}^{-1}$ , T3:  $6.3 \pm 0.7$   $\text{m}\cdot\text{s}^{-1}$ ,  $6.0 \pm 0.6$   $\text{m}\cdot\text{s}^{-1}$ ;  $p < 0.05$ ). Similarly, no significant interactions were observed for group across time levels for PWV values,  $F(3, 27) = 0.39$ ,  $p = 0.76$ ,  $\eta^2 = 0.04$  suggesting that values of PWV of different levels of time were similar between participants (figure 28). Between-subjects analysis displayed an insignificant difference in PWV between groups,  $F(1, 9) = 0.15$ ,  $p = 0.71$ ,  $\eta^2 = 0.02$ . Figure 29 shows the individual deviation of PWV values from pre-event (T1) to various time-points post-event. PWV remained less than  $8 \text{ m}\cdot\text{s}^{-1}$  for all participants.

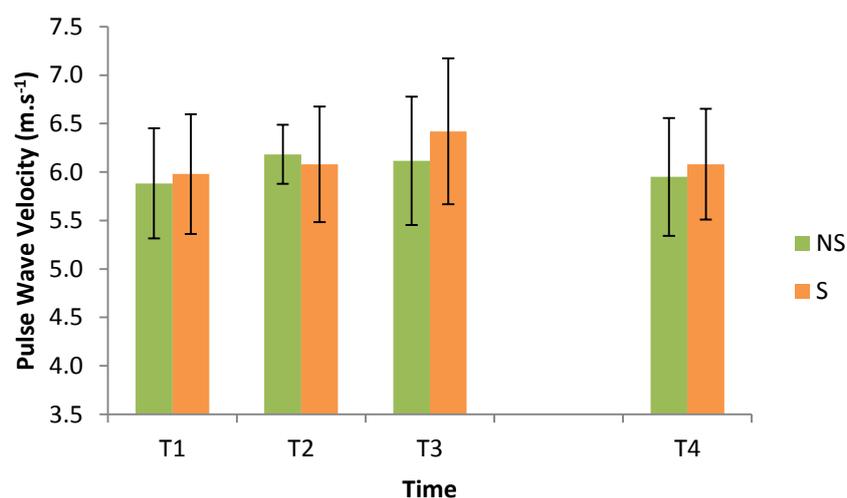


Figure 28. Comparison of pulse wave velocity (PWV) in TRI participants NS (n = 6) and S (n = 5) across 4 time-points (mean  $\pm$  SD,  $p > 0.05$ ).

NS = non-supplementation group; S = supplementation group; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event (in respect to TRI group). Time-points reflect equal variance for NOTRI group conducted at a later period.

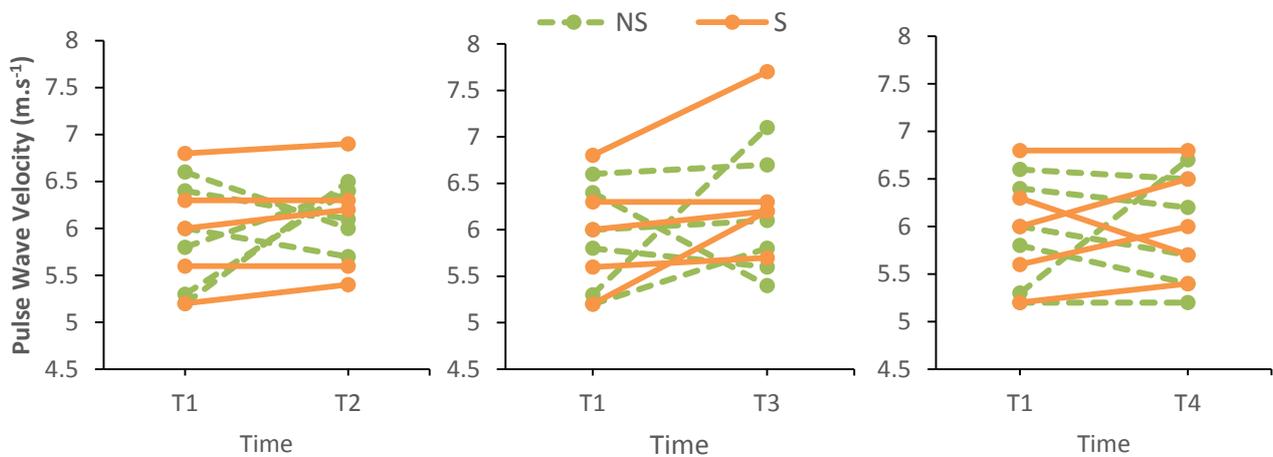


Figure 29. Change in pulse wave velocity values (PWV) for individual participants of NS ( $n = 6$ ) and S ( $n = 5$ ) compared with baseline values (mean  $\pm$  SD,  $p > 0.05$ ).

NS = non-supplementation group; S = supplementation group; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event.

### 6.5.3.3 Arterial Stiffness Biomarkers - Augmentation Index

Levene's test of equality of error variances was violated at T1,  $F(1, 9) = 5.95$ ,  $p = 0.04$  and T3,  $F(1, 9) = 7.96$ ,  $p = 0.02$  and as such, an alpha level of 0.025 was accepted. Within-subject analysis revealed a non-significant main time effect on Aix,  $F(3, 27) = 3.06$ ,  $p = 0.05$ ,  $\eta p^2 = 0.25$ . ANOVA results revealed no significant interaction effect of time and group factors,  $F(3, 27) = 1.39$ ,  $p = 0.27$ ,  $\eta p^2 = 0.13$ . Results show that Aix at different time-points were similar between groups and time alone did not have a significant effect (figure 30). Between-subject analysis found no significant difference between groups for Aix,  $F(1, 9) = 0.29$ ,  $p = 0.6$ ,  $\eta p^2 = 0.03$ . Figure 31 shows the individual deviation of Aix values from pre-event (T1) to various time-points post-event.

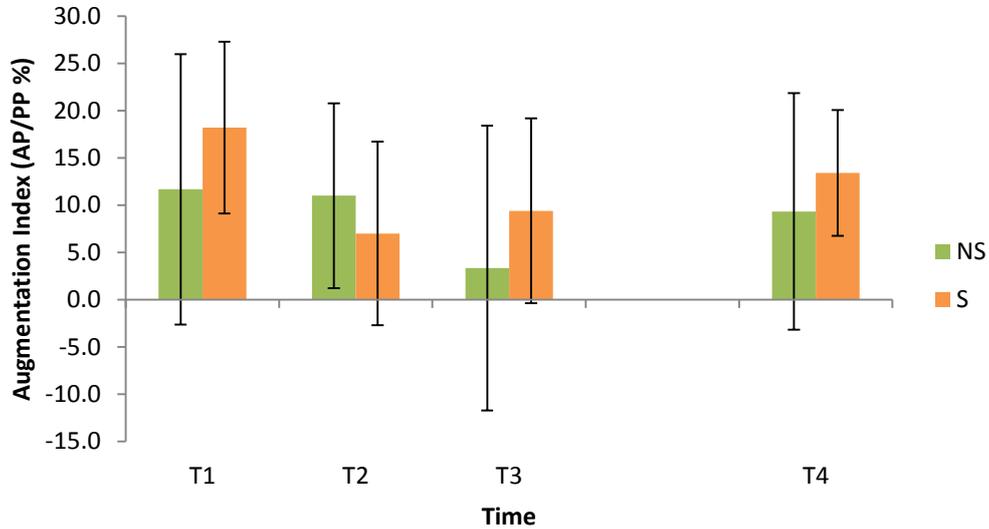


Figure 30. Comparison of augmentation index (Aix) in TRI participants NS (n = 6) and S (n = 5) across 4 time-points (mean  $\pm$  SD,  $p > 0.05$ ).

NS = non-supplementation group; S = supplementation group; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event.

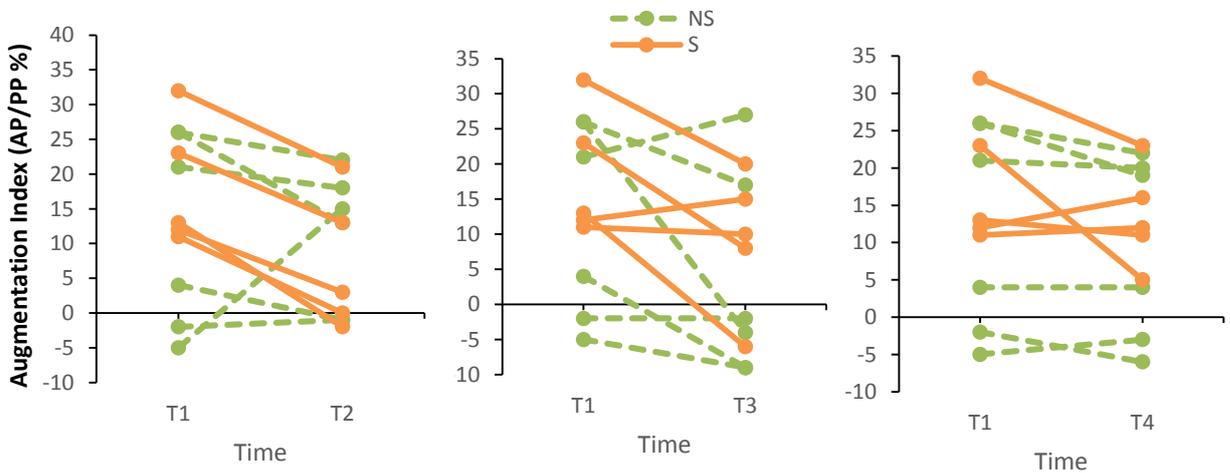


Figure 31. Change in augmentation index (Aix) for individual participants of NS (n = 6) and S (n = 5) compared with baseline values (mean  $\pm$  SD,  $p > 0.05$ ).

NS = non-supplementation group; S = supplementation group; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event.

#### 6.5.3.4 Arterial Stiffness Biomarkers - Augmentation Index Corrected for a Heart Rate of 75 beats·min<sup>-1</sup>

Levene's test was significant for Aix@HR75 at T1, ( $F(1, 9) = 15.24, p = 0.004$ ), T3 ( $F(1, 9) = 5.75, p = 0.04$ ) and T4 ( $F(1, 9) = 14.8, p = 0.004$ ). This finding suggests there was a violation to the assumption of equality of error variances and therefore, a more conservative alpha level of 0.005 was accepted. Within-subjects analysis established no significant main time effect on Aix@HR75,  $F(3, 27) = 1.80, p = 0.17, \eta^2 = 0.17$ . The time and group interaction effect was non-significant,  $F(3, 27) = 0.58, p = 0.06, \eta^2 = 0.06$  indicating that Aix@HR75 did not differ between S and NS groups at different time levels before and after the triathlon as shown in figure 32. Between-subjects analysis found no significant differences in group for Aix@HR75,  $F(1, 9) = 0.21, p = 0.67, \eta^2 = 0.02$ .

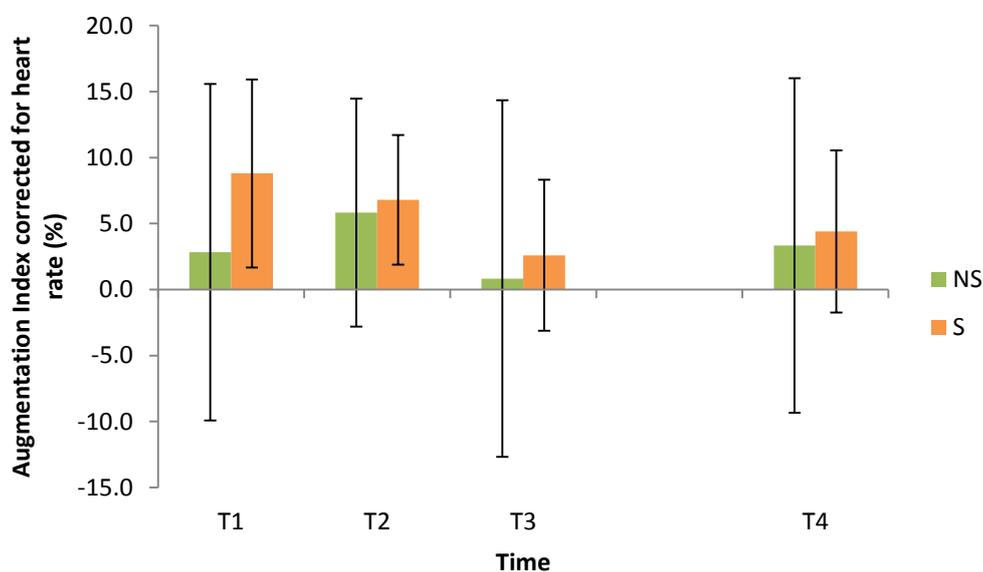


Figure 32. Comparison of augmentation index corrected for heart rate (Aix@HR75) in TRI participants NS ( $n = 6$ ) and S ( $n = 5$ ) across 4 time-points (mean  $\pm$  SD,  $p > 0.05$ ).

NS = non-supplementation group; S = supplementation group; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event.

#### 6.5.3.5 Other Cardiovascular Measurements - Resting Heart Rate

Levene's test of equality of error variances was violated at T2,  $F(1, 9) = 5.86, p = 0.04$ . Due to this violation, an alpha level of 0.025 was accepted for between groups analysis with time level T2. Analysis of within-subjects tests revealed a significant main effect of time on rHR,  $F(3, 27) = 11.39, p < 0.001, \eta^2 = 0.56$ . Pairwise comparisons identified significant differences between T1 and T2, ( $55.5 \pm 6.9$  beats·min<sup>-1</sup> vs.  $68.6 \pm 9.4$  beats·min<sup>-1</sup> respectively, 19% increase,  $p < 0.001$ ) and T2 and T4 ( $58.4 \pm 8.5$  beats·min<sup>-1</sup>, 18% reduction,  $p < 0.005$ ) as presented in figure 33. In addition, differences between T1

and T3 ( $64.6 \pm 8.1$  beats·min<sup>-1</sup>) approached significance with an elevation of 14%,  $p = 0.06$ . A significant time and group interaction existed,  $F(3, 27) = 4.12$ ,  $p < 0.05$ ,  $\eta^2 = 0.31$ . This would suggest that rHR values for S and NS interacted differently after the triathlon event. Bonferroni pairwise comparisons did not find a significant difference between groups among time levels however values for rHR approached significance at T2;  $64 \pm 5$  vs.  $74 \pm 11$ ; TRI and NOTRI respectively,  $p = 0.06$ ). Between-subjects analysis found no significance between groups,  $F(1, 9) = 0.06$ ,  $p = 0.94$ ,  $\eta^2 = 0.001$ .

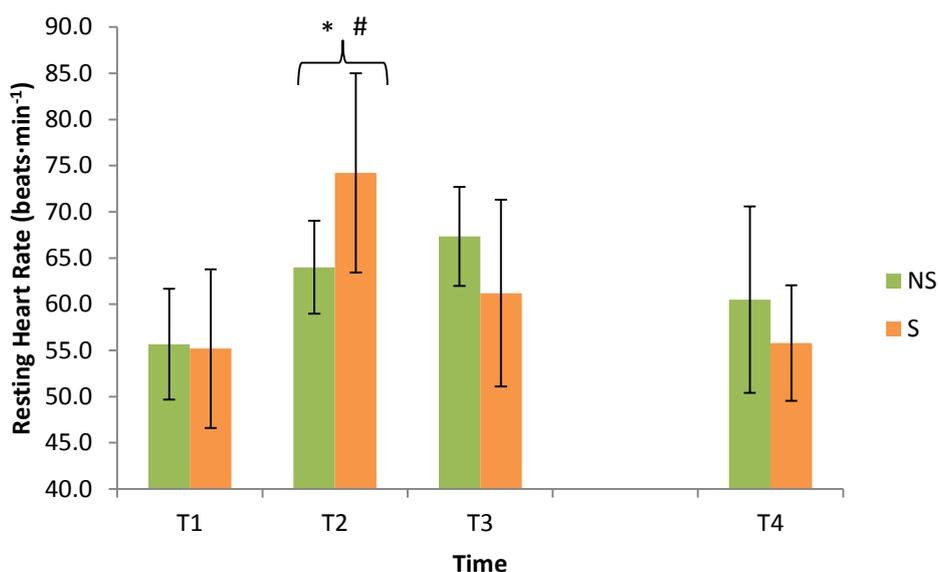


Figure 33. Comparison of resting heart rate (rHR) in TRI participants NS ( $n = 6$ ) and S ( $n = 5$ ) across 4 time-points (mean  $\pm$  SD).

NS = non-supplementation group; S = supplementation group; T1 = 7 days pre-event; T2 = 12-18 hours post-event; T3 = 7 days post-event; T4 = 28 days post-event.

\* significantly different from T1,  $p < 0.001$ .

# significantly different from T4,  $p < 0.005$ .

#### 6.5.3.6 Other Cardiovascular Measurements - Aortic Systolic Blood Pressure

There was a significant main effect of time on aSBP,  $F(3, 27) = 3.43$ ,  $p < 0.05$ ,  $\eta^2 = 0.28$ . After adjustment for multiple comparisons, Bonferroni pairwise analysis did not find significance between time-points,  $p > 0.05$ . There was no significant main effect of group,  $F(1, 9) = 0.03$ ,  $p = 0.87$ ,  $\eta^2 = 0.003$  indicating that values of aSBP were affected by triathlon performance as indicated by time levels but not enough to be significant (table 13). Additionally, supplementation did not have an influence on aSBP across levels of time.

### 6.5.3.7 Other Cardiovascular Measurements - Aortic Diastolic Blood Pressure

Levene's test of equality of error variances was violated at T1,  $F(1, 9) = 5.86$ ,  $p = 0.04$  and as such, an alpha level of 0.025 was accepted for between groups analysis. Within-subjects analysis found no significant main effect of time, although the F statistic approached significance,  $F(3, 27) = 2.738$ ,  $p = 0.06$ ,  $\eta^2 = 0.23$ . Similarly, the time and group interaction for aDBP also approached significance,  $F(3, 27) = 2.93$ ,  $p = 0.05$ ,  $\eta^2 = 0.25$ . There was no significant main effect of group,  $F(1, 9) = 0.09$ ,  $p = 0.77$ ,  $\eta^2 = 0.10$ , indicating supplementation did not significantly affect aDBP through time levels (table 13).

### 6.5.3.8 Other Cardiovascular Measurements - Left Ventricular Ejection Duration

There was a significant main effect of time on ED,  $F(3, 27) = 5.21$ ,  $p < 0.05$ ,  $\eta^2 = 0.37$ . Bonferroni pairwise comparisons did not reveal specific differences between levels of time after adjustment for multiple comparisons. There was no significant interaction effect of time and the groups on ED, indicating values from NS and S participants were in general the same,  $F(3, 27) = 1.17$ ,  $p = 0.34$ ,  $\eta^2 = 0.12$  (table 13).

Table 13. Descriptive statistics of additional cardiovascular measurements between groups.

Measurement	Time-Point	Triathletes		
		NS (n = 6)	S (n = 5)	Overall (n = 11)
aSBP (mmHg)	T1 – 7d Pre	110 ± 12	108 ± 12	109 ± 12
	T2 – 12-18h Post	105 ± 6	100 ± 10	103 ± 8
	T3 – 7d Post	106 ± 10	110 ± 11	108 ± 10
	T4 – 28d Post	101 ± 10	107 ± 9	103 ± 9
aDBP (mmHg)	T1 – 7d Pre	72 ± 4	74 ± 8	73 ± 6
	T2 – 12-18h Post	73 ± 7	67 ± 6	70 ± 7
	T3 – 7d Post	75 ± 6	73 ± 5	74 ± 6
	T4 – 28d Post	69 ± 9	71 ± 5	70 ± 7
LVED (ms)	T1 – 7d Pre	322 ± 15	308 ± 24	302 ± 20
	T2 – 12-18h Post	309 ± 18	284 ± 16	290 ± 17
	T3 – 7d Post	291 ± 22	287 ± 10	298 ± 21
	T4 – 28d Post	304 ± 19	301 ± 23	316 ± 20

NS = non-supplementation group; S = supplementation group; aSBP = aortic systolic blood pressure; aDBP = aortic diastolic blood pressure; LVED = left ventricular ejection duration.

## 6.6 Discussion

### 6.6.1 Arterial Stiffness in Endurance Trained and Recreationally Active Individuals

A major finding in this investigation was that indices of arterial stiffness were similar at baseline between endurance-trained and recreationally active participants. There was no difference in PWV

between ultra-endurance trained and recreationally active controls despite significant differences in the confounding factor, rHR. This finding contributes to the limited span of literature concerning ultra-endurance athletes and arterial health. Previous investigations have observed differences in large artery compliance between ultra-endurance athletes and recreationally active individuals. Burr et al., (2013) observed a significant reduction in large artery compliance, but non-significant differences between small artery compliance in habitual ultra-marathon runners, compared with age-matched recreationally active controls. Similar results were observed in marathon runners with different measurements; Vlachopoulos et al. (2010) observed no difference in Aix, and a significantly elevated PWV in marathoners;  $0.56 \text{ m}\cdot\text{s}^{-1}$  above recreationally active controls. On the other hand, Radtke et al. (2013) found comparable results to the present study, whereby no significant differences in brachial-ankle PWV were observed between recreational, marathon, and ultra-endurance athletes.

Methodological issues exist in deriving aortic stiffness from pulse waves of peripheral limbs due to the inherent contribution of peripheral arteries in the estimation (Laurent et al., 2006; Rowley et al., 2011). Data from our study comply with others finding a non-existent difference in peripheral artery compliance, assessed in this study by wave reflections, between habitual endurance athletes and recreationally active individuals (Burr et al., 2014; Knez et al., 2008; Radtke et al., 2013; Vlachopoulos et al., 2010). The current findings give evidence that 9 months of ultra-endurance training does not provide significantly different arterial stiffness differences from recreationally active individuals. This would indicate that contrary to epidemiological and previous longitudinal studies, ultra-endurance training is not a stimulus for unfavourable adaptation of the CV system, specifically arterial compliance (O'Keefe et al., 2012; Schwartz et al., 2014).

### **6.6.2 Cardiovascular factors affecting Arterial Stiffness Measurements**

In agreement with other studies in the area, the current study found that SBP and DBP did not differ between ultra-endurance trained and physically active controls (Burr et al., 2014). However controversy exists as to whether excessive endurance training may elevate SBP (O'Keefe et al., 2012; Vlachopoulos et al., 2012). Additionally, in the current study rHR and LVED were not significantly different between ultra-endurance trained and recreationally active controls at baseline. Therefore, similarities in arterial stiffness measurements between conditions remained unaffected by other CV factors such as CBP, rHR and LVED.

### **6.6.3 Arterial Stiffness in Response to an Ultra-Endurance Exercise Bout**

Another important finding in this investigation relates to changes in arterial stiffness in response to ultra-endurance exercise. Trained participants competing in an iron-distance triathlon had

significantly greater arterial stiffness than recreationally active individuals in the short period following the race, which did not exist prior to, or 28 days post-event. The finding of differences in PWV, but not Aix or Aix@HR75 in this short period, indicates that those who participated in the exercise were subjected to a low level increase in regional large and medium artery stiffening. Nevertheless, it is necessary to note that the triathletes experienced almost a 3-fold decrease in mean Aix values 7 days post-event, which was not equivalent in the control group. Importantly PWV did not exceed the European Society of Hypertension's clinical threshold value of  $12 \text{ m}\cdot\text{s}^{-1}$  for CV mortality risk in any individuals of this study (Mancia et al., 2009). However, according to current criteria, no participants presented a serious CV risk, although triathletes may have been exposed to an increased risk of CV events in the short period after ultra-endurance exercise.

Differential responses in PWV and Aix likely originate in the methodological calculation of arterial stiffness. Aix is derived from wave reflections at the radial artery which is heavily influenced by peripheral arteries and arterioles, whereas PWV gives a regional stiffness of the aortic-femoral tract (Laurent et al., 2006). There are also differential physiological responses to exercise in peripheral arteries and large to middle sized arteries. Peripheral arteries are likely to be more heavily influenced by nitric oxide (NO) production than central arteries (Joannides et al., 1995). However, Sugawara et al. (2004) found that nitric oxide synthase (NOS) inhibition has no effect on the decrease of middle sized arterial stiffness post-exercise.

The augmented PWV, indicative of large arterial stiffening, observed between triathletes and recreationally active conditions was not great enough effect to elicit a significant increase between time-points when the triathlon group were treated separately. Indeed, the maximal PWV increase of  $0.4 \text{ m}\cdot\text{s}^{-1}$  for triathletes was observed between 7 days pre-event and 7 days post-event. However, pilot testing (chapter 5.2) revealed that differences up to  $0.7 \text{ m}\cdot\text{s}^{-1}$  between 2 testing sessions can be expected. Significant alterations have been found recently by Burr et al., (2012) who identified a reduction large artery compliance, indicative of an augmented arterial stiffening in response to an ultra-endurance footrace. Recently, only 40 minutes of downhill running has been found to induce delayed elevations to PWV (and muscle soreness) which peaked 2 days post-exercise, and remained elevated 3 days post-exercise (Burr Boulter & Beck, in press). It is not unusual for long-distance triathlons to contain substantial components of eccentric exercise, as with downhill running. Eccentric exercise can elicit a delayed onset of muscular soreness (DOMS) which has previously been associated with similar delayed increases in PWV and inflammatory markers (Barnes, Trombold, Dhindsa, Lin & Tanaka, 2010). Lack of change in wave reflections, derived from the radial artery, pre and post-exercise is not an uncommon finding in response to endurance exercise, and may even be reduced post-event (Burr et al., 2012; Burr et al., in press; Vlachopoulos et al., 2010). Previous investigations have

observed significant reductions in Aix immediately post-endurance exercise from  $12.2 \pm 12.5\%$  to  $-5.8 \pm 11.1\%$ , with large and similar standard deviations to the present study (Vlachopoulos et al., 2010). Arterial stiffness and therefore CV risk remained unaffected by ultra-endurance exercise in this study.

The current study is the first, to our knowledge, to investigate arterial stiffness further than 1 day of ultra-endurance exercise, and indicates that an excessive exercise bout does not cause long-term alterations in arterial stiffness. An insignificant low level elevation in large artery stiffness was observed which was largest 7 days post-exercise than 1 day and 28 days post-exercise. Therefore, this study implies there is no serious elevations to CV risk after ultra-endurance exercise. However, differences in markers of artery stiffness between conditions warrants further study with more participants and more frequent testing appointments.

#### **6.6.4 Cardiovascular factors affecting Arterial Stiffness Measurements**

Functional CV variables were assessed concomitantly with arterial stiffness measurements to gain an understanding of mechanisms and identify factors influencing arterial stiffness measurements. Central SBP and DBP did not differ between any time-points within the triathletes, indicating no long-term changes in BP. However, a reduced SBP may have been observed in the immediate period post-exercise if measurements were obtained earlier, as identified by Halliwill et al. (2013) and Vlachopoulos et al. (2010). Additionally, rHR was elevated only at 1 day post-event (13 beats·min<sup>-1</sup> increase from baseline), though remained elevated 7 days later (9 beats·min<sup>-1</sup> increase from baseline). This may be partially explained by a sustained cardiac baroreflex resetting from prolonged exercise, complex central nervous system (CNS) patterns, and a compensatory response for metabolic requirements (Hart et al., 2010; Laforgia, Withers & Gore, 2006; Raine et al., 2001; Sala-Mercado et al., 2006). LVED was not significantly different between time-points. No significant differences were observed in arterial stiffness between time-points, though elevations in rHR may be at least partially responsible for slight changes in arterial stiffness measures. The combination of rHR being different between triathletes and controls 1 day post-event, and dissimilar LVED 7 days post-event, could potentially explain the differences in PWV between conditions at these time-points. Changes to systolic ejection rate and duration influence the extent of vessel recoil during diastole, affecting the level of arterial compliance (Salvi et al., 2013). Since significant alterations in arterial stiffness were not observed within triathletes, cardiac function may predominantly be affected in the short period post-ultra-endurance exercise, reducing the pressure within the arteries.

#### **6.6.5 Potential Mechanisms of Action for Arterial Stiffness Response to Ultra-Endurance Exercise**

Possible explanations for increased large artery stiffness due to a bout of excessive exercise include functional and structural mechanisms. It is conceivable that the prolonged rather than cyclic

inflammatory responses to excessive exercise may stimulate detrimental structural remodelling, contributing to long-term arterial stiffness. Sustained shear stress and oxidative stress induced by prolonged endurance exercise may induce fibrotic changes, decrease arterial wall elasticity, and promote pro-atherogenic phenotypes and plaque accumulation (Laughlin, Newcomer & Bender, 2008; O'Keefe et al., 2012; Schwartz et al., 2014). These harmful changes are associated with an increased risk of CV events and SCD, therefore it is important to understand whether ultra-endurance athletes are predisposed to such conditions (O'Keefe et al., 2012). Conversely, transient responses of arterial stiffness are likely mediated by functional alterations such as impaired endothelial function and adjusted sympathetic vasoconstrictor tone (Dawson et al., 2008; Failla et al., 1999; Sinder, Delp, Reyes, Wu & Muller-Delp, 2009). Elevated RONS production and oxidative stress caused by increasing metabolic reactions may induce endothelial dysfunction. Likely mechanisms responsible include reduced NO bioavailability due to RONS production, such as superoxide ( $O_2^-$ ) and subsequent peroxynitrite (ONOO<sup>-</sup>), as well as the reduction of NO cofactor tetrahydrobiopterin (BH<sub>4</sub>) bioavailability (Sinder, Delp, Reyes, Wu & Muller-Delp, 2009). Sympathetic tone is elevated with exercise which is in part as a response of the metaboreflex, which increases cardiac output and the release of vasoconstrictive substances, mediating blood flow distribution (Boushel, 2010; Davies et al., 2007; Sala-Mercado et al., 2006). Sustained arterial and cardiac catecholamine exposure such as norepinephrine as well as increased sympathetic tone likely influences arterial stiffness by increasing HR, resulting in overall shortening of the cardiac cycle. Vessel recoil may then be shortened during diastole therefore restricting the extent of arterial compliance (Heffernan, et al., 2013; Salvi et al., 2013).

It was originally speculated that participants in the current study, with no previous ultra-endurance exposure, would present amplified findings of arterial stiffness post-event compared with other studies using experienced ultra-endurance athletes. This reasoning was formed due to the enhanced inflammatory response and oxidative stress protection observed in those habituated to endurance exercise (Bloomer et al., 2006; Jówko et al., 2011); though the training period alone may be adequate to elicit similar adaptations. Additionally the basis was formed on the assumption that multiple previous exposure to ultra-endurance exercise bouts would have elicited most structural and functional adaptations, specifically the CV system, resulting in a lessened response (Neilan, Jannuzi et al., 2006; Sahlén et al., 2009). However, the opposite may also hold true, in that repeated exposure may result in an elevated vulnerability for detrimental vascular effects. Excessive exercise such as several ultra-endurance bouts may accelerate atherosclerosis (Knez et al., 2008; Mastaloudis et al., 2001). Shear stress induced arterial remodelling, in the presence of atherosclerosis may become uncontrolled and contribute to plaque vulnerability (Schwartz et al., 2014; Silver & Vita, 2006). This

would result in a greater arterial stiffening response than would potentially be seen in a healthy vessel, without atherosclerotic plaque accumulation. The lack of arterial stiffening response post-event in the current study provides evidence against our postulation. Further investigation is warranted to compare a single excessive exercise bout in those with and without previous exposure to ultra-endurance exercise

#### **6.6.6 Supplementation in Response to an Ultra-Endurance Exercise Event**

The present study indicates that ingestion of multiple supplements with antioxidant and anti-inflammatory properties prior to ultra-endurance exercise did not influence markers of arterial stiffness. Urinary biomarkers of oxidative stress and glutathione obtained pre-event were not influenced by a 12 week supplementation with multiple exogenous antioxidants. It was suggested that increasing exogenous antioxidant availability would increase the endogenous antioxidant reserve (measured in this study as urinary glutathione levels), and reduce oxidative stress experienced during the event (Bloomer, Goldfarb & McKenzie, 2006; Mastaloudis et al., 2004). In turn, it was hypothesised that this would provide a protective mechanism to the endothelial cells of the vasculature. Supplementation did not attenuate or augment markers of arterial stiffness before or after the event. This finding could be a result of inadequate oxidative stress however previous literature has provided significant associations between ultra-endurance and increased RONS production (Neubauer, Konig & Wagner, 2008; Knez, Jenkins & Coombes, 2007). Our study implies that antioxidant supplementation is unnecessary and redundant in protecting exercise-induced arterial stiffness, largely due to the absence of arterial stiffening within all triathletes. Furthermore the lack of differences in SBP, DBP, rHR or LVED between intervention groups throughout the study supports the absence of CV effect of the supplementation strategy employed. Future implications on exercise-induced arterial stiffness of whole food antioxidant strategies with polyphenols should be investigated further.

#### **6.6.7 Methodological Issues and Future Research**

Due to the heterogeneity of the sample and lack of current understanding regarding the changes in arterial stiffness following prolonged endurance exercise, this study aimed to provide an insight for more substantial subsequent investigations. Previous research has found that white European men had an Aix on average 7.8% lower than women (Wojchiechowska et al., 2006). However, a possible differential effect of sex on the results of the current study could not be inferred due to the disparity in distribution of male and female participants. Additionally, the large scale study by Wojchiechowska et al. (2006) identified a non-linear increase in Aix with age. This suggests samples with wide age dispersions, as adopted in the current study, may have very different values. Furthermore exercise-induced adaptations have been shown to differ among young and older individuals. Peripheral arteries are suggested to be more affected in younger individuals, whereas larger elastic arteries are involved

in older individuals (McDonnell et al., 2013). Unfortunately, the sample size in the current study was not large enough to offer a substantial insight into this differentiation. A more homogenous sample would have added specificity to the study but would not have reflected the whole population.

According to the recent expert document by Van Bortel et al. (2012) regarding cfPWV, it is advised that investigators should take the mean of 2 measurements in a single session. A third measurement should be obtained if PWV differs by greater than  $0.5\text{m}\cdot\text{s}^{-1}$ , whereby the investigator should then use take the median value to avoid outliers. This procedure was not conducted in the present study which followed procedures for a single measurement within quality control parameters. Following the recent consensus would have resulted in more precise measurements and would have reduced the risk of investigator errors/type I errors. Furthermore, the SphygmoCor equipment is an apparatus predominantly used in the clinical environment, mainly with already diseased individuals including renal disease, hypertension, hyperlipidaemia, and hypercholesterolemia (Mattace-Raso et al., 2006; Pauca et al., 2001; Wilkinson et al., 2010). Its use in the athletic population has not currently been validated, and its reproducibility in said individuals has not been investigated.

Another limitation of the present study was that hydration status and plasma volume were not assessed. These factors have been found to influence cardiac efficiency, stroke volume, and subsequent BP and arterial stiffness indices (Di Iorio, Nazzaro, Cucciniello & Bellizzi, 2010). Nevertheless, it is likely that the time for recovery before measurements were taken allowed adequate replenishment of hydration. In addition, obtaining measures of oxidative stress post-exercise and recruiting larger numbers of participants would contribute to the understanding of underlying mechanisms with the use of correlational analysis.

Studies in this area of research would benefit from knowledge of nutritional and training practices, as well as further assessment of race information. Nutrition intake prior to, during and post-race would impact performance, and recovery. Specifically caffeine, glucose, alcohol, and salt consumption have been associated with changes in arterial stiffness and wave reflections, these food stuffs were not considered (Draaijer et al., 1993; Papaionnou, Karatzi, Papamichael & Lekakis, 2005; Pase Grima & Sarris, 2010; van den Elzen et al., 2005; Zieman, Melenovsky & Kass, 2005). Additionally, impaired glucose tolerance was not assessed which enhances the production of AGEs, altering the mechanical properties of arterial stiffness (Henry et al., 2003). Though the current study observed no significant differences in race duration, race intensity was not considered. Presumably increasing exercise intensity would influence measures of arterial stiffness (Sharman et al., 2005), which warrants further research. Furthermore, although triathletes followed the same training protocols prior to the race, training practices post-event were undetermined which should be controlled for in future research. It

is also possible that triathletes made notable lifestyle changes in the period post-event which could interact with arterial stiffness measurements, therefore future studies should attempt to control for this factor.

The influence of alcohol on parameters measured in this study was not entirely controlled for. Light to moderate levels of alcohol consumption provides a cardioprotective effect, however irregular consumption of large volumes (binge drinking) elicits harmful influences to the CV system and arterial stiffness (Beilin, 2002; Murray et al., 2002). Therefore, it was urged for participants in this study to refrain from alcohol ingestion prior to measurements. However, it is more than likely that at least some triathletes felt the need to become intoxicated post-event due to the sense of enormity of achievement. Hence post-event measurements may be unfairly influenced.

Non-invasive measurements of arterial stiffness, as used in this study, are calculated using models of the circulation. Therefore, such methods increase the possibility for estimation errors. However, invasive procedures are far more unfeasible in field-based studies given the methodological challenges for example the cost and location of equipment. Additionally, during pulse wave analysis the calculation of  $Aix@HR75$  is corrected for HR based on relatively little evidence (Wilkinson et al., 2000; Wilkinson et al., 2002), despite LVED better predicting changes in the gold standard measurement PWV (Salvi et al., 2013).

Future investigations should examine the dose response of exercise to arterial stiffness indices and equally to investigate the associated time response to different doses and intensities of exercise. Furthermore, examining arterial stiffness measurements prior to involvement in ultra-endurance exercise, and after consecutive annual time-points would provide more valuable evidence for the exercise dose debate. This could have been achieved in the present study by attaining arterial stiffness measurements at the beginning of the training programme (month 0 rather than month 9), and comparing changes to a control population of recreational or sedentary controls; both of which populations should be considered. In addition, the accumulative effects of prolonged endurance exercise should be assessed in relation to vascular health. Multi-day ultra-endurance events provide an opportunity to investigate this, with potentially inadequate recovery periods in comparison to the present study.

## 6.7 Conclusion

In light of recent cross sectional and epidemiological investigations, a longitudinal study was deemed necessary to assess the causal relationship between ultra-endurance event participation and the health of the vascular system. The evidence from this preliminary study suggest an iron-distance triathlon does not cause long-term negative impact to the arteries in non-elite athletes. Additionally, antioxidant and anti-inflammatory supplementation did not influence arterial stiffness.

This pilot study aimed to provide insight to the CV response to ultra-endurance training and an ultra-endurance event, with specific emphasis on arterial stiffness. A supplementary aim was to determine whether antioxidant supplementation influenced the arterial stiffening response to this prolonged exercise. No differences were observed regarding arterial stiffness indices between endurance trained and recreationally active individuals prior to ultra-endurance exercise exposure. The preliminary results, however, showed a delayed central arterial stiffening may occur one day and one week following a single day ultra-endurance event. Importantly, all measurements were found to be similar one month post-event; implying only a transient exercise-induced elevation to arterial stiffness. The mechanisms involved in this response likely include a combination of both functional and structural pathways which require further elucidation. It is possible that the more immediate arterial stiffening post-exercise is associated with the ventricular dysfunction observed in some individuals, in particular, the vulnerability of the right ventricle to dysfunction post-exercise due to the contribution of increased afterload. The effect of the change was very small and remained far clear of clinical cut-off values and so is unlikely to contribute significantly to an increased CV risk for the general population. It should be noted, that when the triathlon group were treated separately no significant differences were observed between time-points. Additionally, 12-weeks of prior antioxidant and anti-inflammatory supplementation provides no additive protection against changes in arterial stiffening above natural strategies. Accordingly, contrary to current speculation, this study provides evidence to suggest there is no causal relationship between a single-day ultra-endurance event and long-term arterial stiffening. For individuals rarely participating in such events, this evidence is reassuring and contradicts several cross-sectional analyses of myocardial scarring and atherosclerotic plaque accumulation in the endurance athlete. This novel study is the first to our knowledge to investigate arterial stiffness in individuals participating in their first ultra-endurance event.

The finding of short-term arterial stiffness post-event gives warrant to future studies to investigate the accumulative influence of prolonged exercise. Multi-day ultra-endurance events could highlight this and so should be identified as a focus for further investigation, although the lower intensity of such events is likely to attenuate this response. Additionally, the intensive training during the period

leading to an ultra-endurance event, should not be ignored in future studies, particularly in those with CV risk factors.

**Key points:**

- No difference in arterial stiffness between ultra-endurance trained and recreationally active individuals at baseline.
- The increase in arterial stiffness observed in the short period after ultra-endurance exercise likely returns to baseline within 1 month after the event.
- Antioxidant supplementation is unnecessary and redundant in protecting exercise-induced arterial stiffness, largely due to the absence of arterial stiffening within all triathletes.
- Further research should investigate the potential accumulative influence of prolonged exercise, for example, multi-day ultra-endurance events as well as the intensive training period leading to the event.

## **Chapter Seven: General Conclusion**

## 7.1 General Conclusion

The health benefits of exercise to the CV system are proven, whereas the potential risks of exercise are theoretical. As such, no clear link exists between “excessive” exercise and CV damage. Nonetheless, evidence from cross-sectional studies suggest there is a potentially damaging effect of long term ultra-endurance participation. The lack of longitudinal investigation, however, regarding the physiological adaptations occurring from recreational activity to ultra-endurance participation limit these findings. Accordingly, the aim of this study was to examine the longitudinal cardiovascular-related physiological changes over an incremental training period in recreationally active individuals. Additionally, it was aimed to investigate the difference in arterial stiffness in relation to athletic status. Finally, this was the first study to examine arterial stiffness after an iron-distance triathlon in individuals previously unaccustomed to ultra-endurance exercise.

Nine months of endurance training was a sufficient training period to allow recreationally active individuals to complete an ironman-distance triathlon. Normal changes over 6 months of triathlon-specific endurance training included enhancements to cardiorespiratory fitness and other training-related parameters. Although much is known of the athlete’s heart, few studies have highlighted the change regarding the electrical patterns of the heart in adaptation to an intensive training programme, particularly in those previously unaccustomed to such exercise. The current study provides information of such changes with reference to time-frames. The frequency of sinus bradycardia findings increased most over the 6 month period relative to other criteria, followed by isolated voltage criteria for LVH and early repolarisation. High volumes of endurance training induced ‘abnormal’ ECG changes in a minority of individuals, as well as the more recognised training-related changes of the ‘athlete’s heart’. It is important to ascertain whether these findings occur due to an accumulative pathophysiological cascade of events or, more likely, whether they occur secondary to ongoing adaptations of the athlete’s heart. Understanding this phenomenon will provide useful monitoring information for new ultra-endurance athletes and contribute to the excessive exercise dose debate. Given that the extent of cardiac adaptations is strongly associated with training hours and fitness it can be expected that those participating in ultra-endurance exercise are those most likely to show an overlap in training-related ECG changes and those associated with pathology. Importantly, in the current study, relatively few individuals displayed abnormal ECG characteristics, despite participating in an intensive long-term training programme.

Preliminary evidence for transiently increased arterial stiffness was found post iron-distance triathlon. However, no evidence was found to expect long-term changes in arterial stiffness post-event. Additionally antioxidant supplementation prior to the event was unnecessary in protecting against exercise-induced arterial stiffness, but likely provides benefits to other systems. It is unlikely that the

extent of arterial stiffening after prolonged exercise would place individuals at high risk of CV event, however it does give an indication of the direction of change. Moreover, the evidence displayed in the current study is reassuring for current ultra-endurance athletes and prospective athletes alike due to the lack of long-term arterial stiffness changes due to such events with adequate recovery.

In conclusion, this study demonstrates that in previously recreationally active individuals undertaking intensive exercise training, relatively few individuals undergo cardiac remodelling resulting in abnormal electrical conduction similar to pathological disease. This abnormal remodelling may relate to a pathological condition or more likely, normal extensive remodelling of the athlete's heart. Furthermore, it is suggested that first participation in an iron-distance triathlon results in no long-term arterial stiffening. The safety of individuals participating in sport is paramount, and although most ultra-endurance athletes are not competing to improve health per se, knowledge of the risks involved should be provided for all athletes. The beneficial impact of exercise on various physiological and psychological systems of the human body are proven. The potentially unfavourable risks of exercise on cardiovascular health remain predominantly theoretical and limited to those with pre-existing conditions, yet require further research. It is understood that the potential risks involved with exercise would affect a minority of individuals, even to the extent of ultra-endurance, and is likely accountable to genetics, illness, underlying risk factors, drugs and training/ recovery mismatch. For these reasons, exercise should be promoted for all until significant evidence provides information to disagree with current consensus. Individuals are encouraged to listen to the body's stress signals and not engage in strenuous activity during these periods, whilst those with CV risk factors or involved with drugs should consult a specialist to undergo both pre-participation screening and monitoring.

## Reference List

- Achten, J., Venables, M. C. & Jeukendrup, A. E. (2003). Fat oxidation rates are higher during running compared with cycling over a wide range of intensities. *Metabolism*, 52(6), 747-752.
- Albano, A. K., Thompson, K. D. & Kapur, N. K. (2012) Acute coronary thrombosis in Boston marathon runners. *The New England Journal of Medicine*, 366(2), 184-185.
- American College of Sports Medicine. (2013). *ACSM's guidelines for exercise testing and prescription*. Baltimore: Lippincott Williams & Wilkins.
- Arbab-Zadeh, A., Perhonen, M., Howden, E., Peshock, R. M., Zhang, R., Adams-Huet, B., et al. (2014). Cardiac remodeling in response to 1 Year of intensive endurance training. *Circulation*, 130(24), 2152-2161.
- Astorino, T. A. (2009). Alterations in VO<sub>2</sub>max and the VO<sub>2</sub> plateau with manipulation of sampling interval. *Clinical Physiology and Functional Imaging*, 29(1), 60-67.
- Azam, S., Hadi, N., Khan, N. U. & Hadi, S. M. (2003). Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. *Medical Science Monitor*, 9(9), 325-330.
- Baggish, A. L., & Wood, M. J. (2011). Athlete's heart and cardiovascular care of the athlete scientific and clinical update. *Circulation*, 123(23), 2723-2735.
- Bagrov, A. Y. & Lakatta, E. G. (2004). The dietary sodium-blood pressure plot "stiffens". *Hypertension*, 44(1), 22-24.
- Barclay, J. K. & Hansel, M. (1991). Free radicals may contribute to oxidative skeletal muscle fatigue. *Canadian Journal of Physiology and Pharmacology*, 69(2), 279-284.
- Barnes, J. N., Trombold, J. R., Dhindsa, M., Lin, H. F. & Tanaka, H. (2010). Arterial stiffening following eccentric exercise-induced muscle damage. *Journal of Applied Physiology*, 109(4), 1102-1108.
- Bassett, D. R. & Howley, E. T. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Medicine and Science in Sports and Exercise*, 32(1), 70-84.
- Baumgartner, T. A. (1989). Norm-referenced measurement: reliability. In M. Safrit & T. Woods (Eds.), *Measurement concepts in physical education and exercise science*. pp (45–72). Champaign, IL: Human Kinetics.
- Bergholm, R., Mäkimattila, S., Valkonen, M., Liu, M. L., Lahdenperä, S., Taskinen, M. R., et al. (1999). Intense physical training decreases circulating antioxidants and endothelium-dependent vasodilatation in vivo. *Atherosclerosis*, 145(2), 341-349.
- Bergman, B. C., Wolfel, E. E., Butterfield, G. E., Lopaschuk, G. D., Casazza, G. A., Horning, M. A., et al. (1999). Active muscle and whole body lactate kinetics after endurance training in men. *Journal of Applied Physiology*, 87(5), 1684-1696.
- Blacher, J., Asmar, R., Djane, S., London, G. M. & Safar, M. E. (1999). Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension*, 33(5), 1111-1117.

- Bloomer, R. J., Goldfarb, A. H. & McKenzie, M. J. (2006). Oxidative stress response to aerobic exercise: comparison of antioxidant supplements. *Medicine and Science in Sports and Exercise*, 38(6), 1098.
- Borg, G. (1982) Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*, 14 (5), 377-81
- Bouchard, C., Sarzynski, M. A., Rice, T. K., Kraus, W. E., Church, T. S., Sung, Y. J., et al. (2011). Genomic predictors of the maximal O<sub>2</sub> uptake response to standardized exercise training programs. *Journal of Applied Physiology*, 110(5), 1160-1170.
- Boushel, R. (2010). Muscle metaboreflex control of the circulation during exercise. *Acta Physiologica*, 199(4), 367-383.
- Boutouyrie, P., Briet, M., Collin, C., Vermeersch, S. & Pannier, B. (2009). Assessment of pulse wave velocity. *Artery Research*, 3(1), 3-8.
- Boveris, A. & Chance, B. (1973). The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochemical Journal*, 134(3), 707-716.
- Brooks, G. A. (2007). Lactate. *Sports Medicine*, 37(4-5), 341-343.
- Brosnan, M., La Gerche, A., Kalman, J., Lo, W., Fallon, K., MacIsaac, A., et al. (2014). Comparison of frequency of significant electrocardiographic abnormalities in endurance versus nonendurance athletes. *The American Journal of Cardiology*, 113(9), 1567-1573.
- Bruce, C. R., Thrush, A. B., Mertz, V. A., Bezaire, V., Chabowski, A., Heigenhauser, G. J., et al. (2006). Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *American Journal of Physiology - Endocrinology and Metabolism*, 291(1), 99-107.
- Bruton, A., Conway, J. H. & Holgate, S. T. (2000). Reliability: what is it, and how is it measured? *Physiotherapy*, 86(2), 94-99.
- Burr, J. F., Bouler, M. & Beck, K. (in press). Arterial stiffness results from eccentrically biased downhill running exercise. *Journal of Science and Medicine in Sport*.
- Burr, J. F., Bredin, S. S. D., Phillips, A., Foulds, H., Cote, A., Charlesworth, S., et al. (2012). Systemic arterial compliance following ultra-marathon. *International Journal of Sports Medicine*, 33(3), 224.
- Burr, J. F., Drury, C. T., Phillips, A. A., Ivey, A., Ku, J. & Warburton, D. E. (2014). Long-term ultra-marathon running and arterial compliance. *Journal of Science and Medicine in Sport*, 17(3), 322-325.
- Caro, C. G., Pedley, T. J., Schroter, R. C., Seed, W. A. & Parker, K. H. (1978). *The mechanics of the circulation*. Oxford: Oxford University Press.

- Cecelja, M. & Chowienczyk, P. (2009). Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension a systematic review. *Hypertension*, *54*(6), 1328-1336.
- Chen, C. H., Nevo, E., Fetits, B., Pak, P. H., Yin, F. C., Maughan, W. L., et al. (1997). Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure Validation of generalized transfer function. *Circulation*, *95*(7), 1827-1836.
- Chiu, Y. C., Arand, P. W., Shroff, S. G., Feldman, T. & Carroll, J. D. (1991). Determination of pulse wave velocities with computerized algorithms. *American Heart Journal*, *121*(5), 1460-1470.
- Chirinos, J. A., Zambrano, J. P., Chakko, S., Veerani, A., Schob, A., Willens, H. J., et al. (2005). Aortic pressure augmentation predicts adverse cardiovascular events in patients with established coronary artery disease. *Hypertension*, *45*(5), 980-985.
- Coleman, A., Freeman, P., Steel, S. & Shennan, A. (2005). Validation of the Omron MX3 Plus oscillometric blood pressure monitoring device according to the European Society of Hypertension international protocol. *Blood Pressure Monitoring*, *10*(3), 165-168.
- Corrado, D. & McKenna, W. J. (2007). Appropriate interpretation of the athlete's electrocardiogram saves lives as well as money. *European Heart Journal*, *28*(16), 1920-1922.
- Corrado, D., Basso, C., Pavei, A., Michieli, P., Schiavon, M. & Thiene, G. (2006). Trends in sudden cardiovascular death in young competitive athletes after implementation of a preparticipation screening program. *The Journal of the American Medical Association*, *296*(13), 1593-1601.
- Corrado, D., Basso, C., Rizzoli, G., Schiavon, M. & Thiene, G. (2003). Does sports activity enhance the risk of sudden death in adolescents and young adults?. *Journal of the American College of Cardiology*, *42*(11), 1959-1963.
- Corrado, D., Basso, C., Schiavon, M., Pelliccia, A. & Thiene, G. (2008). Pre-participation screening of young competitive athletes for prevention of sudden cardiac death. *Journal of the American College of Cardiology*, *52*(24), 1981-1989.
- Corrado, D., Migliore, F., Basso, C. & Thiene, G. (2006). Exercise and the risk of sudden cardiac death. *Herz Kardiovaskuläre Erkrankungen*, *31*(6), 553-558.
- Corrado, D., Pelliccia, A., Heidbuchel, H., Sharma, S., Link, M., Basso, C., et al. (2010). Recommendations for interpretation of 12-lead electrocardiogram in the athlete. *European Heart Journal*, *31*(2), 243-259.
- Corrado, G., Bagnardi, V., Zambon, A. & La Vecchia, C. (2004). A meta-analysis of alcohol consumption and the risk of 15 diseases. *Preventive Medicine*, *38*(5), 613-619.
- Coyle, E. F., Sidossis, L. S., Horowitz, J. F. & Beltz, J. D. (1992). Cycling efficiency is related to the percentage of type I muscle fibers. *Medicine and Science in Sports and Exercise*, *24*(7), 782-788.

- Cruickshank, K., Riste, L., Anderson, S. G., Wright, J. S., Dunn, G. & Gosling, R. G. (2002). Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance an integrated index of vascular function?. *Circulation*, *106*(16), 2085-2090.
- Cunha, R. S., Pannier, B., Benetos, A., Siché, J. P., London, G. M., Mallion, J. M., et al. (1997). Association between high heart rate and high arterial rigidity in normotensive and hypertensive subjects. *Journal of Hypertension*, *15*(12), 1423-1430.
- Dai, D. F., Rabinovitch, P. S. & Ungvari, Z. (2012). Mitochondria and cardiovascular aging. *Circulation Research*, *110*(8), 1109-1124.
- Davies, K. J., Quintanilha, A. T., Brooks, G. A., Dallman, P. R. & Packer, L. (1982). Free radicals and tissue damage produced by exercise. *Biochemical and Biophysical Research Communications*, *107*(4), 1198-1205.
- Davies, T., Frenneaux, M., Campbell, R. & White, M. (2007). Human arterial responses to isometric exercise: the role of the muscle metaboreflex. *Clinical Science*, *112*, 441-447.
- Davis, J. M., Zhao, Z., Stock, H. S., Mehl, K. A., Buggy, J. & Hand, G. A. (2003). Central nervous system effects of caffeine and adenosine on fatigue. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *284*(2), 399-404.
- Dawson, E. A., Whyte, G. P., Black, M. A., Jones, H., Hopkins, N., Oxborough, D., et al. (2008). Changes in vascular and cardiac function after prolonged strenuous exercise in humans. *Journal of Applied Physiology*, *105*(5), 1562-1568.
- Dempsey, J. A., Romer, L., Rodman, J., Miller, J. & Smith, C. (2006). Consequences of exercise-induced respiratory muscle work. *Respiratory Physiology & Neurobiology*, *151*(2), 242-250.
- Deschamps, A. M. & Murphy, E. (2009). Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats. *American Journal of Physiology-Heart and Circulatory Physiology*, *297*(5), 1806-1813.
- DeSouza, C. A., Shapiro, L. F., Clevenger, C. M., Dinenna, F. A., Monahan, K. D., Tanaka, H., et al. (2000). Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation*, *102*(12), 1351-1357.
- DeVan, A. E. & Seals, D. R. (2012). Vascular health in the ageing athlete. *Experimental Physiology*, *97*(3), 305-310.
- Ding, F. H., Fan, W. X., Zhang, R. Y., Zhang, Q., Li, Y. & Wang, J. G. (2011). Validation of the noninvasive assessment of central blood pressure by the SphygmoCor and Omron devices against the invasive catheter measurement. *American Journal of Hypertension*, *24*(12), 1306-1311.
- Doherty, M., Nobbs, L. & Noakes, T. D. (2003). Low frequency of the " plateau phenomenon" during maximal exercise in elite British athletes. *European Journal of Applied Physiology*, *89*(6), 619-623.

- Dolinsky, V. W., Jones, K. E., Sidhu, R. S., Haykowsky, M., Czubyrt, M. P., Gordon, T., et al. (2012). Improvements in skeletal muscle strength and cardiac function induced by resveratrol during exercise training contribute to enhanced exercise performance in rats. *The Journal of Physiology*, 590(11), 2783-2799.
- Douglas, P. S., O'Toole, M. L., Miller, W. D. B. & Reichel, N. (1990). Different effects of prolonged exercise on the right and left ventricles. *Journal of the American College of Cardiology*, 15(1), 64-69.
- Draaijer, P., Kool, M. J., Maessen, J. M., van Bortel, L. M., de Leeuw, P. W., van Hooff, J. P. & Leunissen, K. M. (1993). Vascular distensibility and compliance in salt-sensitive and salt-resistant borderline hypertension. *Journal of Hypertension*, 11(11), 1199-1208.
- Drezner, J. A. (2008). Contemporary approaches to the identification of athletes at risk for sudden cardiac death. *Current Opinion in Cardiology*, 23(5), 494-501.
- Drezner, J. A. (2012). Standardised criteria for ECG interpretation in athletes: a practical tool. *British Journal of Sports Medicine*, 46(1), 6-8.
- Drezner, J. A., Ashley, E., Baggish, A. L., Börjesson, M., Corrado, D., Owens, D. S., et al. (2013). Abnormal electrocardiographic findings in athletes: recognising changes suggestive of cardiomyopathy. *British Journal of Sports Medicine*, 47(3), 137-152.
- Drezner, J. A., Asif, I. M., Owens, D. S., Prutkin, J. M., Salerno, J. C., Fean, R., et al. (2012). Accuracy of ECG interpretation in competitive athletes: the impact of using standardised ECG criteria. *British Journal of Sports Medicine*, 46(5), 335-340.
- Drost, E. A. (2011). Validity and reliability in social science research education. *Research and Perspectives*, 38(1), 105-123.
- Duncan, G. E., Howley, E. T. & Johnson, B. N. (1997). Applicability of VO<sub>2</sub>max criteria: discontinuous versus continuous protocols. *Medicine and Science in Sports and Exercise*, 29(2), 273-278.
- Durnin, J. V. G. A. & Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition*, 32(1), 77-97.
- Ector, J., Ganame, J., van der Merwe, N., Adriaenssens, B., Pison, L., Willems, R., et al. (2007). Reduced right ventricular ejection fraction in endurance athletes presenting with ventricular arrhythmias: a quantitative angiographic assessment. *European Heart Journal*, 28(3), 345-353.
- Edwards, D. G. & Lang, J. T. (2005). Augmentation index and systolic load are lower in competitive endurance athletes. *American Journal of Hypertension*, 18(5), 679-683.

- Failla, M., Grappiolo, A., Emanuelli, G., Vitale, G., Frascini, N., Bigoni, M., et al. (1999). Sympathetic tone restrains arterial distensibility of healthy and atherosclerotic subjects. *Journal of Hypertension*, *17*(8), 1117-1123.
- Faude, O., Kindermann, W. & Meyer, T. (2009). Lactate threshold concepts. *Sports Medicine*, *39*(6), 469-490.
- Feugier, P. & Chevalier, J. M. (2004). Endofibrosis of the iliac arteries: an underestimated problem. *Acta Chirurgica Belgica*, *104*(6), 635-40.
- Field, A. (2009). *Discovering statistics using IBM SPSS statistics* (3<sup>rd</sup> ed.). London: Sage Publications LTD.
- Figueroa, A., Hooshmand, S., Figueroa, M. & Bada, A. M. (2010). Cardiovascular baroreflex and aortic hemodynamic responses to isometric exercise and post-exercise muscle ischemia in resistance trained men. *Scandinavian Journal of Medicine & Science in Sports*, *20*(2), 305-309.
- Fisher-Wellman, K. & Bloomer, R. J. (2009). Acute exercise and oxidative stress: a 30 year history. *Dynamic Medicine*, *8*(1), 1.
- Foster, C., Florhaug, J. A., Franklin, J., Gottschall, L., Hrovatin, L. A., Parker, S., et al. (2001). A new approach to monitoring exercise training. *The Journal of Strength & Conditioning Research*, *15*(1), 109-115.
- Fukuba, Y. & Whipp, B. J. (1999). A metabolic limit on the ability to make up for lost time in endurance events. *Journal of Applied Physiology*, *87*(2), 853-861.
- Gates, P. E., Tanaka, H., Hiatt, W. R. & Seals, D. R. (2004). Dietary sodium restriction rapidly improves large elastic artery compliance in older adults with systolic hypertension. *Hypertension*, *44*(1), 35-41.
- Gati, S., Sheikh, N., Ghani, S., Zaidi, A., Wilson, M., Raju, H., et al. (2013). Should axis deviation or atrial enlargement be categorised as abnormal in young athletes? The athlete's electrocardiogram: time for re-appraisal of markers of pathology. *European Heart Journal*, *34*(47), 3641-3648.
- Gellish, R. L., Goslin, B. R., Olson, R. E., McDonald, A., Russi, G. D. & Moudgil, V. K. (2007). Longitudinal modeling of the relationship between age and maximal heart rate. *Medicine and Science in Sports and Exercise*, *39*(5), 822-829.
- George, K., Spence, A., Naylor, L. H., Whyte, G. P., & Green, D. J. (2011). Cardiac adaptation to acute and chronic participation in endurance sports. *Heart*, *97*(24), 1999-2004.
- George, K., Whyte, G. P., Green, D. J., Oxborough, D., Shave, R. E., Gaze, D. & Somauroo, J. (2012). The endurance athletes heart: acute stress and chronic adaptation. *British Journal of Sports Medicine*, *46*(1), 29-36.
- Ghasemi, A. & Zahediasl, S. (2012). Normality tests for statistical analysis: a guide for non-statisticians. *International Journal of Endocrinology and Metabolism*, *10*(2), 486.

- Ghosh, S., Vivar, J. C., Sarzynski, M. A., Sung, Y.J., Timmons, J. A., Bouchard, C., et al. (2013). Integrative pathway analysis of a genome-wide association study of  $VO_{2max}$  response to exercise training. *Journal of Applied Physiology*, *115*(9), 1343-1359.
- Gibala, M. J., Little, J. P., Van Essen, M., Wilkin, G. P., Burgomaster, K. A., Safdar, A., et al. (2006). Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *The Journal of Physiology*, *575*(3), 901-911.
- Girden, E. R. (1992) *ANOVA: Repeated measures*. Newbury Park: Sage Publications.
- Gleeson, M., Nieman, D. C. & Pederson, B. K. (2004). Exercise, nutrition and immune function. *Journal of Sports Science*, *22*(1), 115-125.
- Gliemann, L., Schmidt, J. F., Olesen, J., Biensø, R. S., Peronard, S. L., Grandjean, S. U., et al. (2013). Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. *The Journal of Physiology*, *591*(20), 5047-5059.
- Goldin, A., Beckman, J. A., Schmidt, A. M., & Creager, M. A. (2006). Advanced glycation end products sparking the development of diabetic vascular injury. *Circulation*, *114*(6), 597-605.
- Goldstein, E. R., Ziegenfuss, T., Kalman, D., Kreider, R., Campbell, B., Wilborn, C., et al. (2010). International society of sports nutrition position stand: caffeine and performance. *Journal of the International Society Sports Nutrition*, *7*(1), 5.
- Gómez-Marcos, M. A., Recio-Rodríguez, J. I., Patino-Alonso, M. C., Agudo-Conde, C., Gómez-Sánchez, L., Rodríguez-Sánchez, E., et al. (2011). Relationship between intima-media thickness of the common carotid artery and arterial stiffness in subjects with and without type 2 diabetes: a case-series report. *Cardiovascular Diabetology*, *10*(3), 1258-1266.
- Goodpaster, B. H., He, J., Watkins, S. & Kelley, D. E. (2001). Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *The Journal of Clinical Endocrinology & Metabolism*, *86*(12), 5755-5761.
- Goto, C., Higashi, Y., Kimura, M., Noma, K., Hara, K., Nakagawa, K., et al. (2003). Effect of different intensities of exercise on endothelium-dependent vasodilation in humans role of endothelium-dependent nitric oxide and oxidative stress. *Circulation*, *108*(5), 530-535.
- Green, D. J., Bilsborough, W., Naylor, L. H., Reed, C., Wright, J., O'Driscoll, G. & Walsh, J. H. (2005). Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *The Journal of Physiology*, *562*(2), 617-628.
- Green, D. J., Rowley, N. Spence, A., Carter, H., Whyte, G., George, K., et al. (2013). Why isn't flow-mediated dilation enhanced in athletes? *Medicine and Science in Sports and Exercise*, *45*(1), 75-82.

- Green, D. J., Spence, A., Halliwill, J. R., Cable, N. T. & Thijssen, D. H. (2011). Exercise and vascular adaptation in asymptomatic humans. *Experimental Physiology*, *96*(2), 57-70.
- Gross, M., Steffes, M., Jacobs, D. R., Yu, X., Lewis, L., Lewis, C. E., et al. (2005). Plasma F<sub>2</sub>-isoprostanes and coronary artery calcification: the CARDIA study. *Clinical Chemistry* *51*(1), 125-131.
- Gurovich, A. N., Beck, D. T. & Braith, R. W. (2009). Aortic pulse wave analysis is not a surrogate for central arterial pulse wave velocity. *Experimental Biology and Medicine*, *234*(11), 1339-1344.
- Halliwell, B. (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *The American Journal of Medicine*, *91*(3), 14-22.
- Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology*, *141*(2), 312-322.
- Hamid, A. A., Aiyelaagbe, O. O., Usman, L. A., Ameen, O. M. & Lawal, A. (2010). Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry*, *4*(8), 142-151.
- Hart, E. C., Rasmussen, P., Secher, N. H., George, K. P., Cable, N. T., Volianitis, S., et al. (2010). The carotid baroreflex is reset following prolonged exercise in humans. *Acta Physiologica*, *200*(4), 291-299.
- Hawkins, S. A., Marcell, T. J., Victoria, J. S. & Wiswell, R. A. (2001). A longitudinal assessment of change in VO<sub>2</sub>max and maximal heart rate in master athletes. *Medicine and Science in Sports and Exercise*, *33*(10), 1744-1750.
- Hawley, J. A. (2002). Adaptations of skeletal muscle to prolonged, intense endurance training. *Clinical and Experimental Pharmacology and Physiology*, *29*(3), 218-222.
- Heffernan, K. S., Lefferts, W. K., Kasprovicz, A. G., Tarzia, B. J., Thijssen, D. H. & Brutsaert, T. D. (2013). Manipulation of arterial stiffness, wave reflections, and retrograde shear rate in the femoral artery using lower limb external compression. *Physiological Reports*, *1*(2), 1-11.
- Helge, J. W., Lundby, C., Christensen, D. L., Langfort, J., Messonnier, L., Zacho, M., et al. (2003). Skiing across the Greenland icecap: divergent effects on limb muscle adaptations and substrate oxidation. *Journal of Experimental Biology*, *206*(6), 1075-1083.
- Hickson, R. C., Hagberg, J. M., Ehsani, A. A. & Holloszy, J. O. (1980). Time course of the adaptive responses of aerobic power and heart rate to training. *Medicine and Science in Sports and Exercise*, *13*(1), 17-20.
- Higashi, Y. & Yoshizumi, M. (2004). Exercise and endothelial function: role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacology & Therapeutics*, *102*(1), 87-96.

- Hoffman, M. D., Lebus, D. K., Ganong, A. C., Casazza, G. A. & Loan, M. V. (2010). Body composition of 161-km ultramarathoners. *International Journal of Sports Medicine*, 31(02), 106-109.
- Hoffman, M. D., Ong, J. C., & Wang, G. (2010). Historical analysis of participation in 161 km ultramarathons in North America. *The International Journal of the History of Sport*, 27(11), 1877-1891.
- Hopkins, W. G. (2000). Measures of reliability in sports medicine and science. *Sports Medicine*, 30(1), 1-15.
- Horvath, I. G., Nemeth, A., Lenkey, Z., Alessandri, N., Tufano, F., Kis, P., et al. (2010). Invasive validation of a new oscillometric device (Arteriograph) for measuring augmentation index, central blood pressure and aortic pulse wave velocity. *Journal of Hypertension*, 28(10), 2068-2075.
- Howley, E. T., Bassett, D. R. & Welch, H. G. (1995). Criteria for maximal oxygen uptake: review and commentary. *Medicine and Science in Sports and Exercise*, 27(9), 1292-1301.
- Humes, R. (2014). *Adolescent Cardiac Issues, An Issue of Pediatric Clinics*. Amsterdam: Elsevier Health Sciences.
- Intengan, H. D. & Schiffrin, E. L. (2001). Vascular remodeling in hypertension roles of apoptosis, inflammation, and fibrosis. *Hypertension*, 38(3), 581-587.
- Iwasaki, K. I., Zhang, R., Zuckerman, J. H. & Levine, B. D. (2003). Dose-response relationship of the cardiovascular adaptation to endurance training in healthy adults: how much training for what benefit? *Journal of Applied Physiology*, 95(4), 1575-1583.
- Jensen, F. B. (2009). The dual roles of red blood cells in tissue oxygen delivery: oxygen carriers and regulators of local blood flow. *Journal of Experimental Biology*, 212(21), 3387-3393.
- Jeukendrup, A. E. & Martin, J. (2001). Improving cycling performance. *Sports Medicine*, 31(7), 559-569.
- Jeukendrup, A. E., Saris, W. H. M. & Wagenmakers, A. J. M. (1998). Fat metabolism during exercise: a review. *International Journal of Sports Medicine*, 19(6), 371-9.
- Ji, L. L., Gomez-Cabrera, M. C. & Vina, J. (2006). Exercise and hormesis: activation of cellular antioxidant signalling pathway. *Annals of the New York Academy of Sciences*, 1067(1), 425-435.
- Joannides, R., Haefeli, W. E., Linder, L., Richard, V., Bakkali, E. H., Thuillez, C., et al. (1995). Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation*, 91(5), 1314-1319.
- Jobson, S. A., Nevill, A. M., George, S. R., Jeukendrup, A. E. & Passfield, L. (2008). Influence of body position when considering the ecological validity of laboratory time-trial cycling performance. *Journal of Sports Sciences*, 26(12), 1269-1278.
- Johnson, B. D., Mather, K. J. & Wallace, J. P. (2011). Mechanotransduction of shear in the endothelium: basic studies and clinical implications. *Vascular Medicine*, 16(5), 365-377.

- Jones, A. M. & Carter, H. (2000). The effect of endurance training on parameters of aerobic fitness. *Sports Medicine*, 29(6), 373-386.
- Jówko, E., Sacharuk, J., Balasińska, B., Ostaszewski, P., Charmas, M. & Charmas, R. (2011). Green tea extract supplementation gives protection against exercise-induced oxidative damage in healthy men. *Nutrition Research*, 31(11), 813-821.
- Joyner, M. J. & Coyle, E. F. (2008). Endurance exercise performance: the physiology of champions. *The Journal of Physiology*, 586(1), 35-44.
- Karamanoglu, M. M. A. R., O'Rourke, M. F., Avolio, A. P. & Kelly, R. P. (1993). An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *European Heart Journal*, 14(2), 160-167.
- Karatzis, E., Papaioannou, T. G., Aznaouridis, K., Karatzi, K., Stamatelopoulos, K., Zampelas, A., et al. (2005). Acute effects of caffeine on blood pressure and wave reflections in healthy subjects: should we consider monitoring central blood pressure?. *International Journal of Cardiology*, 98(3), 425-430.
- Kasch, F. W., Boyer, J. L., Schmidt, P. K., Wells, R. H., Wallace, J. P., Verity, L. S., et al. (1999). Ageing of the cardiovascular system during 33 years of aerobic exercise. *Age and Ageing*, 28(6), 531-536.
- Keating, J. & Matyas, T. (1998). Unreliable inferences from reliable measurements. *The Australian Journal of Physiotherapy*, 44(1), 5-10.
- Kenney, L. W., Wilmore, J. & Costill, D. (1999). *Physiology of Sport and Exercise* (5<sup>th</sup> ed.). Champaign, IL: Human Kinetics.
- Kiens, B. (2006). Skeletal muscle lipid metabolism in exercise and insulin resistance. *Physiological Reviews*, 86(1), 205-243.
- Kingwell, B. A., Berry, K. L., Cameron, J. D., Jennings, G. L. & Dart, A. M. (1997). Arterial compliance increases after moderate-intensity cycling. *American Journal of Physiology-Heart and Circulatory Physiology*, 273(5), H2186-H2191.
- Kingwell, B. A., Cameron, J. D., Gillies, K. J., Jennings, G. L. & Dart, A. M. (1995). Arterial compliance may influence baroreflex function in athletes and hypertensives. *American Journal of Physiology*, 268(1), 411-411.
- Knechtle, B., Knechtle, P. & Lepers, R. (2011). Participation and performance trends in ultra-triathlons from 1985 to 2009. *Scandinavian Journal of Medicine & Science in Sports*, 21(6), 82-90.
- Knechtle, B., Wirth, A., Baumann, B., Knechtle, P., Rosemann, T. & Oliver, S. (2010). Differential correlations between anthropometry, training volume, and performance in male and female Ironman triathletes. *The Journal of Strength & Conditioning Research*, 24(10), 2785-2793.

- Knechtle, B., Wirth, A., Knechtle, P., Rosemann, T., Rüst, C. A. & Bescos, R. (2011). A comparison of fat mass and skeletal muscle mass estimation in male ultra-endurance athletes using bioelectrical impedance analysis and different anthropometric methods. *Nutricion Hospitalaria*, *26*(6), 1420-7.
- Knez, W. L., Coombes, J. S. & Jenkins, D. G. (2006). Ultra-endurance exercise and oxidative damage. *Sports Medicine*, *36*(5), 429-441.
- Knez, W. L., Jenkins, D. G. & Coombes, J. S. (2007). Oxidative stress in half and full Ironman triathletes. *Medicine and Science in Sports and Exercise*, *39*(2), 283.
- Knez, W. L., Sharman, J. E., Jenkins, D. G. & Coombes, J. S. (2008). Central hemodynamics in ultra-endurance athletes. *Journal of Science and Medicine in Sport*, *11*(4), 390-395.
- Kulig, J. & Koplan, B. A. (2010). Wolff-Parkinson-White syndrome and accessory pathways. *Circulation*, *122*(15), 480-483.
- La Gerche, A., Burns, A. T., Mooney, D. J., Inder, W. J., Taylor, A. J., Bogaert, J., et al. (2012). Exercise-induced right ventricular dysfunction and structural remodelling in endurance athletes. *European Heart Journal*, *33*(8), 998-1006.
- La Gerche, A., Connelly, K. A., Mooney, D. J., Maclsaac, A. I. & Prior, D. L. (2008). Biochemical and functional abnormalities of left and right ventricular function after ultra-endurance exercise. *Heart*, *94*(7), 860-866.
- La Gerche, A., Robberecht, C., Kuiperi, C., Nuyens, D., Willems, R., de Ravel, T., et al. (2010). Lower than expected desmosomal gene mutation prevalence in endurance athletes with complex ventricular arrhythmias of right ventricular origin. *Heart*, *96*(16), 1268-1274.
- LaForgia, J., Withers, R. T. & Gore, C. J. (2006). Effects of exercise intensity and duration on the excess post-exercise oxygen consumption. *Journal of Sports Sciences*, *24*(12), 1247-1264.
- Landers, G. J., Blanksby, B. A., Ackland, T. R. & Smith, D. (2000). Morphology and performance of world championship triathletes. *Annals of Human Biology*, *27*(4), 387-400.
- Lantelme, P., Mestre, C., Lievre, M., Gressard, A. & Milon, H. (2002). Heart rate an important confounder of pulse wave velocity assessment. *Hypertension*, *39*(6), 1083-1087.
- Laughlin, M. H., Newcomer, S. C. & Bender, S. B. (2008). Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *Journal of Applied Physiology*, *104*(3), 588-600.
- Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., et al. (2001). Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension*, *37*(5), 1236-1241.

- Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., et al. (2006). Expert consensus document on arterial stiffness: methodological issues and clinical applications. *European Heart Journal*, *27*(21), 2588-2605.
- Lee, D. C., Pate, R. R., Lavie, C. J. & Blair, S. N. (2012). Running and all-cause mortality risk - Is more better. *Medicine and Science in Sport and Exercise*, *44*(6), 990-994.
- Lee, D. C., Sui, X., Ortega, F. B., Kim, Y. S., Church, T. S., Winett, R. A., et al. (2011). Comparisons of leisure-time physical activity and cardiorespiratory fitness as predictors of all-cause mortality in men and women. *British Journal of Sports Medicine*, *45*(6), 504-510.
- Lee, S., Kuk, J. L., Davidson, L. E., Hudson, R., Kilpatrick, K., Graham, T. E., et al. (2005). Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without type 2 diabetes. *Journal of Applied Physiology*, *99*(3), 1220-1225.
- Lehoux, S., Esposito, B., Merval, R. & Tedgui, A. (2005). Differential regulation of vascular focal adhesion kinase by steady stretch and pulsatility. *Circulation*, *111*(5), 643-649.
- Lepers, R. (2008). Analysis of Hawaii ironman performances in elite triathletes from 1981 to 2007. *Medicine and Science in Sports and Exercise*, *40*(10), 1828.
- Liu, M. L., Bergholm, R., Mäkimattila, S., Lahdenperä, S., Valkonen, M., Hilden, H., et al. (1999). A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. *American Journal of Physiology-Endocrinology and Metabolism*, *276*(6), 1083-1091.
- Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition*, *78*(3), 5175-5205.
- London, G. M., Blacher, J., Pannier, B., Guérin, A. P., Marchais, S. J. & Safar, M. E. (2001). Arterial wave reflections and survival in end-stage renal failure. *Hypertension*, *38*(3), 434-438.
- Lucia, A., Rabadán, M., Hoyos, J., Hernández-Capilla, M., Pérez, M., San Juan, A. F., et al. (2006). Frequency of the VO<sub>2</sub>max plateau phenomenon in world-class cyclists. *International Journal of Sports Medicine*, *27*(12), 984-992.
- Mackenzie, I. S., Wilkinson, I. B. & Cockcroft, J. R. (2002). Assessment of arterial stiffness in clinical practice. *QJM*, *95*(2), 67-74.
- Maeda, S., Tanabe, T., Otsuki, T., Sugawara, J., Ajisaka, R., & Matsuda, M. (2008). Acute exercise increases systemic arterial compliance after 6-month exercise training in older women. *Hypertension Research*, *31*(2), 377-381.
- Mancia, G., Laurent, S., Agabiti-Rosei, E., Ambrosioni, E., Burnier, M., Caulfield, M. J., et al. (2009). Reappraisal of European guidelines on hypertension management: a European Society of Hypertension Task Force document. *Blood Pressure*, *18*(6), 308-347.

- Mangoni, A. A., Mircoli, L., Giannattasio, C., Ferrari, A. U. & Mancia, G. (1996). Heart rate-dependence of arterial distensibility in vivo. *Journal of Hypertension*, *14*(7), 897-901.
- Mann, T. N., Lamberts, R. P. & Lambert, M. I. (2014). High responders and low responders: factors associated with individual variation in response to standardized training. *Sports Medicine*, *44*(8), 1113-1124.
- Margaritis, I., Tessier, F., Richard, M. J. & Marconnet, P. (1997). No evidence of oxidative stress after a triathlon race in highly trained competitors. *International Journal of Sports Medicine*, *18*(03), 186-190.
- Margonis, K., Fatouros, I. G., Jamurtas, A. Z., Nikolaidis, M. G., Douroudos, I., Chatzinikolaou, A., et al. (2007). Oxidative stress biomarkers responses to physical overtraining: implications for diagnosis. *Free Radical Biology and Medicine*, *43*(6), 901-910.
- Maron, B. J. & Pelliccia, A. (2006). The heart of trained athletes cardiac remodeling and the risks of sports, including sudden death. *Circulation*, *114*(15), 1633-1644.
- Maron, B. J. (2003). Sudden death in young athletes. *New England Journal of Medicine*, *349*(11), 1064-1075.
- Maron, B. J., Thompson, P. D., Puffer, J. C., McGrew, C. A., Strong, W. B., Douglas, P. S., et al. (1996). Cardiovascular preparticipation screening of competitive athletes: a statement for health professionals from the sudden death committee (clinical cardiology) and congenital cardiac defects committee (cardiovascular disease in the young), American Heart Association. *Circulation*, *94*(4), 850-856.
- Martin Bland, J. & Altman, D. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, *327*(8476), 307-310.
- Martin Bland, J. & Altman, D. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, *327*(8476), 307-310.
- Martin, W. D., Dalsky, G. P., Hurley, B. F., Matthews, D. E., Bier, D. M., Hagberg, J. M., et al. (1993). Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. *American Journal of Physiology*, *265*(5), 708-714.
- Mastaloudis, A., Leonard, S. W. & Traber, M. G. (2001). Oxidative stress in athletes during extreme endurance exercise. *Free Radical Biology and Medicine*, *31*(7), 911-922.
- Mastaloudis, A., Morrow, J. D., Hopkins, D. W., Devaraj, S. & Traber, M. G. (2004). Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners. *Free Radical Biology and Medicine*, *36*(10), 1329-1341.
- Matelot, D., Schnell, F., Kervio, G., Du Boullay, N. T. & Carré, F. (2013). Athlete's bradycardia may be a multifactorial mechanism. *Journal of Applied Physiology*, *114*(12), 1755-1756.

- Mattace-Raso, F. U., van der Cammen, T. J., Hofman, A., van Popele, N. M., Bos, M. L., Schalekamp, M. A., et al. (2006). Arterial stiffness and risk of coronary heart disease and stroke the rotterdam study. *Circulation*, *113*(5), 657-663.
- Maughan, R. J. (1993). An evaluation of a bioelectrical impedance analyser for the estimation of body fat content. *British Journal of Sports Medicine*, *27*(1), 63-66.
- McCrea, C. E., Skulas-Ray, A. C., Chow, M. & West, S. G. (2012). Test–retest reliability of pulse amplitude tonometry measures of vascular endothelial function: implications for clinical trial design. *Vascular Medicine*, *17*(1), 29-36.
- McDonnell, B. J., Maki-Petaja, K. M., Munnery, M., Wilkinson, I. B., Cockcroft, J. R. & McEniery, C. M. (2013). Habitual exercise and blood pressure: age dependency and underlying mechanisms. *American Journal of Hypertension*, *26*(3), 334-341.
- Meilhac, O., Ramachandran, S., Chiang, K., Santanam, N. & Parthasarathy, S. (2001). Role of arterial wall antioxidant defence in beneficial effects of exercise on atherosclerosis in mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *21*(10), 1681-1688.
- Mihl, C., Dassen, W. R. M. & Kuipers, H. (2008). Cardiac remodelling: concentric versus eccentric hypertrophy in strength and endurance athletes. *Netherlands Heart Journal*, *16*(4), 129-133.
- Mitchell, G. F., Parise, H., Benjamin, E. J., Larson, M. G., Keyes, M. J., Vita, J. A., et al. (2004). Changes in arterial stiffness and wave reflection with advancing age in healthy men and women the Framingham Heart Study. *Hypertension*, *43*(6), 1239-1245.
- Miyachi, M., Donato, A. J., Yamamoto, K., Takahashi, K., Gates, P. E., Moreau, K. L., et al. (2003). Greater age-related reductions in central arterial compliance in resistance-trained men. *Hypertension*, *41*(1), 130-135.
- Miyashita, H. (2012). Clinical assessment of central blood pressure. *Current Hypertension Reviews*, *8*(2), 80-90.
- Moens, A. L., Goovaerts, I., Claeys, M. J. & Vrints, C. J. (2005). Flow-Mediated Vasodilation A Diagnostic Instrument, or an Experimental Tool?. *CHEST Journal*, *127*(6), 2254-2263.
- Möhlenkamp, S., Lehmann, N., Breuckmann, F., Bröcker-Preuss, M., Nassenstein, K., Halle, M., et al. (2008). Running: the risk of coronary events. Prevalence and prognostic relevance of coronary atherosclerosis in marathon runners. *European Heart Journal*, *29*(15), 1903-1910.
- Moncada, S. & Higgs, E. A. (2006). Nitric oxide and the vascular endothelium. *Handbook of Experimental Physiology*, *176*(1), 213-254.
- Montgomery, H. E., Clarkson, P., Dollery, C. M., Prasad, K., Losi, M. A., Hemingway, H., et al. (1997). Association of angiotensin-converting enzyme gene I/D polymorphism with change in left ventricular mass in response to physical training. *Circulation*, *96*(3), 741-747.

- Murase, T., Haramizu, S., Ota, N. & Hase, T. (2009). Suppression of the aging-associated decline in physical performance by a combination of resveratrol intake and habitual exercise in senescence-accelerated mice. *Biogerontology*, *10*(4), 423-434.
- Murphy, E. & Steenbergen, C. (2007). Cardioprotection in females: a role for nitric oxide and altered gene expression. *Heart Failure Reviews*, *12*(3-4), 293-300.
- Murray, R. P., Connett, J. E., Tyas, S. L., Bond, R., Ekuma, O., Silversides, C. K. & Barnes, G. E. (2002). Alcohol volume, drinking pattern, and cardiovascular disease morbidity and mortality: is there a U-shaped function?. *American Journal of Epidemiology*, *155*(3), 242-248.
- Myerburg, R. J. & Vetter, V. L. (2007). Electrocardiograms should be included in preparticipation screening of athletes. *Circulation*, *116*(22), 2616-2626.
- Neilan, T. G., Januzzi, J. L., Lee-Lewandrowski, E., Ton-Nu, T. T., Yoerger, D. M., Jassal, D. S., et al. (2006). Myocardial injury and ventricular dysfunction related to training levels among nonelite participants in the Boston marathon. *Circulation*, *114*(22), 2325-2333.
- Neilan, T. G., Yoerger, D. M., Douglas, P. S., Marshall, J. E., Halpern, E. F., Lawlor, D., et al. (2006). Persistent and reversible cardiac dysfunction among amateur marathon runners. *European Heart Journal*, *27*(9), 1079-1084.
- Neubauer, O., Kern, N., Nics, L. & Wagner, K. H. (2008). No indications of persistent oxidative stress in response to an ironman triathlon. *Medicine and Science in Sport and Exercise*, *40*(12), 2119-2128.
- Neubauer, O., König, D. & Wagner, K. H. (2008). Recovery after an Ironman triathlon: sustained inflammatory responses and muscular stress. *European Journal of Applied Physiology*, *104*(3), 417-426.
- Neubauer, O., Reichhold, S., Nics, L., Hoelzl, C., Valentini, J., Stadlmayr, B., et al. (2010). Antioxidant responses to an acute ultra-endurance exercise: impact on DNA stability and indications for an increased need for nutritive antioxidants in the early recovery phase. *British Journal of Nutrition*, *104*(08), 1129-1138.
- Neumayr, G., Pfister, R., Mitterbauer, G., Eibl, G. & Hoertnagl, H. (2005). Effect of competitive marathon cycling on plasma N-terminal pro-brain natriuretic peptide and cardiac troponin T in healthy recreational cyclists. *The American Journal of Cardiology*, *96*(5), 732-735.
- Nichols, W. W. & O'Rourke, M. F. (1998). *McDonald's blood flow in arteries: theoretical, experimental and clinical principles*. (4<sup>th</sup> ed.). London: Arnold.
- Nichols, W., O'Rourke, M. & Vlachopoulos, C. (2011). *McDonald's blood flow in arteries: theoretical, experimental and clinical principles* (6<sup>th</sup> ed.). London: Hodder Arnold.

- Nieman, D. C., Henson, D. A., McAnulty, S. R., McAnulty, L., Swick, N. S., Utter, A. C. et al. (2002). Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. *Journal of Applied Physiology*, 92(5), 1970-1977.
- O'Rourke, M. F., Staessen, J. A., Vlachopoulos, C. & Duprez, D. (2002). Clinical applications of arterial stiffness; definitions and reference values. *American Journal of Hypertension*, 15(5), 426-444.
- Ogita, H. & Liao, J. K. (2004). Endothelial function and oxidative stress. *Endothelium*, 11(2), 123-132.
- O'Keefe, J. H., Patil, H. R., Lavie, C. J., Magalski, A., Vogel, R. A. & McCullough, P. A. (2012). Potential adverse cardiovascular effects from excessive endurance exercise. In *Mayo Clinic Proceedings*, 87(6), 587-595.
- O'Keefe, J. H., Schnohr, P. & Lavie, C. J. (2013). The dose of running that best confers longevity. *Heart*, 99(8), 588-590.
- O'Toole, M. L., Douglas, P. S. & Hiller, W. D. B. (1989). Lactate, oxygen uptake, and cycling performance in triathletes. *International Journal of Sports Medicine*, 10(06), 413-418.
- Otsuki, T., Maeda, S., Lemitsu, M., Saito, Y., Tanimura, Y., Ajisaka, R., et al. (2007). Vascular endothelium-derived factors and arterial stiffness in strength-and endurance-trained men. *American Journal of Physiology - Heart and Circulatory Physiology*, 292(2), 786-791.
- Paffenbarger Jr, R. S., Hyde, R., Wing, A. L. & Hsieh, C. C. (1986). Physical activity, all-cause mortality, and longevity of college alumni. *New England Journal of Medicine*, 314(10), 605-613.
- Papadakis, M., Carre, F., Kervio, G., Rawlins, J., Panoulas, V. F., Chandra, N., et al. (2011). The prevalence, distribution, and clinical outcomes of electrocardiographic repolarization patterns in male athletes of African/Afro-Caribbean origin. *European Heart Journal*, 32(18), 2304-2313.
- Papadakis, M., Whyte, G. & Sharma, S. (2008). Preparticipation screening for cardiovascular abnormalities in young competitive athletes. *BMJ*, 337, 806-811.
- Papaioannou, T. G., Karatzis, E. N., Karatzi, K. N., Gialafos, E. J., Protogerou, A. D., Stamatelopoulos, K. S., et al. (2007). Hour-to-hour and week-to-week variability and reproducibility of wave reflection indices derived by aortic pulse wave analysis: implications for studies with repeated measurements. *Journal of Hypertension*, 25(8), 1678-1686.
- Pase, M. P., Grima, N. A. & Sarris, J. (2010). The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. *The American Journal of Clinical Nutrition*, 93(2), 446-454.
- Pauca, A. L., O'Rourke, M. F. & Kon, N. D. (2001). Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension*, 38(4), 932-937.
- Pauca, A. L., Wallenhaupt, S. L., Kon, N. D. & Tucker, W. Y. (1992). Does radial artery pressure accurately reflect aortic pressure? *Chest Journal*, 102(4), 1193-1198.

- Pedley, T. J., Schroter, R. C., Seed, W. A. & Parker, K. H. (1978). *The mechanics of the circulation*. Oxford: Oxford University Press.
- Pelliccia, A. & Maron, B. J. (1995). Preparticipation cardiovascular evaluation of the competitive athlete: perspectives from the 30-year Italian experience. *The American Journal of Cardiology*, 75(12), 827-829.
- Pelliccia, A., Maron, B. J., Culasso, F., Di Paolo, F. M., Spataro, A., Biffi, A., et al. (2000). Clinical significance of abnormal electrocardiographic patterns in trained athletes. *Circulation*, 102(3), 278-284.
- Pelliccia, A., Maron, B. J., Culasso, F., Di Paolo, F. M., Spataro, A., Biffi, A., et al. (2000). Clinical significance of abnormal electrocardiographic patterns in trained athletes. *Circulation*, 102(3), 278-284.
- Pelliccia, A., Maron, B. J., Di Paolo, F. M., Biffi, A., Quattrini, F. M., Pisicchio, C., et al. (2005). Prevalence and clinical significance of left atrial remodeling in competitive athletes. *Journal of the American College of Cardiology*, 46(4), 690-696.
- Pelliccia, A., Di Paolo, F. M., Corrado, D., Buccolieri, C., Quattrini, F. M., Pisicchio, C., et al. (2006). Evidence for efficacy of the Italian national pre-participation screening programme for identification of hypertrophic cardiomyopathy in competitive athletes. *European Heart Journal*, 27(18), 2196-2200.
- Peters, E. M. (2003). Nutritional aspects in ultra-endurance exercise. *Current Opinion in Clinical Nutrition & Metabolic Care*, 6(4), 427-434.
- Petersen, S. E., Wiesmann, F., Hudsmith, L. E., Robson, M. D., Francis, J. M., Selvanayagam, J. B., et al. (2006). Functional and structural vascular remodeling in elite rowers assessed by cardiovascular magnetic resonance. *Journal of the American College of Cardiology*, 48(4), 790-797.
- Pini, R., Cavallini, M. C., Palmieri, V., Marchionni, N., Di Bari, M., Devereux, R. B., et al. (2008). Central But Not Brachial Blood Pressure Predicts Cardiovascular Events in an Unselected Geriatric Population The ICARE Dicomano Study. *Journal of the American College of Cardiology*, 51(25), 2432-2439.
- Powers, S. K., Deruisseau, K. C., Quindry, J. & Hamilton, K. L. (2004). Dietary antioxidants and exercise. *Journal of Sports Sciences*, 22(1), 81-94.
- Powers, S. K. & Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological Reviews*, 88(4), 1243-1276.
- Powers, S. K., Nelson, W. B. & Hudson, M. B. (2011). Exercise-induced oxidative stress in humans: cause and consequences. *Free Radical Biology and Medicine*, 51(5), 942-950.

- Predel, H. G. (2014). Marathon run: cardiovascular adaptation and cardiovascular risk. *European Heart Journal*, (35)44, 3091-3096.
- Prior, B. M., Yang, H. T. & Terjung, R. L. (2004). What makes vessels grow with exercise training?. *Journal of Applied Physiology*, 97(3), 1119-1128.
- Qin, X. J., He, W., Hai, C. X., Liang, X. & Liu, R. (2008). Protection of multiple antioxidants Chinese herbal medicine on the oxidative stress induced by adriamycin chemotherapy. *Journal of Applied Toxicology*, 28(3), 271-282.
- Quinn, T. J., Sprague, H. A., Van Huss, W. D. & Olson, H. W. (1990). Caloric expenditure, life status, and disease in former male athletes and non-athletes. *Medicine and Science in Sports and Exercise*, 22(6), 742-750.
- Rakobowchuk, M., Stuckey, M. I., Millar, P. J., Gurr, L. & MacDonald, M. J. (2009). Effect of acute sprint interval exercise on central and peripheral artery distensibility in young healthy males. *European journal of Applied Physiology*, 105(5), 787-795.
- Reference Values for Arterial Stiffness' Collaboration. (2010). Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *European Heart Journal*, 31(19), 2338-2350.
- Ring, M., Eriksson, M. J., Zierath, J. R. & Caidahl, K. (2014). Arterial stiffness estimation in healthy subjects: a validation of oscillometric (Arteriograph) and tonometric (SphygmoCor) techniques. *Hypertension Research*, 37(1), 999-1007.
- Ristow, M., Zarse, K., Oberbach, A., Klötting, N., Birringer, M., Kiehn topf, M. et al. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 106(210), 8665-8670.
- Robergs, R. A., Ghiasvand, F. & Parker, D. (2004). Biochemistry of exercise-induced metabolic acidosis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 287(3), 502-516.
- Rossiter, H. B., Kowalchuk, J. M. & Whipp, B. J. (2006). A test to establish maximum O<sub>2</sub> uptake despite no plateau in the O<sub>2</sub> uptake response to ramp incremental exercise. *Journal of Applied Physiology*, 100(3), 764-770.
- Rossow, L., Fahs, C. A., Guerra, M., Jae, S. Y., Heffernan, K. S. & Fernhall, B. (2010). Acute effects of supramaximal exercise on carotid artery compliance and pulse pressure in young men and women. *European Journal of Applied Physiology*, 110(4), 729-737.
- Rowe, W. J. (1992). Extraordinary unremitting endurance exercise and permanent injury to normal heart. *The Lancet*, 340(8821), 712-714.

- Rowley, N. J., Dawson, E. A., Birk, G. K., Cable, N. T., George, K., Whyte, G., et al. (2011). Exercise and arterial adaptation in humans: uncoupling localized and systemic effects. *Journal of Applied Physiology*, *110*(5), 1190-1195.
- Rowley, N. J., Dawson, E. A., Hopman, M. T., George, K. P., Whyte, G. P., Thijssen, D. H. & Green, D. J. (2012). Conduit diameter and wall remodeling in elite athletes and spinal cord injury. *Medicine and Science in Sports and Exercise*, *44*(5), 844-849.
- Rüst, C. A., Knechtle, B., Knechtle, P., Rosemann, T. & Lepers, R. (2011). Personal best times in an Olympic distance triathlon and in a marathon predict Ironman race time in recreational male triathletes. *Open Access Journal of Sports Medicine*, *2*, 121-129.
- Sahlén, A., Gustafsson, T. P., Svensson, J. E., Marklund, T., Winter, R., Linde, C., et al. (2009). Predisposing factors and consequences of elevated biomarker levels in long-distance runners aged ≥55 years. *The American Journal of Cardiology*, *104*(10), 1434-1440.
- Sala-Mercado, J. A., Hammond, R. L., Kim, J. K., Rossi, N. F., Stephenson, L. W. & O'Leary, D. S. (2006). Muscle metaboreflex control of ventricular contractility during dynamic exercise. *American Journal of Physiology-Heart and Circulatory Physiology*, *290*(2), 751-757.
- Salvi, P., Palombo, C., Salvi, G. M., Labat, C., Parati, G., & Benetos, A. (2013). Left ventricular ejection time, not heart rate, is an independent correlate of aortic pulse wave velocity. *Journal of Applied Physiology*, *115*(11), 1610-1617.
- Scalbert, A., Johnson, I. T. & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition*, *81*(1), 2155-2175.
- Scharhag, J., Löllgen, H. & Kindermann, W. (2013). Competitive sports and the heart: benefit or risk? *Deutsches Ärzteblatt International*, *110*(1-2), 14.
- Scharhag, J., Schneider, G., Urhausen, A., Rochette, V., Kramann, B. & Kindermann, W. (2002). Athlete's heart: right and left ventricular mass and function in male endurance athletes and untrained individuals determined by magnetic resonance imaging. *Journal of the American College of Cardiology*, *40*(10), 1856-1863.
- Scharhag-Rosenberger, F., Walitzek, S., Kindermann, W. & Meyer, T. (2012). Differences in adaptations to 1 year of aerobic endurance training: individual patterns of nonresponse. *Scandinavian Journal of Medicine & Science in Sports*, *22*(1), 113-118.
- Schnohr, P., Marott, J. L., Lange, P. & Jensen, G. B. (2013). Longevity in male and female joggers: the Copenhagen City Heart Study. *American Journal of Epidemiology*, *177*(7), 683-689.
- Schnohr, P., O'Keefe, J. H., Marott, J. L., Lange, P. & Jensen, G. B. (in press). Dose of jogging and long term mortality-the Copenhagen City heart study. *Journal of the American College of Cardiology*.

- Schütz, U. H., Schmidt-Trucksäss, A., Knechtle, B., Machann, J., Wiedelbach, H., Ehrhardt, M., et al. (2012). The Transeurope footrace project: longitudinal data acquisition in a cluster randomized mobile MRI observational cohort study on 44 endurance runners at a 64-stage 4,486 km transcontinental ultramarathon. *BMC Medicine*, *10*(1), 78.
- Schwartz R. S., Kraus, S. M., Schwartz, J. G., Wickstrom, K. K., Peichel, G., Garberich, R. F., et al. (2014). Increased coronary artery plaque volume among male marathon runners. *Missouri Medicine*, *111*(2), 85–90.
- Seals, D. R., DeSouza, C. A., Donato, A. J. & Tanaka, H. (2008). Habitual Exercise and Arterial Aging. *Journal of Applied Physiology*, *105*(4), 1323-1332.
- Seals, D. R., Walker, A. E., Pierce, G. L. & Lesniewski, L. A. (2009). Habitual exercise and vascular ageing. *The Journal of Physiology*, *587*(23), 5541-5549.
- Seifried, H. E., Anderson, D. E., Fisher, E. I. & Milner, J. A. (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. *The Journal of Nutritional Biochemistry*, *18*(9), 567-579.
- Sharma, S., Whyte, G., Elliott, P., Padula, M., Kaushal, R., Mahon, N., et al. (1999). Electrocardiographic changes in 1000 highly trained junior elite athletes. *British Journal of Sports Medicine*, *33*(5), 319-324.
- Sharman, J. E., Lim, R., Qasem, A. M., Coombes, J. S., Burgess, M. I., Franco, J., et al. (2006). Validation of a generalized transfer function to noninvasively derive central blood pressure during exercise. *Hypertension*, *47*(6), 1203-1208.
- Shave, R., Baggish, A., George, K., Wood, M., Scharhag, J., Whyte, G., et al. (2010). Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. *Journal of the American College of Cardiology*, *56*(3), 169-176.
- Shave, R. E., Dawson, E., Whyte, G., George, K., Ball, D., Gaze, D. C., et al. (2002). Evidence of exercise-induced cardiac dysfunction and elevated cTnT in separate cohorts competing in an ultra-endurance mountain marathon race. *International Journal of Sports Medicine*, *23*(07), 489-494.
- Shave, R., George, K. P., Atkinson, G. R. E. G., Hart, E. M. M. A., Middleton, N., Whyte, G., et al. (2007). Exercise-induced cardiac troponin T release: a meta-analysis. *Medicine and Science in Sports and Exercise*, *39*(12), 2099-2106.
- Shokawa, T., Imazu, M., Yamamoto, H., Toyofuku, M., Tasaki, N., Okimoto, T., et al. (2005). Pulse wave velocity predicts cardiovascular mortality: findings from the Hawaii-Los Angeles-Hiroshima study. *Circulation Journal: Japanese Circulation Society*, *69*(3), 259-264.

- Siebenhofer, A., Kemp, C., Sutton, A. & Williams, B. (1999). The reproducibility of central aortic blood pressure measurements in healthy subjects using applanation tonometry and sphygmocardiography. *Journal of Human Hypertension*, 13(9), 625-629.
- Sierksma, A., Lebrun, C. E., van der Schouw, Y. T., Grobbee, D. E., Lamberts, S. W., Hendriks, H. F. et al. (2004). Alcohol consumption in relation to aortic stiffness and aortic wave reflections: a cross-sectional study in healthy postmenopausal women. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 24(2), 342-348.
- Silver, A. E. & Vita, J. A. (2006). Shear Stress–Mediated Arterial Remodeling in Atherosclerosis Too Much of a Good Thing?. *Circulation*, 113(24), 2787-2789.
- Sindler, A. L., Delp, M. D., Reyes, R., Wu, G. & Muller-Delp, J. M. (2009). Effects of ageing and exercise training on eNOS uncoupling in skeletal muscle resistance arterioles. *The Journal of Physiology*, 587(15), 3885-3897.
- Skinner, J. S. & McLellan, T. H. (1980). The transition from aerobic to anaerobic metabolism. *Research Quarterly for Exercise and Sport*, 51(1), 234-248.
- Sokolow, M. & Lyon, T. P. (1949). The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. *American Heart Journal*, 37(2), 161-186.
- Somauroo, J. D., Pyatt, J. R., Jackson, M., Perry, R. A. & Ramsdale, D. R. (2001). An echocardiographic assessment of cardiac morphology and common ECG findings in teenage professional soccer players: reference ranges for use in screening. *Heart*, 85(6), 649-654.
- Spurway, N. & Jones, A. M. (2007). Lactate testing. In E. M. Winter, A. M. Jones, R. R. Davison, P. D. Bromley & T. H. Mercer (Eds.), *Sport and Exercise Physiology Testing Guidelines: Volume II—Exercise & Clinical Testing: The British Association of Sport and Exercise Sciences Guide*. (pp. 112-119). Abingdon: Routledge.
- Stehouwer, C. D. A., Henry, R. M. A. & Ferreira, I. (2008). Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. *Diabetologia*, 51(4), 527-539.
- Steinberg, D. (2009). The LDL modification hypothesis of atherogenesis: an update. *Journal of Lipid Research*, 50(1), 376-381.
- Stoner, L., Young, J. M. & Fryer, S. (2012). Assessments of arterial stiffness and endothelial function using pulse wave analysis. *International Journal of Vascular Medicine*, 2012(1), 1-9.
- St-Pierre, J., Buckingham, J. A., Roebuck, S. J. & Brand, M. D. (2002). Topology of superoxide production from different sites in the mitochondrial electron transport chain. *The Journal of Biological Chemistry*, 277(6), 44784-44790.
- Sugawara, J., Hayashi, K., Yokoi, T. & Tanaka, H. (2008). Age-associated elongation of the ascending aorta in adults. *Journal of the American College of Cardiology: Cardiovascular Imaging*, 1(6), 739-748.

- Tabachnick, B. G. & Fidell, S. F. (2013) *Using multivariate statistics* (6<sup>th</sup> Ed). Essex: Pearson Education Ltd
- Tanaka, H., DeSouza, C. A. & Seals, D. R. (1998). Absence of age-related increase in central arterial stiffness in physically active women. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *18*(1), 127-132.
- Tanaka, H., Dinunno, F. A., Monahan, K. D., Clevenger, C. M., DeSouza, C. A. & Seals, D. R. (2000). Aging, habitual exercise, and dynamic arterial compliance. *Circulation*, *102*(11), 1270-1275.
- Tanda, G. & Knechtle, B. (2013). Marathon performance in relation to body fat percentage and training indices in recreational male runners. *Open Access Journal of Sports Medicine*, *4*(1), 141-149.
- Tarnopolsky, M. A., Rennie, C. D., Robertshaw, H. A., Fedak-Tarnopolsky, S. N., Devries, M. C. & Hamadeh, M. J. (2007). Influence of endurance exercise training and sex on intramyocellular lipid and mitochondrial ultrastructure, substrate use, and mitochondrial enzyme activity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *292*(3), 1271-1278.
- Taylor, H. L., Buskirk, E. & Henschel, A. (1955). Maximal oxygen intake as an objective measure of cardio-respiratory performance. *Journal of Applied Physiology*, *8*(1), 73–80.
- Tee, J. C., Bosch, A. N. & Lambert, M. I. (2007). Metabolic consequences of exercise-induced muscle damage. *Sports Medicine*, *37*(10), 827-836.
- Thijssen, D. H., Cable, N. T. & Green, D. J. (2012). Impact of exercise training on arterial wall thickness in humans. *Clinical Science*, *122*(7), 311-322.
- Thompson, P. D. (2007). Cardiovascular adaptations to marathon running. *Sports Medicine*, *37*(4-5), 444-447.
- Tinken, T. M., Thijssen, D. H. J., Black, M. A., Cable, N. T. & Green, D. J. (2008). Time course of change in vasodilator function and capacity in response to exercise training in humans. *Journal of Physiology*, *586*(20), 5003-5012.
- Tinken, T. M., Thijssen, D. H., Hopkins, N., Dawson, E. A., Cable, N. T. & Green, D. J. (2010). Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension*, *55*(2), 312-318.
- Van den Elzen, A. P., Sierksma, A., Oren, A., Vos, L. E., Witteman, J. C., Grobbee, D. E., et al. (2005). Alcohol intake and aortic stiffness in young men and women. *Journal of Hypertension*, *23*(4), 731-735.
- Vappou, J., Luo, J., Okajima, K., Di Tullio, M. & Konofagou, E. (2011). Aortic pulse wave velocity measured by pulse wave imaging (PWI): a comparison with applanation tonometry. *Artery Research*, *5*(2), 65-71.

- Venables, M. C. & Jeukendrup, A. E. (2008). Endurance training and obesity: effect on substrate metabolism and insulin sensitivity. *Medicine and Science in Sports and Exercise*, 40(3), 495-502.
- Vihma, T. (2010). Effects of weather on the performance of marathon runners. *International Journal of Biometeorology*, 54(3), 297-306.
- Virmani, R., Burke, A. P., Farb, A. & Kark, J. A. (1997). Causes of sudden death in young and middle-aged competitive athletes. *Cardiology Clinics*, 15(3), 439-466.
- Viskin, S. (2009). The QT interval: too long, too short or just right. *Heart Rhythm*, 6(5), 711-715.
- Vlachopoulos, C., Aznaouridis, K. & Stefanadis, C. (2010). Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *Journal of the American College of Cardiology*, 55(13), 1318-1327.
- Vlachopoulos, C., Hirata, K. & O'Rourke, M. F. (2003). Effect of caffeine on aortic elastic properties and wave reflection. *Journal of Hypertension*, 21(3), 563-570.
- Vlachopoulos, C., Kardara, D., Anastasakis, A., Baou, K., Terentes-Printzios, D., Tousoulis, et al. (2010). Arterial stiffness and wave reflections in marathon runners. *American Journal of Hypertension*, 23(9), 974-979.
- Völgyi, E., Tylavsky, F. A., Lyytikäinen, A., Suominen, H., Alén, M. & Cheng, S. (2008). Assessing body composition with DXA and bioimpedance: effects of obesity, physical activity, and age. *Obesity*, 16(3), 700-705.
- Vollaard, N. B., Constantin-Teodosiu, D., Fredriksson, K., Rooyackers, O., Jansson, E., Greenhaff, P. L., et al. (2009). Systematic analysis of adaptations in aerobic capacity and submaximal energy metabolism provides a unique insight into determinants of human aerobic performance. *Journal of Applied Physiology*, 106(5), 1479-1486.
- Warburton, D. E., Nicol, C. W. & Bredin, S. S. (2006). Health benefits of physical activity: the evidence. *Canadian Medical Association Journal*, 174(6), 801-809.
- Weber, T., Auer, J., O'Rourke, M. F., Kvas, E., Lassnig, E., Berent, R. & Eber, B. (2004). Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation*, 109(2), 184-189.
- Wen, C. P., Wai, J. P. M., Tsai, M. K., Yang, Y. C., Cheng, T. Y. D., Lee, M. C., et al. (2011). Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. *The Lancet*, 378(9798), 1244-1253.
- Westerhof, N. & Westerhof, B. E. (2012). Wave transmission and reflection of waves "The myth is in their use". *Artery Research*, 6(1), 1-6.
- Westerhof, N., Lankhaar, J. W. & Westerhof, B. E. (2009). The arterial windkessel. *Medical & Biological Engineering & Computing*, 47(2), 131-141.

- White, W. B., Berson, A. S., Robbins, C., Jamieson, M. J., Prisant, L. M., Roccella, E., et al. (1993). National standard for measurement of resting and ambulatory blood pressures with automated sphygmomanometers. *Hypertension*, *21*(4), 504-509.
- Whyte, G. P., George, K., Shave, R., Middleton, N. & Nevill, A. M. (2008). Training induced changes in maximum heart rate. *International Journal of Sports Medicine*, *29*(02), 129-133.
- Widlansky, M. E., Gokce, N., Keane, J. F. & Vita, J. A. (2003). The clinical implications of endothelial dysfunction. *Journal of the American College of Cardiology*, *42*(7), 1149-1160.
- Wilcox, R. R. (2012). *Introduction to robust estimation and hypothesis testing*. London: Academic Press.
- Wilhelm, M., Rimensberger, C., Carlen, F., Brugger, N., Krause, R. & Seiler, C. (2013). Cardiac adaptations in non-elite ultra-endurance athletes: focus on the right ventricle and arrhythmias. *European Heart Journal*, *34*(1), 1786.
- Wilkinson, I. B., Fuchs, S. A., Jansen, I. M., Spratt, J. C., Murray, G. D., Cockcroft, J. R., et al. (1998). Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *Journal of Hypertension*, *16*(12), 2079-2084.
- Wilkinson, I. B., MacCallum, H., Flint, L., Cockcroft, J. R., Newby, D. E. & Webb, D. J. (2000). The influence of heart rate on augmentation index and central arterial pressure in humans. *The Journal of Physiology*, *525*(1), 263-270.
- Wilkinson, I. B., McEniery, C. M., Schillaci, G., Boutouyrie, P., Segers, P., Donald, A., et al. (2010). ARTERY Society guidelines for validation of non-invasive haemodynamic measurement devices: Part 1, arterial pulse wave velocity. *Artery Research*, *4*(2), 34-40.
- Wilkinson, I. B., Mohammad, N. H., Tyrrell, S., Hall, I. R., Webb, D. J., Paul, V. E., et al. (2002). Heart rate dependency of pulse pressure amplification and arterial stiffness. *American Journal of Hypertension*, *15*(1), 24-30.
- Williams, A. G., Day, S. H., Folland, J. P., Gohlke, P., Dhamrait, S. U. & Montgomery, H. E. (2005). Circulating angiotensin converting enzyme activity is correlated with muscle strength. *Medicine and Science in Sports and Exercise*, *37*(6), 944-948.
- Williams, B., Lacy, P. S., Thom, S. M., Cruickshank, K., Stanton, A., Collier, D., et al. (2006). Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) Study. *Circulation*, *113*(9), 1213-1225.
- Williams, C. A. & Bale, P. (1998). Bias and limits of agreement between hydrodensitometry, bioelectrical impedance and skinfold calipers measures of percentage body fat. *European Journal of Applied Physiology and Occupational Physiology*, *77*(3), 271-277.
- Wilmore, J. & Costill, D. (2005). *Physiology of Sport and Exercise* (3<sup>rd</sup> ed.). Champaign, IL: Human Kinetics.

- Wilmore, J. H., Stanforth, P. R., Gagnon, J., Leon, A. S., Rao, D. C., Skinner, J. S., et al. (1996). Endurance exercise training has a minimal effect on resting heart rate: the HERITAGE Study. *Medicine and Science in Sports and Exercise*, 28(7), 829-835.
- Wilson, M., O'Hanlon, R., Prasad, S., Deighan, A., MacMillan, P., Oxborough, D., et al. (2011). Diverse patterns of myocardial fibrosis in lifelong, veteran endurance athletes. *Journal of Applied Physiology*, 110(6), 1622-1626.
- Wilson, M., O'Hanlon, R., Prasad, S., Deighan, A., MacMillan, P., Oxborough, D., et al. (2011). Diverse patterns of myocardial fibrosis in lifelong, veteran endurance athletes. *Journal of Applied Physiology*, 110(6), 1622-1626.
- Wojciechowska, W., Staessen, J. A., Nawrot, T., Cwynar, M., Seidlerová, J., Stolarz, K., et al. (2006). Reference values in white Europeans for the arterial pulse wave recorded by means of the SphygmoCor device. *Hypertension Research*, 29(7), 475-483.
- Wortley, G. & Islas, A. A. (2011). The problem with ultra-endurance athletes. *British Journal of Sports Medicine*, 45(14), 1085-1085.
- Zaryski, C. & Smith, D. J. (2005). Training principles and issues for ultra-endurance athletes. *Current Sports Medicine Reports*, 4(3), 165-170.
- Zhao, Z., Luo, Y., Li, G., Zhu, L., Wang, Y. & Zhang, X. (2013). Thoracic aorta vasoreactivity in rats under exhaustive exercise: effects of Lycium barbarum polysaccharides supplementation. *Journal of the International Society of Sports Nutrition*, 10(1), 47-54.
- Zhou, S., Robson, S. J., King, M. J. & Davie, A. J. (1997). Correlations between short-course triathlon performance and physiological variables determined in laboratory cycle and treadmill tests. *The Journal of Sports Medicine and Physical Fitness*, 37(2), 122-130.
- Zieman, S. J., Melenovsky, V. & Kass, D. A. (2005). Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 25(5), 932-943.
- Zierath, J. R., & Hawley, J. A. (2004). Skeletal muscle fiber type: influence on contractile and metabolic properties. *PLoS biology*, 2(10), 348.
- Zuo, J. L., Li, Y., Yan, Z. J., Zhang, R. Y., Shen, W. F., Zhu, D. L., et al. (2010). Validation of the central blood pressure estimation by the SphygmoCor system in Chinese. *Blood Pressure Monitoring*, 15(5), 268-274.

## Acknowledgements

I am incredibly thankful for the opportunity to undergo this research project; it has been a great experience to work so closely with Mike and Justin over the last couple of years. I should also thank Justin for enticing me to undertake the event, the hard work was definitely worth it. Likewise, I have tremendous appreciation for the laboratory technicians and the 30 plus investigators who ensured the study ran as smoothly as possible, with special mentions to Brad, John, Craig and Lucy; the help and advice has been invaluable.

The individuals who volunteered huge amounts of time and effort to participate in this study were a pleasure to work with and I'm very happy to have shared the journey with you all.

My family have provided enormous support throughout this process and I am so appreciative for them, seeing me over the finish line quite literally.

## Appendices

### Appendix 1: Study 2 Participant Information Sheet for Triathletes

UNIVERSITY OF HERTFORDSHIRE

ETHICS COMMITTEE FOR STUDIES INVOLVING THE USE OF HUMAN PARTICIPANTS  
(‘ETHICS COMMITTEE’)

## FORM EC6: PARTICIPANT INFORMATION SHEET

### Title of Research

The Influence of Ultra-Endurance Exercise on the Cardiovascular and Related Physiological Systems.

### Introduction

You are being invited to take part in a research study. Before you decide whether to do so, it is important that you understand the research that is being done and what your involvement will include. Please take the time to read the following information carefully and discuss it with others if you wish. Do not hesitate to ask us anything that is not clear or for any further information you would like to help you make your decision. Please do take your time to decide whether or not you wish to take part. Thank you for reading this.

### What is the purpose of this study?

Participation trends in ultra-endurance events have increased several fold in the past decade. As such, there has been an increased interest in providing scientific research in the area. An important aspect of this research is the health benefits/disadvantages that occur as a consequence of participating in an extreme endurance event and also the accumulative effect of excessive training. In addition, limited research focuses on individuals who undertake longer distance events from a relatively 'novice' or 'recreational' background. The heart is of particular interest to healthcare practitioners due to the problems that are associated with pathological functioning and exercise such as exercise-induced sudden cardiac death.

Emerging data has suggested that excessive endurance training may induce pathologic structural remodelling of the heart and large arteries in a small subgroup of individuals (Mastaloudis et al., 2001). Epidemiological studies have identified an increased relative risk of cardiovascular disease, hypertension, and risk of death in those participating in the most aerobic exercise indicative of a detrimental, accumulative, effect of excess exercise over time (Quinn et al., 1990; Paffenbarger et al., 1986). However, it has been suggested that these responses may in fact be mitigated in ultra-endurance athletes as a result of exercise-induced adaptations (Knez et al., 2006). This statement is supported by many studies presenting a transient cardiovascular dysfunction post ultra-endurance exercise, however, Neilan, et al. (2006) recently found a more prolonged dysfunction in a small number of subjects.

As you are aware, we are currently undertaking a longitudinal research study to investigate the effects of habitual endurance training on a UK cohort of recreationally active individuals who will be taking part in their first iron-distance triathlon (which involves a 3.8km swim, 180km cycle, and 42.2km run, in succession in one day). The study has previously been granted ethical approval for Phase 1 (protocol number: LS6/9/12SRA multi-disciplinary

investigation in the effects of sustained aerobic training in healthy, recreational individuals preparing for a long-distance triathlon). During Phase 1, you have been monitored for a number of parameters ranging from cardiovascular and physiological variables, immunological status, nutritional and bone mineral density variables, psychological 'mental toughness', functional movement and biomechanical assessment.

The aim of the forthcoming study will be to investigate the cardiovascular and cardiac-related response to completing an ironman distance triathlon; including the short-term and more long-term consequences. Several aspects will be considered including nutrition, supplementation, training, sleep, and where available the use of clinical feedback such as oxidative stress, inflammation, and hormonal function (through the use of Genova Diagnostics kits which has received ethical approval (Study 1: protocol number: LMS/SF/UH/00010 The influence of Dietary Intake on Functional Nutrition Biomarkers and Exercise Performance in Recreationally Trained Individuals. Study 2: protocol number: LMS/SF/UH/00011 The Effect of Alpha Lipoic Acid and Probiotic Supplementation on Gastrointestinal Permeability, Endotoxemia and Exercise Performance in recreationally Trained Individuals).

### **Aims of project**

#### **The key aims of the project include:**

1. To investigate the effects of prolonged endurance exercise on cardiovascular and cardiac-related variables in multiple subgroups.
2. To investigate the effects of dietary intake and prolonged endurance exercise on cardiovascular and cardiac-related variables.
3. To investigate the relationship between various diagnostic biomarkers and cardiovascular/cardiac-related variables over a period of prolonged endurance exercise.

### **Do I have to take part?**

It is completely up to you whether or not you decide to take part in this study. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. Agreeing to join the study does not mean that you have to complete it. You are free to withdraw at any stage without giving a reason. A decision to withdraw at any time, or a decision not to take part at all, will not affect the rest of the treatment/care that you receive.

### **What will happen to me if I take part?**

If you decide to take part in this study you will be asked to:

1. Attend the University of Hertfordshire 1 week prior to the race to carry out non-invasive assessments of the cardiovascular system which includes the following measurements:  
12 lead electrocardiogram (ECG)  
Pulse wave analysis, using SphygmoCor  
Pulse wave velocity, using SphygmoCor
2. Less than 16 hours post-event (ie. the next day) you will be asked to attend a location in Cayella, nearby to the race finish, whereby pulse wave analysis and pulse wave velocity measurements will be carried out.
3. 1 week post-event you will be asked to attend the University of Hertfordshire for another cardiovascular assessment which repeats (1) above.
4. 4 weeks post-event you will be asked to attend the University of Hertfordshire for one last cardiovascular assessment which repeats (1) above.

Each session you attend will take around 30 minutes of your time.

### **Preparation**

- Please avoid alcoholic substances and caffeinated substances such as coffee/tea, chocolate and energy drinks 12 hours prior to your appointment.
- Please refrain from exercising 12 hours prior to your appointment.
- Provide the investigator with current medication and/or supplementation on each occasion.

### **What is electrocardiography (ECG) and what is required of me?**

ECG is a non-invasive procedure used to record the electrical activity of the heart over a short period of time.

### **During the procedure**

- You will be asked to lie down and an investigator will clean several areas of your upper body. Small patches called electrodes will be attached to these areas which will be connected by cables to the ECG apparatus.
- You are required to remain still during the procedure which should not take long.
- When the procedure is finished the wires will be disconnected by the investigator and you will be free to remove the electrodes.

### **What is SphygmaCor and what is required of me?**

A SphygmaCor is a tool used to provide a comprehensive yet non-invasive assessment of the cardiovascular system. By recording various pulse waveforms and using several equations the equipment is able to calculate various important cardiovascular parameters.

### **Pulse Wave Analysis (PWA)**

Pulse waveforms are obtained through a measurement taken at the wrist. Central blood pressures among other parameters are then calculated using several carefully designed equations.

### **During the procedure**

- Your peripheral blood pressure will be obtained prior to the procedure. A cuff will be placed around your arm which will inflate fairly quickly, then deflate at a slower rate.
- You will be asked to sit with your arm resting on a stable surface with your palm facing upwards.
- The investigator will place a tonometer (probe) over the lower part of your wrist until an acceptable 10 second reading has been obtained.

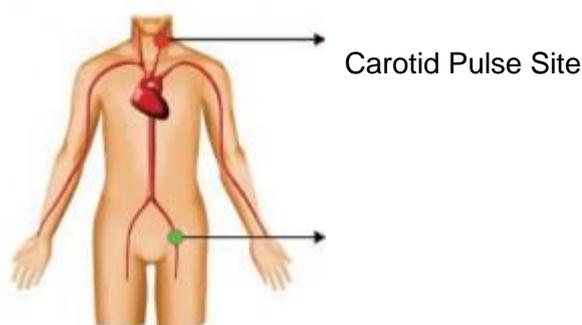


### **Pulse Wave Velocity (PWV)**

PWV is used to estimate arterial stiffness by measuring the velocity of the blood pressure waveform between two superficial artery sites. Pulse waveforms are obtained through measurements taken at the carotid and femoral site. A 3 cable ECG will be recorded simultaneously to act as a timing reference.

### **During the procedure**

- Your peripheral blood pressure will be obtained prior to the procedure. A cuff will be placed around your arm which will inflate fairly quickly, then deflate at a slower rate.



- You will be asked to lie face up on a stable surface.
- Three electrodes will be placed on your chest which will be connected by cables to the SphygmoCor equipment.
- The investigator will place a tonometer (probe) over the carotid artery until an acceptable 11 second reading has been obtained. (See diagram above for site location.)
- The investigator will place a tonometer (probe) over the femoral artery until an acceptable 11 second reading has been obtained. (See diagram above for site location.)

In addition, you will be asked to keep a record of training sessions over the 4 week period after the race. A template for this will be provided.

### **What are the possible disadvantages, risks or side effects of taking part?**

#### **Electrocardiography (ECG)**

Participants may be required to shave the chest area where electrodes are will be placed. You may experience slight discomfort upon removal of the electrodes. No electricity is sent through the body, so there is no risk of shock.

#### **SphygmoCor**

There should be no pain associated with the use of this equipment, if there is any discomfort please inform the investigator.

**Exercise Training/ Race Performance** – as part of undertaking this study, you have already entered the 2013 Barcelona Challenge Triathlon, which is an ultra-distance event. You are already undertaking progressive endurance training under the guidance of an Accredited Sport and Exercise physiologist, where the risks of exertional fatigue, musculoskeletal injury and soreness have been previously outlined, and you have been made aware that you undertake such training at your own liability and responsibility. Such risks are minimized if you adhere to the progressive nature of the training, and discuss any concerns with the lead researcher.

In entering the race you have already agreed to the terms and conditions outlined by the race director, including rules and regulations for competing in this event. Participation in this event is undertaken at your own liability and responsibility as previously outlined at the beginning of Phase 1. Competing in an ultra-endurance race is both physically and mentally challenging, and carries the risk of fatigue, dehydration, exhaustion, gastrointestinal distress, nausea, dizziness, collapse and musculoskeletal injury. However, the potential for these risks have been reduced in that all participants are recreationally trained, and by October 2013 will be specifically race trained (so long as you maintain the training programme). All participants will be covered by medical services provided during and immediately after the race event. All participants will have signed a race event waiver to this effect.

### **What are the possible benefits of taking part?**

By participating in the exercise training appropriately you will benefit in terms of suitable preparation for the 2013 Barcelona Challenge Triathlon. All participants will also benefit in terms of personal knowledge of the test data once analysed. Participants will be debriefed post study and if requested will receive feedback in terms of cardiovascular function. If necessary participants will be advised to take further action with their general practitioner.

### **How will my taking part in this study be kept confidential?**

The information that is obtained during the study will follow ethical and legal practice and handled in strict confidence. The information obtained, however, will be used for statistical analysis with your right to privacy retained. All information will be anonymised; that is, all figures and numbers will not be traceable to you and personal details will be removed. All data you provide will be stored on a password protected computer.

### **What will happen to the results of the research study?**

If you agree to take part, your results will be stored on a password protected computer and disk drive, with your name and other details removed. All paper data will be locked in a filing cabinet in a room which will be locked when it is not being occupied by a member of the research team at University of Hertfordshire. Hopefully, we expect that the data will be published in a scientific journal. On publication of results it will not be possible to identify individual participants and all personal data will be destroyed upon completion.

### **Who has reviewed this study?**

This research study has been reviewed by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favorable opinion by the Hertfordshire University Ethics Committee. The study design and suitability has been discussed with Dr. Michael Roberts and Dr. Justin Roberts.

### **Who can I contact if I have any questions?**

If you would like further information or would like to discuss any details personally, please get in touch with me, in writing, by phone or by email:

#### **Principal Investigator:**

Tony Dawkins  
52 Goldings Road, Loughton, Essex, IG10 2QN  
07906272430  
[tonydawkins@hotmail.co.uk](mailto:tonydawkins@hotmail.co.uk)

**Although we hope it is not the case, if you have any complaints or concerns about any aspect of the way you have been approached or treated during the course of this study, please write to the University Secretary and Registrar.**

**Thank you very much for reading this information and giving consideration to taking part in this study.**

## Appendix 2: Study 2 Participant Information Sheet for Control Group

UNIVERSITY OF HERTFORDSHIRE

ETHICS COMMITTEE FOR STUDIES INVOLVING THE USE OF HUMAN PARTICIPANTS  
(‘ETHICS COMMITTEE’)

### FORM EC6: PARTICIPANT INFORMATION SHEET

#### Title of Research

The Influence of Ultra-Endurance Exercise on the Cardiovascular and Related Physiological Systems.

#### Introduction

You are being invited to take part in a research study. Before you decide whether to do so, it is important that you understand the research that is being done and what your involvement will include. Please take the time to read the following information carefully and discuss it with others if you wish. Do not hesitate to ask us anything that is not clear or for any further information you would like to help you make your decision. Please do take your time to decide whether or not you wish to take part. Thank you for reading this.

#### What is the purpose of this study?

Participation trends in ultra-endurance events have increased several fold in the past decade. As such, there has been an increased interest in providing scientific research in the area. An important aspect of this research is the health benefits/disadvantages that occur as a consequence of participating in an extreme endurance event and also the accumulative effect of excessive training. In addition, limited research focuses on individuals who undertake longer distance events from a relatively 'novice' or 'recreational' background. The heart is of particular interest to healthcare practitioners due to the problems that are associated with pathological functioning and exercise such as exercise-induced sudden cardiac death.

Emerging data has suggested that excessive endurance training may induce pathologic structural remodelling of the heart and large arteries in a small subgroup of individuals (Mastaloudis et al., 2001). Epidemiological studies have identified an increased relative risk of cardiovascular disease, hypertension, and risk of death in those participating in the most aerobic exercise indicative of a detrimental, accumulative, effect of excess exercise over time (Quinn et al., 1990; Paffenbarger et al., 1986). However, it has been suggested that these responses may in fact be mitigated in ultra-endurance athletes as a result of exercise-induced adaptations (Knez et al., 2006). This statement is supported by many studies presenting a transient cardiovascular dysfunction post ultra-endurance exercise, however, Neilan, et al. (2006) recently found a more prolonged dysfunction in a small number of subjects.

The aim of the forthcoming study will be to investigate the cardiovascular and cardiac-related response to completing an ironman distance triathlon; including the short-term and more long-term consequences. You are asked to take part in this study to act as part of the control population, you will not be required to complete an ultra-endurance event.

## **Aims of project**

### **The key aims of the project include:**

4. To investigate the effects of prolonged endurance exercise on cardiovascular and cardiac-related variables in multiple subgroups.
5. To investigate the effects of dietary intake and prolonged endurance exercise on cardiovascular and cardiac-related variables.
6. To investigate the relationship between various diagnostic biomarkers and cardiovascular/cardiac-related variables over a period of prolonged endurance exercise.

### **Do I have to take part?**

It is completely up to you whether or not you decide to take part in this study. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. Agreeing to join the study does not mean that you have to complete it. You are free to withdraw at any stage without giving a reason. A decision to withdraw at any time, or a decision not to take part at all, will not affect the rest of the treatment/care that you receive.

### **What will happen to me if I take part?**

If you decide to take part in this study you will be asked to:

5. Attend the university of Hertfordshire 4 times over a 5 week period to carry out non-invasive assessments of the cardiovascular system which includes the following measurements:
  - 12 lead electrocardiogram (ECG)
  - Pulse wave analysis, using SphygmoCor
  - Pulse wave velocity, using SphygmoCor

Measurements will be obtained at baseline, one week, two weeks and five weeks.

### **Preparation**

- Please avoid alcoholic substances and caffeinated substances such as coffee/tea, chocolate and energy drinks 12 hours prior to your appointment.
- Please refrain from exercising 12 hours prior to your appointment.
- Provide the investigator with current medication and/or supplementation on each occasion.

### **What is electrocardiography (ECG) and what is required of me?**

ECG is a non-invasive procedure used to record the electrical activity of the heart over a short period of time.

#### **During the procedure**

- You will be asked to lie down and an investigator will clean several areas of your upper body. Small patches called electrodes will be attached to these areas which will be connected by cables to the ECG apparatus.
- You are required to remain still during the procedure which should not take long.
- When the procedure is finished the wires will be disconnected by the investigator and you will be free to remove the electrodes.

### **What is SphycmaCor and what is required of me?**

A SphygmaCor is a tool used to provide a comprehensive yet non-invasive assessment of the cardiovascular system. By recording various pulse waveforms and using

several equations the equipment is able to calculate various important cardiovascular parameters.

### **Pulse Wave Analysis (PWA)**

Pulse waveforms are obtained through a measurement taken at the wrist. Central blood pressures among other parameters are then calculated using several carefully designed equations.

#### During the procedure

- Your peripheral blood pressure will be obtained prior to the procedure. A cuff will be placed around your arm which will inflate fairly quickly, then deflate at a slower rate.
- You will be asked to sit with your arm resting on a stable surface with your palm facing upwards.
- The investigator will place a tonometer (probe) over the lower part of your wrist until an acceptable 10 second reading has been obtained.

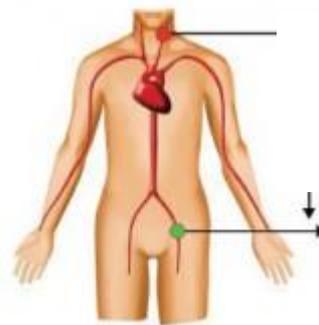


### **Pulse Wave Velocity (PWV)**

PWV is used to estimate arterial stiffness by measuring the velocity of the blood pressure waveform between two superficial artery sites. Pulse waveforms are obtained through measurements taken at the carotid and femoral site. A 3 cable ECG will be recorded simultaneously to act as a timing reference.

#### During the procedure

- Your peripheral blood pressure will be obtained prior to the procedure. A cuff will be placed around your arm which will inflate fairly quickly, then deflate at a slower rate.
- You will be asked to lie face up on a stable surface.
- Three electrodes will be placed on your chest which will be connected by cables to the SphygmoCor equipment.
- The investigator will place a tonometer (probe) over the carotid artery until an acceptable 11 second reading has been obtained. (See diagram above for site location.)
- The investigator will place a tonometer (probe) over the femoral artery until an acceptable 11 second reading has been obtained. (See diagram above for site location.)



Carotid Pulse Site

In addition, template will be provided for you to keep a record of training sessions over the testing period.

### **What are the possible disadvantages, risks or side effects of taking part?**

#### **Electrocardiography (ECG)**

Participants may be required to shave the chest area where electrodes are will be placed. You may experience slight discomfort upon removal of the electrodes. No electricity is sent through the body, so there is no risk of shock.

**SphygmoCor**

There should be no pain associated with the use of this equipment, if there is any discomfort please inform the investigator.

**What are the possible benefits of taking part?**

All participants will benefit in terms of personal knowledge of the test data once analysed. Participants will be debriefed post study and if requested will receive feedback in terms of cardiovascular function. If necessary participants will be advised to take further action with their general practitioner.

**How will my taking part in this study be kept confidential?**

The information that is obtained during the study will follow ethical and legal practice and handled in strict confidence. The information obtained, however, will be used for statistical analysis with your right to privacy retained. All information will be anonymised; that is, all figures and numbers will not be traceable to you and personal details will be removed. All data you provide will be stored on a password protected computer.

**What will happen to the results of the research study?**

If you agree to take part, your results will be stored on a password protected computer and disk drive, with your name and other details removed. All paper data will be locked in a filing cabinet in a room which will be locked when it is not being occupied by a member of the research team at University of Hertfordshire. Hopefully, we expect that the data will be published in a scientific journal. On publication of results it will not be possible to identify individual participants and all personal data will be destroyed upon completion.

**Who has reviewed this study?**

This research study has been reviewed by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favorable opinion by the Hertfordshire University Ethics Committee. The study design and suitability has been discussed with Dr. Michael Roberts and Dr. Justin Roberts.

**Who can I contact if I have any questions?**

If you would like further information or would like to discuss any details personally, please get in touch with me, in writing, by phone or by email:

**Principal Investigator:**

Tony Dawkins  
52 Goldings Road, Loughton, Essex, IG10 2QN  
07906272430  
[tonydawkins@hotmail.co.uk](mailto:tonydawkins@hotmail.co.uk)

**Although we hope it is not the case, if you have any complaints or concerns about any aspect of the way you have been approached or treated during the course of this study, please write to the University Secretary and Registrar.**

**Thank you very much for reading this information and giving consideration to taking part in this study.**

Appendix 3: Study 2 Consent Form

FORM EC3

University of Hertfordshire

CONSENT FORM FOR STUDIES INVOLVING HUMAN PARTICIPANTS

I, the undersigned [please give your name here, in BLOCK CAPITALS]

of [please give contact details here, sufficient to enable the investigator to get in touch with you, such as a postal or email address]

hereby freely agree to take part in the study entitled :

The Influence of Ultra-Endurance Exercise on the Cardiovascular and Related Physiological Systems.

1 I confirm that I have been given a Participant Information Sheet (a copy of which is attached to this form) giving particulars of the study, including its aim(s), methods and design, the names and contact details of key people and, as appropriate, the risks and potential benefits, and any plans for follow-up studies that might involve further approaches to participants. I have been given details of my involvement in the study. I have been told that in the event of any significant change to the aim(s) or design of the study I will be informed, and asked to renew my consent to participate in it.

2 I have been assured that I may withdraw from the study at any time without disadvantage or having to give a reason.

3 I have been given information about the risks of my suffering harm or adverse effects. I have been told about the aftercare and support that will be offered to me in the event of this happening, and I have been assured that all such aftercare or support would be provided at no cost to myself.

4 I have been told how information relating to me (data obtained in the course of the study, and data provided by me about myself) will be handled: how it will be kept secure, who will have access to it, and how it will or may be used.

5 I confirm that my questions regarding involvement with this study have been answered to my satisfaction.

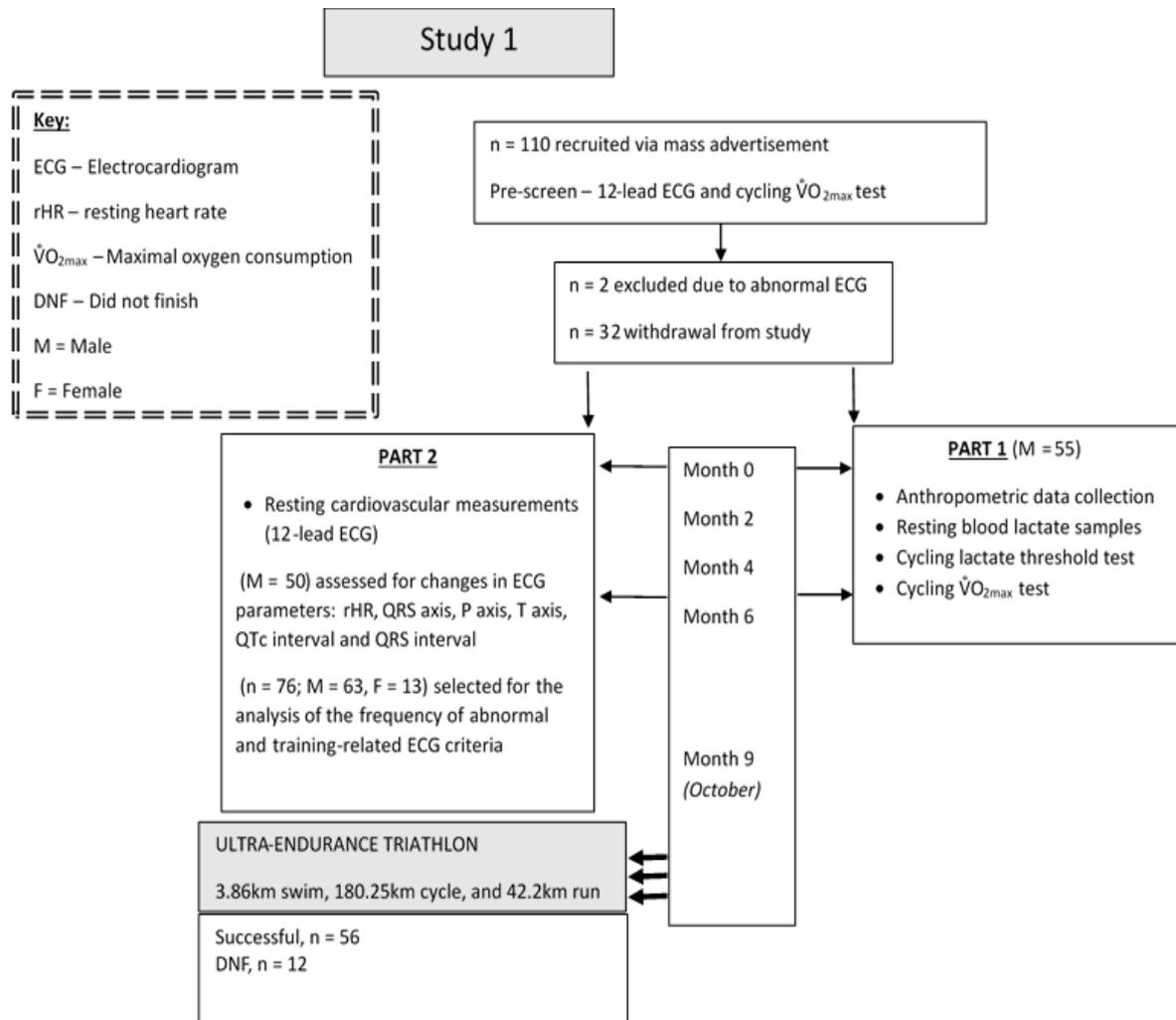
Signature of participant.....Date.....

Signature of (principal) investigator..... Date.....

Name of (principal) investigator [in BLOCK CAPITALS please]

.....

### Appendix 4: Flow Diagram of Study 1 Method



## Appendix 5: Study 1 Descriptive Data

### Part 1

Descriptive statistics of physiological data over 6 month period of a progressive training programme, mean  $\pm$  SD.

Measurement	Age Category	Month 0	Month 2	Month 4	Month 6
Relative $\dot{V}O_{2max}$ (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	18 to 32	49.7 $\pm$ 6.2	52.6 $\pm$ 6.7	53.4 $\pm$ 5.2	54.6 $\pm$ 6.1
	33 to 38	46.9 $\pm$ 6.0	50.4 $\pm$ 5.9	50.4 $\pm$ 6.2	52.0 $\pm$ 5.6
	39 to 50	43.4 $\pm$ 4.7	47.7 $\pm$ 4.8	49.0 $\pm$ 5.7	49.6 $\pm$ 4.4
	Total (n = 55)	46.6 $\pm$ 6.1	<sup>a</sup> 50.1 $\pm$ 6.1	<sup>a</sup> 50.9 $\pm$ 5.9	<sup>a</sup> 52.0 $\pm$ 5.6
Absolute $\dot{V}O_{2max}$ (L·min <sup>-1</sup> )	18 to 32	3.666 $\pm$ 0.399	3.867 $\pm$ 0.487	3.928 $\pm$ 0.467	3.981 $\pm$ 0.411
	33 to 38	3.855 $\pm$ 0.523	4.103 $\pm$ 0.575	4.077 $\pm$ 0.512	4.173 $\pm$ 0.431
	39 to 50	3.529 $\pm$ 0.403	3.885 $\pm$ 0.579	3.998 $\pm$ 0.550	3.968 $\pm$ 0.530
	Total (n = 55)	3.676 $\pm$ 0.454	<sup>a</sup> 3.946 $\pm$ 0.549	<sup>a</sup> 3.999 $\pm$ 0.506	<sup>a</sup> 4.035 $\pm$ 0.464
Maximal Heart Rate (beats·min <sup>-1</sup> )	18 to 32	189 $\pm$ 9	187 $\pm$ 10	187 $\pm$ 9	187 $\pm$ 10
	33 to 38	182 $\pm$ 6	182 $\pm$ 6	180 $\pm$ 7	181 $\pm$ 9
	39 to 50	181 $\pm$ 9	180 $\pm$ 8	179 $\pm$ 9	178 $\pm$ 8
	Total (n = 55)	184 $\pm$ 9	183 $\pm$ 9	<sup>a</sup> 182 $\pm$ 9	182 $\pm$ 9
Max Power (Watts)	18 to 32	275 $\pm$ 35	284 $\pm$ 29	295 $\pm$ 32	299 $\pm$ 33
	33 to 38	279 $\pm$ 33	299 $\pm$ 34	309 $\pm$ 36	318 $\pm$ 34
	39 to 50	270 $\pm$ 40	286 $\pm$ 48	294 $\pm$ 44	303 $\pm$ 41
	Total (n = 55)	274 $\pm$ 36	<sup>a</sup> 289 $\pm$ 38	<sup>a,b</sup> 299 $\pm$ 38	<sup>a,b,c</sup> 306 $\pm$ 37
Body Fat (%)	18 to 32	16.5 $\pm$ 4.9	15.9 $\pm$ 5.1	15.4 $\pm$ 4.5	14.9 $\pm$ 5.0
	33 to 38	18.3 $\pm$ 5.9	17.3 $\pm$ 5.3	16.7 $\pm$ 5.4	15.8 $\pm$ 5.8
	39 to 50	20.7 $\pm$ 5.6	19.6 $\pm$ 5.3	18.8 $\pm$ 5.1	17.8 $\pm$ 5.1
	Total (n = 55)	18.6 $\pm$ 5.7	17.7 $\pm$ 5.3	17.1 $\pm$ 5.1	16.3 $\pm$ 5.4
Weight	18 to 32	73.9 $\pm$ 7.4	74.1 $\pm$ 7.3	74.1 $\pm$ 7.1	73.5 $\pm$ 7.3
	33 to 38	82.4 $\pm$ 9	81.5 $\pm$ 8.4	81.5 $\pm$ 8.3	80.3 $\pm$ 7.7
	39 to 50	81.9 $\pm$ 11.6	81.6 $\pm$ 11.7	80.6 $\pm$ 11.6	80.3 $\pm$ 11.4
	Total (n = 55)	79.4 $\pm$ 10.2	79.1 $\pm$ 9.9	78.8 $\pm$ 9.7	78.1 $\pm$ 9.5

$\dot{V}O_{2max}$  = maximal volume of oxygen consumption

Age category, 18 to 32 (n = 18); 33 to 38 (n = 17); 39 to 50 (n = 20). <sup>a</sup> significantly different from month 0, p < 0.05; <sup>b</sup> significantly different from month 2, p < 0.05; <sup>c</sup> significantly different from month 4, p < 0.05.

## Part 2

Quartile representation of 12-lead ECG variables over the course of a 6 month incremental training programme with Friedman test significance and rank (n = 50).

ECG variable	Time-point	Median (IQR)	Rank
Resting Heart Rate (beats·min <sup>-1</sup> )	Month 0	59 (52 to 65)	3.21
	Month 2	<sup>a</sup> 56 (48 to 61)	2.41
	Month 4	<sup>a</sup> 53 (47 to 61)	2.34
	Month 6	<sup>a</sup> 50 (47 to 58)	2.04
QRS Complex Axis (°)	Month 0	52 (44 to 62)	2.58
	Month 2	52 (35 to 65)	2.36
	Month 4	53 (40 to 61)	2.27
	Month 6	55 (42 to 62)	2.79
P Wave Axis (°)	Month 0	76 (54 to 84)	2.48
	Month 2	72 (59 to 84)	2.32
	Month 4	76 (60 to 84)	2.56
	Month 6	75 (61 to 87)	2.64
T Wave Axis (°)	Month 0	61 (46 to 70)	2.60
	Month 2	58 (42 to 69)	2.48
	Month 4	56 (41 to 70)	2.34
	Month 6	59 (45 to 74)	2.58
QTc Interval (ms)	Month 0	409 (393 to 424)	2.63
	Month 2	422 (404 to 444)	3.16
	Month 4	<sup>b</sup> 404 (388 to 420)	2.28
	Month 6	<sup>b</sup> 402 (384 to 418)	1.93
QTc dispersion (ms)	Month 0	32 (26 to 44)	2.46
	Month 2	31 (26 to 43)	2.64
	Month 4	30 (20 to 44)	2.36
	Month 6	32 (25 to 43)	2.54
QRS Interval (ms)	Month 0	102 (98 to 106)	2.46
	Month 2	101 (96 to 105)	2.31
	Month 4	102 (97 to 106)	2.43
	Month 6	103 (98 to 105)	2.80

QTc = QT interval corrected for a heart rate of 60 beats·min<sup>-1</sup>. Significance level set to 0.008 after Bonferroni adjustment for multiple comparisons (0.05/6).

<sup>a</sup> significantly different from month 0, p < 0.008; <sup>b</sup> significantly different from month 2, p < 0.008.

## Appendix 6: Study 2 Supplementation Information

Daily supplementation was provided by Biocare LTD for study 2 (chapter six). Ingredients for each supplement are listed below, whilst nutritional information regarding each supplement is provided below.

- An adult multinutrient – Adult multivitamin and minerals formula (Biocare Ltd., UK) - 1 capsule per day (containing a broad spectrum multinutrient with high bioavailability, taken with breakfast).
- An essential fatty acid formula – Mega EPA (Biocare Ltd., UK) – 2 capsules per day containing a total of 524mg of eicosapentaenoic acid (EPA) and 375mg of docosahexaenoic acid (DHA), taken with meals.
- An antioxidant formula – Antioxidant Complex (Biocare Ltd., UK) – 1 capsule per day containing 150mg quercetin, 50mg turmeric extract, 15mg lycopene powder, 15mg of green tea extract, 15mg of grapeseed extract and 8mg lutein powder, taken with meals.
- A probiotic formula – Bio-acidophilus (Biocare Ltd., UK) – 2 capsules per day containing a total of 80mg.d<sup>-1</sup> lactobacillus acidophilus. Taken with food in the afternoon/ evening.

Phase 3 supplementation information for individual products supplemented daily 12 weeks prior to iron-distance triathlon.

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### Adult Multivitamin and Minerals Formula

Ingredient	Amount per Capsule
Vitamin C	240.9mg
Pantothenic Acid (Vitamin B5)	100mg
Niacin (Vitamin B3)	50mg NE
Vitamin E 75iu natural source	50mg
Thiamine (Vitamin B1)	25mg
Riboflavin (Vitamin B2)	25mg
Vitamin B6 (Cobalamin)	25mg
Magnesium	22mg
Inositol	12mg
P.A.B.A. (para amino benzoic acid)	10mg
Potassium	8.9mg
Zinc	8.5mg
Calcium	6mg
Vitamin A	600mcg RAE

Folic Acid	400mcg
Manganese	300mcg
Molybdenum	98.7mcg
Selenium	50mcg
Chromium	50mcg
Iodine	37.8mcg
Biotin	35mcg
Cobalamin (vitamin B12)	30mcg
Ergocalciferol (vitamin D2)	6.25mcg

**Antioxidant Complex**

Quercetin	150mg
Turmeric Extract	50mg
Lycopene Powder	15mg
Green Tea Extract (Camellia sinensis)	15mg
Grapeseed Extract (Vitaflavan®)	15mg
Lutein Powder	8mg

**Mega EPA**

Fish Oil Concentrate	2333mg
Of which EPA	524mg
Of which DHA	375mg
Natural Mixed Tocopherols	10mg

**Bioacidophilus (Probiotic Formula)**

Fructooligosacharrides	200mg
Lactobacillus acidophilus	80mg
Bifidobacterium bifidum &	12mg
Bifidobacterium lactis	

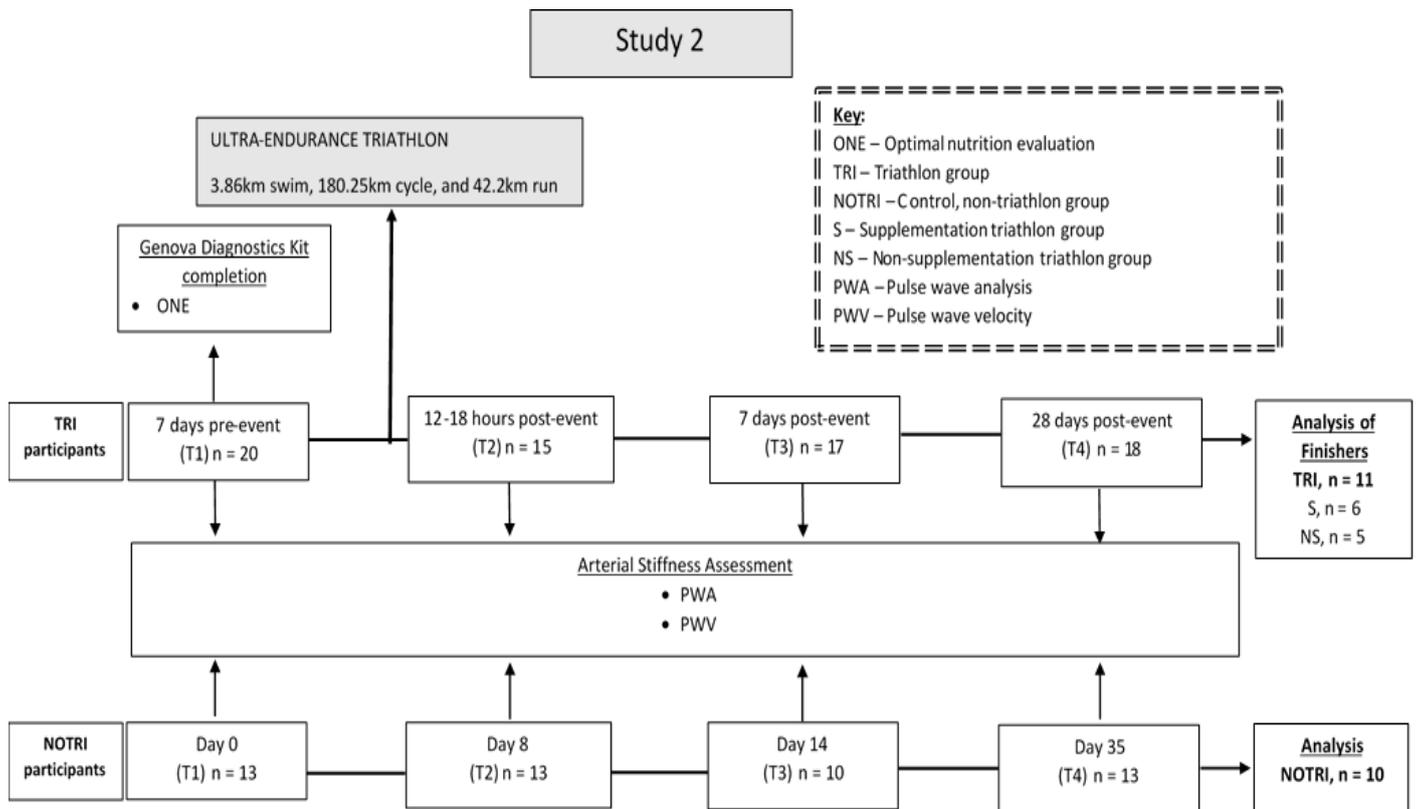
**B Vitamin Complex**

Thiamine (vitamin B1)	50mg
Riboflavin (vitamin B2)	50mg
Niacin (Vitamin B3)	50mg

Pantothenic Acid (vitamin B5)	50mg
Folic acid (vitamin B6)	50mg
Vitamin C	40mg
Choline	30mg
Inositol	30mg
L-Glycine	30mg
P.A.B.A (para amino benzoic acid)	30mg
Magnesium	9.5mg
Folic Acid	400mcg
Biotin	200mcg
Cobalamin (vitamin B12)	50mcg

NE = niacin equivalent, iu = international units, RAE = retinol activity equivalent, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid.

## Appendix 7: Flow Diagram of Study 2 Method



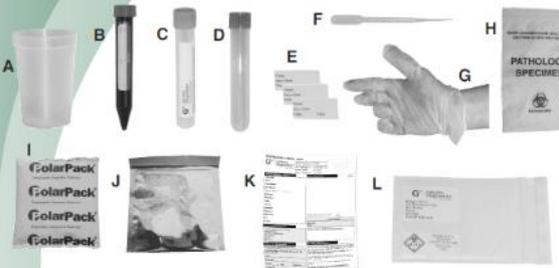
## Appendix 8: Genova Optimal Nutrition Evaluation Details



**Genova Diagnostics<sup>®</sup> Europe**  
Innovative Testing for Optimal Health

### Optimal Nutritional Evaluation

#### Patient Instructions for Urine Collection



**Check Your Kit**

A - 1 Urine collection cup	G - 1 Glove
B - 1 Blue-top amber tube	H - 1 Biohazard bag and absorbent pad
C - 1 Green-top preservative tube	I - 1 Gel freezer brick
D - 1 Green-top urine tube	J - 1 Silver pouch
E - 3 Collection labels	K - 1 Requisition (to be completed and signed)
F - 1 Pipette	L - 1 Mailing envelope

- If any of the items are missing or expired, call the laboratory on 020 8336 7750.
- The appearance of the above kit components are for illustration use only and actual contents may vary slightly from that shown.

### Step 1:

#### Important things to know and consider

- Abnormal kidney function or use of diuretics may influence test results. This test should not be performed on individuals with kidney disorders. In addition, certain medicines may impact test results [e.g. adrenal steroids including corticosteroids, aminoglycoside antibiotics, amphetamines, antihistamines, cephalosporins, cimetidine (Tagamet), fibrates (e.g. Ciprofibrate), penicillin or D-penicillamine, and trimethoprim]. Let your practitioner know about your use of these medications. Do not change use of medications unless instructed to do so by your healthcare provider.
- 4 Days before the test discontinue all of the following (unless instructed otherwise by your practitioner): Non-essential medications including acetaminophen and over-the-counter cold remedies; any vitamins, minerals, amino acids, and herbal supplements taken regularly – including enhanced sports drinks, energy drinks, and vitamin waters.
- 2 days before the test: (unless instructed otherwise by your practitioner) Discontinue creatine, alpha-ketoglutarate, and malic acid supplements, as well as citrate, malate, or orotate forms of minerals.
- 24 hrs before the test: Avoid eating or drinking any products containing aspartame (Nutra-Sweet, Splenda, Canderel) and monosodium glutamate (MSG), and avoid over-consuming any single food. Otherwise, eat your usual diet.
- It is essential to avoid excessive fluid intake for the 24 hours prior to collection. Aim to drink no more than an average fluid intake of 1.5 litres, spread throughout the day.

#### Schedule and prepare for your urine collection

- Female patients should not collect urine during a menstrual period.
- Fast overnight (at least 8 hours) prior to the urine collection.
- Samples must be frozen for at least 2 hours prior to returning. Please note: samples completed on Friday – Sunday should be stored frozen until Monday for returning to the laboratory by overnight delivery.
- Freeze the enclosed gel freezer brick a minimum of 4 hours before sending.
- Please be aware that this test is not suitable for children under the age of 18 months.
- Complete the requisition form with all patient and payment information. Be sure it is signed by the patient/ responsible party in the box labelled "Final Sample Date / Time."

### Step 2:

#### Collecting your urine specimen

Not following these instructions may affect your test results.

**CAUTIONS:** Do not discard powder or liquid in tubes. Avoid contact of the eyes or skin with the powder or liquid in the tubes. For contact with the eyes, wash thoroughly for 15 minutes. For skin contact, wash thoroughly with soap and water. Do not inhale or ingest powder or liquid. For accidental ingestion, contact your healthcare provider at once.



- 1** Write your full name (first and last), date of birth, time and date of collection on each label using a ballpoint pen or pencil only. Attach the labels to all collection tubes.



- 2** After awakening for the day, collect your first morning urine\* into the cup provided. After filling the cup, pass any additional urine into the toilet.  
\*If you wake up to urinate during the night within six hours before your rising time, collect your urine and keep it cool (away from direct heat), then add that sample to the urine you collect when you rise for the day. You may wish to use a disposable cup or other clean container (cleaned with hot water only).



- 3** Do not switch the lids of the tubes. This will invalidate the test. Opening one tube at a time, use the pipette to transfer urine from the collection cup into the tubes until each is nearly full.



- 4** Recap the tubes tightly and shake them to mix thoroughly.



- 5** Place the filled tubes into the biohazard bag and freeze for a minimum of 2 hours prior to sending.

Consult your healthcare provider if you have any questions at any time during this test.

### Step 3:

#### Returning The Samples To The Laboratory

Samples must be returned to Genova Diagnostics by overnight delivery to arrive next day.

**For UK mainland patients only:** Genova Diagnostics recommends using the enclosed courier collection service, or alternative e.g.: Royal Mail, Special Delivery. The mailing envelope is not pre-paid.

**For patients outside of the UK:** please arrange an international courier such as FedEx, DHL, UPS.

Please note: the laboratory is closed at weekends. Samples should not be in transit over the weekend; please arrange for the samples to be sent overnight and to arrive no later than Friday.

- Plan to return the samples Monday – Thursday by overnight delivery only.
- If samples are completed on a Friday - Sunday, please keep them frozen until Monday and return it on Monday for Tuesday.
- Make sure the collection tubes are tightly closed and identified with completed labels. Failure to complete the labels correctly will result in the samples being rejected.
- Put the tubes in the biohazard bag with absorbent paper and seal it securely.
- Place the biohazard bag in the silver pouch with the frozen freezer brick.
- Place the sealed silver pouch inside the mailing envelope with your completed and signed requisition form.
- Seal the envelope.
- Once posting/couriering of samples has been arranged, keep any tracking numbers issued by the courier / Royal Mail for future reference.

## Appendix 9: Study 2 Descriptive Data

Table. Descriptive statistics of participant characteristics and cardiovascular measurements of triathletes and recreationally active participants.

Measurement	Time-Point	Triathletes			Recreationally Active
		NS (n = 6)	S (n = 5)	Overall (n = 11)	NOTRI n = 10
PWV	T1 – 7d Pre	5.9 ± 0.6	6.0 ± 0.6	5.9 ± 0.6	5.6 ± 0.8
	T2 – 12-18h Post	6.2 ± 0.3	6.1 ± 0.6	6.1 ± 0.4	5.5 ± 0.6
	T3 – 7d Post	6.1 ± 0.7	6.4 ± 0.8	6.3 ± 0.7	5.6 ± 0.6
	T4 – 28d Post	6.0 ± 0.6	6.1 ± 0.6	6.1 ± 0.6	5.8 ± 0.8
Aix	T1 – 7d Pre	11.7 ± 14.3	18.2 ± 9.1	14.6 ± 12.1	10.0 ± 10.4
	T2 – 12-18h Post	11.0 ± 9.8	7.0 ± 9.7	9.2 ± 9.5	12.7 ± 7.6
	T3 – 7d Post	3.3 ± 15.1	9.4 ± 9.8	6.1 ± 12.7	7.9 ± 7.9
	T4 – 28d Post	9.3 ± 12.5	13.4 ± 6.7	11.2 ± 10.0	12.2 ± 8.2
Aix@HR75	T1 – 7d Pre	2.8 ± 12.8	8.8 ± 7.1	5.5 ± 10.5	5.5 ± 9.8
	T2 – 12-18h Post	5.8 ± 8.6	6.8 ± 4.9	6.3 ± 6.9	5.8 ± 7.6
	T3 – 7d Post	0.8 ± 13.5	2.6 ± 5.7	1.6 ± 10.3	2.9 ± 8.1
	T4 – 28d Post	3.3 ± 12.7	4.4 ± 6.1	3.8 ± 9.8	5.4 ± 6.9
Resting HR	T1 – 7d Pre	55 ± 6	55 ± 9	56 ± 7	63 ± 13
	T2 – 12-18h Post	64 ± 5	74 ± 11	69 ± 9	56 ± 11
	T3 – 7d Post	67 ± 5	61 ± 10	65 ± 8	60 ± 8
	T4 – 28d Post	61 ± 10	56 ± 6	58 ± 9	61 ± 10
aSBP (mmHg)	T1 – 7d Pre	110 ± 12	108 ± 12	109 ± 12	103 ± 11
	T2 – 12-18h Post	105 ± 6	100 ± 10	103 ± 8	103 ± 11
	T3 – 7d Post	106 ± 10	110 ± 11	108 ± 10	102 ± 8
	T4 – 28d Post	101 ± 10	107 ± 9	103 ± 9	101 ± 7
aDBP (mmHg)	T1 – 7d Pre	72 ± 4	74 ± 8	73 ± 6	70 ± 11
	T2 – 12-18h Post	73 ± 7	67 ± 6	70 ± 7	67 ± 11
	T3 – 7d Post	75 ± 6	73 ± 5	74 ± 6	67 ± 9
	T4 – 28d Post	69 ± 9	71 ± 5	70 ± 7	67 ± 8
pSBP (mmHg)	1 – 7d Pre	124 ± 6	120 ± 11	122 ± 8	119 ± 11
	2 – 18h Post	122 ± 9	117 ± 9	119 ± 9	119 ± 11
	3 – 7d Post	124 ± 9	121 ± 6	122 ± 8	119 ± 8
	4 – 28d Post	16 ± 8	120 ± 6	118 ± 7	118 ± 8
pDBP (mmHg)	1 – 7d Pre	71 ± 5	73 ± 7	72 ± 6	69 ± 10
	2 – 18h Post	72 ± 7	65 ± 6	69 ± 7	66 ± 11
	3 – 7d Post	73 ± 6	73 ± 5	73 ± 5	66 ± 9
	4 – 28d Post	68 ± 9	66 ± 9	69 ± 7	66 ± 8
LVED (ms)	T1 – 7d Pre	322 ± 15	308 ± 24	316 ± 20	301 ± 21
	T2 – 12-18h Post	309 ± 18	284 ± 16	298 ± 21	311 ± 21
	T3 – 7d Post	291 ± 22	287 ± 10	290 ± 17	306 ± 17
	T4 – 28d Post	304 ± 19	301 ± 23	302 ± 20	293 ± 21
Weight (kg)	1 – 7d Pre	70.8 ± 10.6	77.6 ± 9.6	73.9 ± 10.3	72.7 ± 5.6
	2 – 18h Post	70.7 ± 10.6	77.0 ± 9.9	73.5 ± 10.3	72.9 ± 5.5
	3 – 7d Post	70.0 ± 10.6	77.0 ± 9.4	73.2 ± 10.2	73.1 ± 5.7
	4 – 28d Post	70.5 ± 10.1	78.0 ± 10.0	73.9 ± 10.3	72.0 ± 5.6

NS = non-supplementation group; S = supplementation group; aSBP = aortic systolic blood pressure; aDBP = aortic diastolic blood pressure; pSBP = peripheral systolic blood pressure; pDBP = peripheral diastolic blood pressure; LVED = left ventricular ejection duration.

## Appendix 10: Demonstration of SphygmoCor Equipment in Use



The SphygmoCor equipment being used during the pulse wave analysis (PWA) protocol with the participants radial artery of the left arm.



Electrode set up for electrocardiography (ECG) during pulse wave velocity (PWV) measurement.

Electrodes were positioned on the suprasternal notch, the xiphoid process and the lower left side of the abdominal trunk according to the SphygmoCor manual.

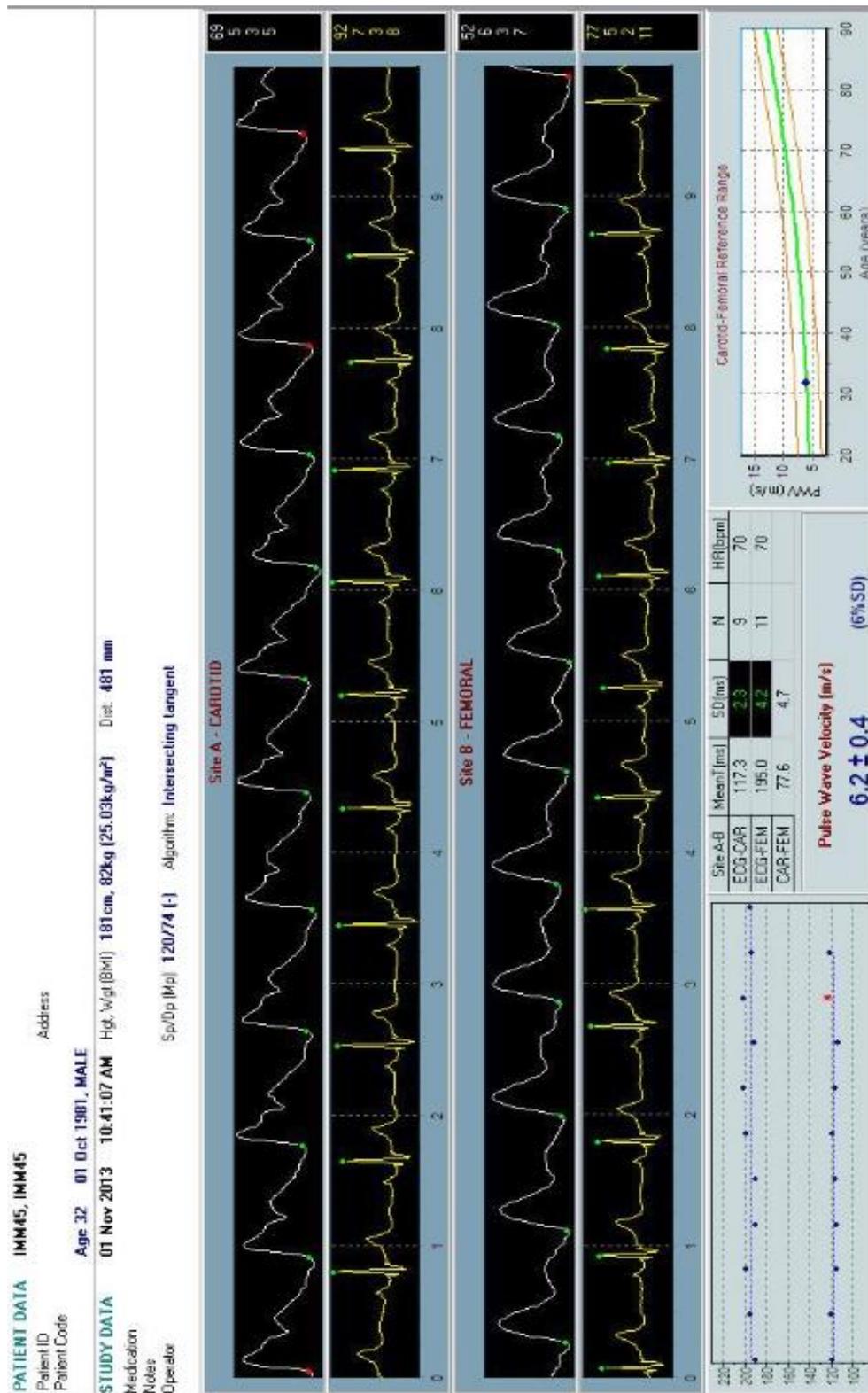
R wave of ECG used as a timing reference for the calculation of PWV since carotid and femoral pressure pulse waves cannot be obtained simultaneously with this procedure.



Partial occlusion of the carotid artery (site A) with the tonometer, attached to the SphygmoCor equipment, during the pulse wave velocity (PWV) procedure.

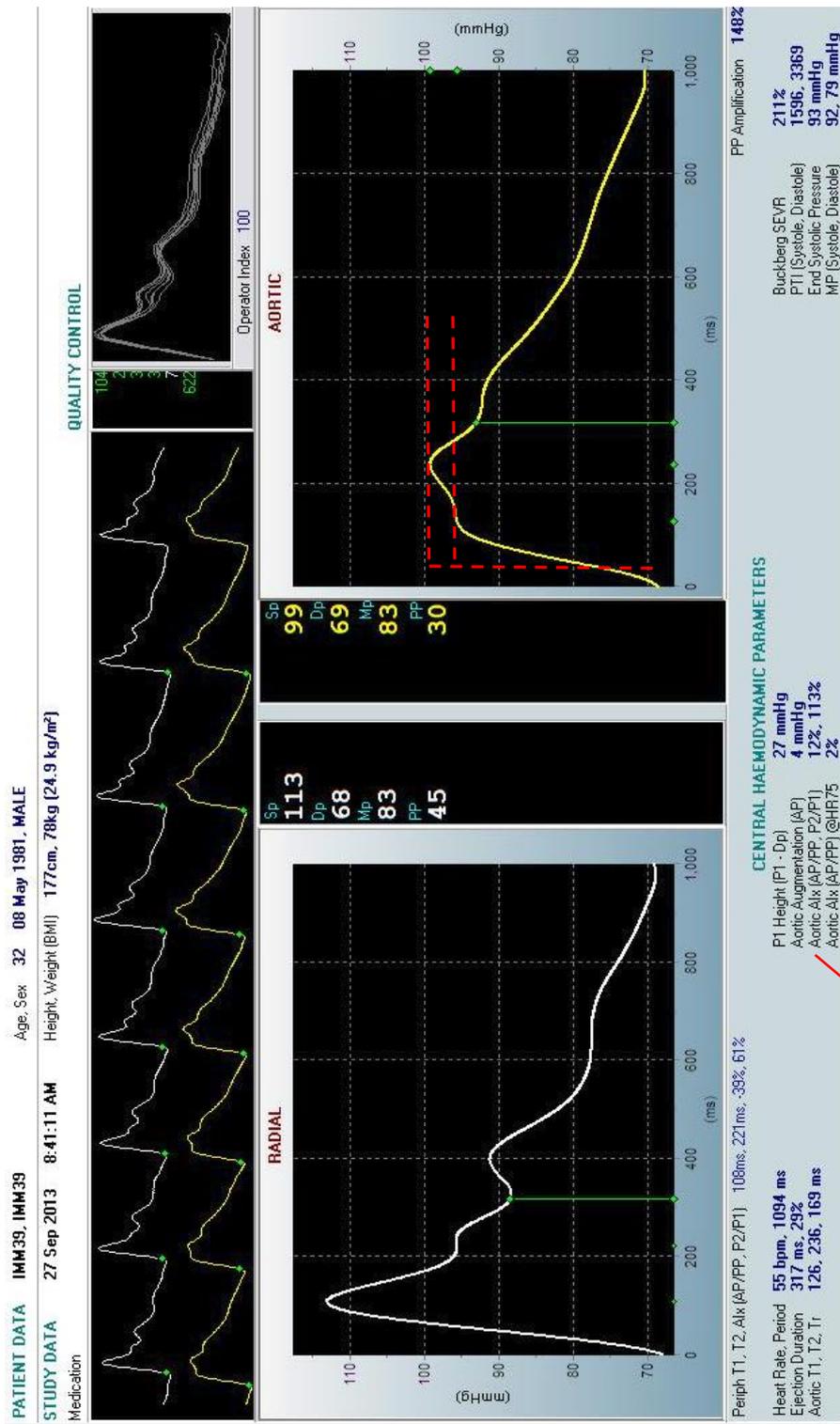
Subsequent measurement of the femoral artery (site B) would be conducted once appropriate site A recordings are obtained.

Appendix 11: Demonstration of pulse wave velocity output from SphygmoCor



Example of pulse wave velocity (PWV) output screen from SphygmoCor apparatus. PWV assessed via the foot-to-foot method. Sequential measurements obtained at the common carotid and femoral artery with simultaneous 3-cable ECG for timing reference (located below both carotid and femoral pulse waves).

Appendix 12: Demonstration of pulse wave analysis output from SphygmoCor



Aortic augmentation index (Aix) =

$$\frac{AP}{PP}$$

Example of pulse wave analysis (PWA) output screen from SphygmoCor apparatus, displaying aortic augmentation index (Aix), Aix corrected for a heart rate of 75 beats·min<sup>-1</sup>, aortic systolic and diastolic blood pressure and ejection duration.

Measurements obtained via applanation tonometry from the right radial artery. The inbuilt generalised transfer function was utilised to produce aortic values from peripheral measurements.