

The favourable effects of community-based, mixed-intensity aerobic interval training on arterial stiffness and structure

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ABSTRACT

Background: Literature suggests that arterial disorders account for up to 80% of cardiovascular disease (CVD)-related deaths and that approximately 40% of the cardioprotective effects of aerobic exercise (AE) are due to the benefits it confers on vascular haemodynamics. Longitudinal laboratory-based studies have demonstrated that AE and interval training can improve numerous indices of arterial health, thereby combating early vascular ageing and reducing CVD risk. However, no study has investigated the arterial health benefits conferred by concurrent aerobic interval exercise carried out in the 'real-world', that is, in pre-existing community settings whereby individuals are required to self-regulate the exercise intensity.

Objective: To determine the effects of a community-based, self-paced mixed-intensity cycling intervention on arterial health indices in healthy, sedentary men.

Method: An 8 week repeated-measures intervention design was adopted. Fifteen apparently healthy, sedentary, young to middle-aged adult males (31.8 ± 6.1 years) participated and were split into intervention ($n=10$) and control groups ($n=5$). The intervention group undertook 45 minutes of self-paced aerobic interval training 3 times a week for 8 weeks. The gymnasium-based indoor cycling intervention was based on principles of AE interspersed with both high-intensity interval training (HIIT) and sprint interval training (SIT) within a single session. Control participants maintained their routine lifestyles for 8 weeks. A range of measures were determined at baseline (PRE), after 4 weeks (MID) and post-intervention (POST). Resting arterial health indices assessed pertained to target organ damage-related tissue biomarkers of early vascular ageing and included bilateral operative arterial stiffness (carotid-femoral pulse wave velocity, cfPWV), wave reflections (augmentation index, $Alx@75$), central haemodynamics (central pulse pressure, cPP), wall thickness (carotid intima-media thickness, cIMT; femoral intima-media thickness, fIMT) and arterial geometry (carotid end-diastolic diameter, cEDD and carotid wall:lumen ratio, cWLR). The $Alx@75$ and cPP were measured using a specialised oscillometric device whilst all other indices were assessed by ultrasonography.

Results: The average heart rate during the self-regulated sessions was $81 \pm 7\%$ HR_{peak}. Significant improvements in VO_{2peak}, arterial health, BMI, waist circumference, resting heart rate and resting blood pressure were observed in the intervention group only. The VO_{2peak} increased by $15.1 \pm 8.3\%$ ($p < 0.001$, $p\text{Eta}^2 = 0.74$) from PRE (33.4 ± 5.4 ml/kg/min) to POST (38.3 ± 5.8 ml/kg/min), right cfPWV decreased by 10.7% (CI 8.07-12.07) from PRE (8.65 ± 0.36 m/s) to POST (7.72 ± 0.43 m/s) ($p < 0.001$, $p\text{Eta}^2 = 0.97$), $Alx@75$ improved by 23.8% (CI 8.7-38.8, $p = 0.006$, $p\text{Eta}^2 = 0.59$), cIMT and fIMT showed 12.2% and 13.1% decreases respectively ($p < 0.05$), cEDD increased by 7.2% ($p < 0.05$) and cWLR decreased by 18.5% ($p < 0.05$). At POST, there were significant between-group differences in VO_{2peak} ($p = 0.034$, $p\text{Eta}^2 = 0.03$), cfPWV ($p < 0.001$, $p\text{Eta}^2 = 0.66$), cPP ($p = 0.015$, $p\text{Eta}^2 = 0.38$), fIMT ($p = 0.046$, $p\text{Eta}^2 = 0.74$), cEDD ($p = 0.022$, $p\text{Eta}^2 = 0.034$) and cWLR ($p = 0.048$, $p\text{Eta}^2 = 0.48$). VO_{2peak} and cfPWV were negatively related at POST ($r = -0.54$, $p < 0.05$).

Conclusions and perspectives: In healthy, previously sedentary, young to middle-aged male adults, self-paced cycling incorporating different modalities of interval training significantly improves cardiorespiratory fitness and tissue biomarkers of early vascular ageing in addition to causing systemic outward arterial remodelling. The adaptations observed are associated with an improved CV risk profile, indicating the high responsiveness of this population to concurrent aerobic interval training. The present results are consistent with those of previous controlled laboratory-based studies and demonstrate the feasibility and effectiveness of a 'real-world' community-based exercise approach to enhance arterial health.

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ATTESTATION OF AUTHORSHIP

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person, (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been accepted for the award of any other degree or diploma of a university or other institution of higher learning.



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ETHICAL APPROVAL

Ethical Approval for this research was granted by the Auckland University of Technology Ethics Committee (AUTEC). The AUTEC reference was 14/242 (Appendix A), with approval granted originally on 26TH August 2014.

NOMENCLATURE

Δ delta	(% improvement)
AE	aerobic exercise
AGEs	advanced glycation end-products
AIT	aerobic interval training
AIx	augmentation index (%)
AIx@75	augmentation index standardised to a heart rate of 75 beats per minute (%)
ANS	autonomic nervous system
AP	augmentation pressure (mmHg)
AS	arterial stiffness
BMI	body mass index (kg/m ²)
BP	blood pressure (mmHg)
BRS	baroreflex sensitivity
cBP	central blood pressure (mmHg)
CCA	common carotid artery
cEDD	carotid end-diastolic diameter (mm)
CFA	common femoral artery
cfPWV	carotid-femoral pulse wave velocity (m/s)
cIMT	carotid intima-media thickness (mm)
cPP	central pulse pressure (mmHg)
CRF	cardiorespiratory fitness
cRI	carotid resistivity index
CV	cardiovascular
CVD	cardiovascular disease
cWLR	carotid wall:lumen ratio
DBP	diastolic blood pressure (mmHg)
ECM	extracellular matrix
eNOS	endothelial-derived nitric oxide synthase
ET-1	endothelin-1
EVA	early vascular ageing
fIMT	femoral intima-media thickness (mm)
FMD	flow-mediated dilation
fRI	femoral resistivity index
FRS	Framingham Risk Score
HDL-C	high-density lipoprotein cholesterol
HIIT	high-intensity interval training
HR	heart rate (beats per minute, bpm)

HR _{peak}	peak individually-determined heart rate (beats per minute)
IMT	intima-media thickness (mm)
IPAQ	International Physical Activity Questionnaire
LDL-C	low-density lipoprotein cholesterol
LMI	Les Mills International
MA/O	middle-aged and older adults
MAP	mean arterial pressure (mmHg)
MCAE	moderate continuous aerobic exercise
MS	metabolic syndrome
NO	nitric oxide
PAR-Q	Physical Activity Readiness Questionnaire
pBP	peripheral blood pressure (mmHg)
PP	pulse pressure (mmHg)
pPP	peripheral pulse pressure (mmHg)
PWV	pulse wave velocity
RAAS	renin-angiotensin-aldosterone system
RE	resistance exercise
RFs	risk factors
RI	resistivity index
RPE	Rating of perceived exertion
RPM™	Raw Power in Motion (intervention exercise)
SBP	systolic blood pressure (mmHg)
SIT	sprint interval training
SNA	sympathetic nerve activity
TC	total cholesterol
TC/HDL	total cholesterol:high-density lipoprotein cholesterol ratio
TGs	triglycerides
TPR	total peripheral resistance
US	ultrasound
VO _{2peak}	peak oxygen consumption (ml/kg/min)
VSMCs	vascular smooth muscle cells
WC	waist circumference (cm)
WLR	wall:lumen ratio

CHAPTER 1: INTRODUCTION

1.1 PROJECT CONTEXT

Cardiovascular disease (CVD) and arterial health

Inadequate risk stratification obstructs the prevention of cardiovascular disease (CVD) (1), the leading cause of mortality in modern societies (2). Traditional risk factors (RFs) such as increasing age, hypertension, dyslipidaemia, obesity, physical inactivity, type II diabetes and tobacco use account for 75–90% of the risk of developing CVD (3). Many of these RFs alter arterial structure, properties and function (4), which is significant given that arterial disorders account for up to 80% of all CVD-related deaths (5). Arterial structure and function are modified during the normal vascular ageing process (6), but in some individuals, this process is accelerated (7) and compounded by atherosclerosis-based pathological vascular ageing (8). This is known as premature or early vascular ageing (EVA), and it increases predisposition to cardiovascular (CV) events (7). Direct damage to arterial walls probably explains the remaining CVD risk which cannot be attributed to the aforementioned traditional risk factors (9) and therefore, their optimal functioning is necessary to maintain the integrity of the arterial system.

Fortunately, most CV disorders progress gradually (10) and many of the specific surrogate indices of arterial health can be measured non-invasively during the sub-clinical stages of CVD, before overt clinical manifestations (11). As most CVD-related disorders are associated with the natural ageing process, risk stratification is improved by the determination of EVA via the assessment of tissue biomarkers associated with target organ damage (12). These biomarkers include arterial stiffness, arterial wall thickness, endothelial function and central pulse pressure (systolic-diastolic blood pressure). The importance of assessing these facets lies in the fact that they are hallmark features of CVD and related conditions which have significant global morbidity and mortality rates. Assessment of circulating biomarkers (traditional CV risk factors) alone only gives a snapshot of arterial wall damage (12). Figure 1.1 depicts the relationships between traditional CV risk factors, biomarkers and EVA status. Ideally, EVA should be prevented by lifestyle interventions which can decrease both traditional CV risk factors and tissue biomarkers.

The composition of each of the three layers of the arterial wall (tunica intima, media and adventitia) varies along the heterogenous arterial tree with large, central, conduit arteries being more elastic and peripheral ones more muscular (13). Progressive and passive damage to the elastic arteries predisposes individuals to CVD (14). Arterial health status is ultimately monitored to enable CVD risk stratification and CV event prediction, to diagnose early stages of CVD-related disorders and to track the progress of pharmacological or non-pharmacological (lifestyle) therapies (12). Due to this increasing awareness of arterial health in the pathogenesis of CVD, there is continuing research into reliable and valid techniques to measure and quantify it (15).

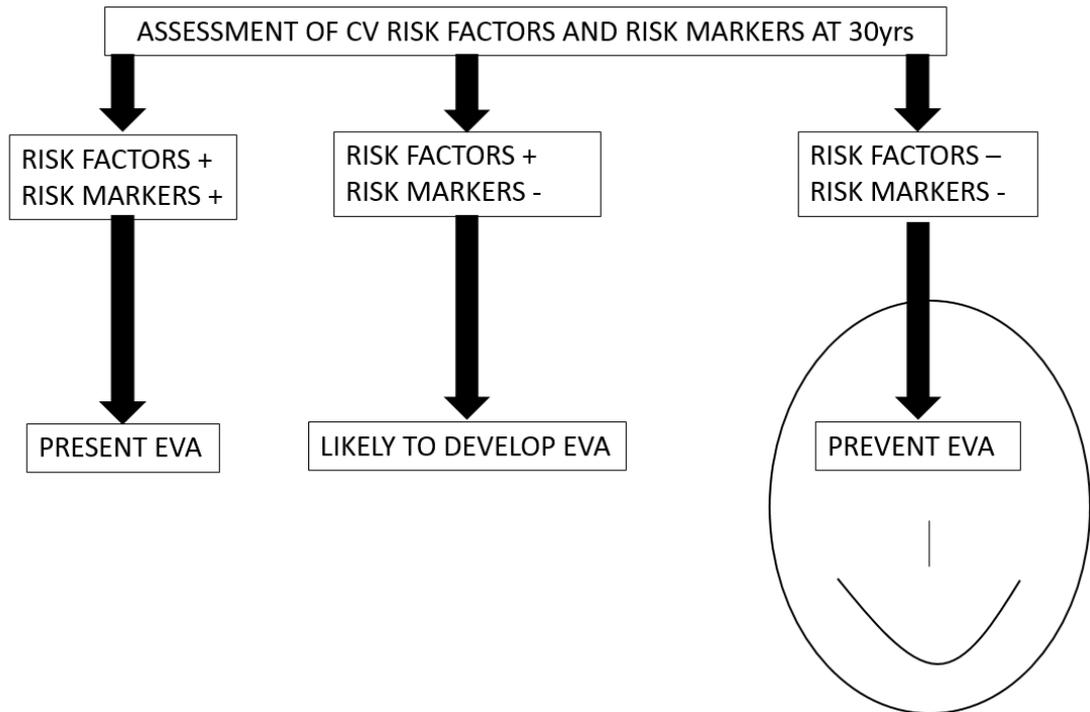


Figure 1.1: Assessment of vascular age status using traditional cardiovascular risk factors and novel biomarkers

Cardiovascular risk stratification and prevention opportunities are optimised if traditional CV risk factors and tissue biomarkers are assessed simultaneously in young adulthood. EVA, early vascular ageing

The umbrella term ‘arterial health’, refers to both structural and functional aspects of the arterial system. In the present study, the term ‘arterial health’ refers to the operative stiffness of arterial walls (pulse wave velocity), wave reflections (augmentation index), central haemodynamics (central pulse pressure), structural arterial wall modifications (intima-media thickness) and arterial geometry (end-diastolic diameter and wall:lumen ratio). Arterial stiffness is the primary determinant of left ventricular afterload (16) and is influenced by both structural and functional aspects but it is important to note that arterial stiffness, structural modifications, pulse wave reflections and vascular geometry are all inter-dependent and act in synergy to determine arterial health.

The concept of arterial stiffness

Blood ejected during ventricular systole is accommodated via vascular expansion (17). To avoid being ruptured, arteries must be compliant enough to undergo a large change in volume for a given pressure change. Arterial stiffness (AS), or wall rigidity, refers to the impaired ability of arteries to constrict and expand in response to pressure changes (18). It occurs heterogeneously in the arterial tree (with an increase in AS peripherally), generating a stiffness gradient and limiting pulsatile flow to the microcirculation (19). This measure, which has a genetic component (20), is associated with CV risk factors and can be used as a marker of potential vascular disease (6).

Early ageing of elastic arteries occurs either passively (related to heart rate and modified wall composition) or actively (related to endothelial function) (21). During natural ageing, the main change in the tunica media is a decreased elastin:collagen ratio, which increases AS, whilst the main change in the intima is thickening, a process associated with atherosclerosis (13). An increase in AS, prematurely or during the natural ageing process, has important implications for overall CV performance as it affects blood pressure, blood flow and diameter change during the cardiac cycle (21).

The assessment of arterial stiffness, wave reflections and central haemodynamics

Several methods and devices are used in the assessment of AS which can be measured either invasively or using non-invasive yet reproducible and relatively inexpensive techniques (22). The main indices of AS other than ultrasound-derived measures of compliance and distensibility are central pulse pressure (cPP), the augmentation index (AIx), (both derived via pressure waveform analysis, PWA) and pulse wave velocity (PWV), the gold-standard measure of AS.

Pulse wave velocity, which describes the speed of blood propagation along the arterial tree is a surrogate marker for AS and independently predicts CV morbidity and mortality when measured over the aorta (18). It is a measure of operative stiffness and is a determinant of ventricular afterload (16). As the most widely used method to determine regional AS, aortic PWV is assessed using Doppler guided by 2D ultrasound to record the time taken for the incident wave to travel between two predetermined points a measured distance apart (23).

The pulse pressure waveform, assessed during pulse wave analysis, is a composite of an incident, forward travelling wave generated by left ventricular contraction, and a reflected wave (generated at sites of impedance mismatch) returning to the heart (Figure 1.2) (24).

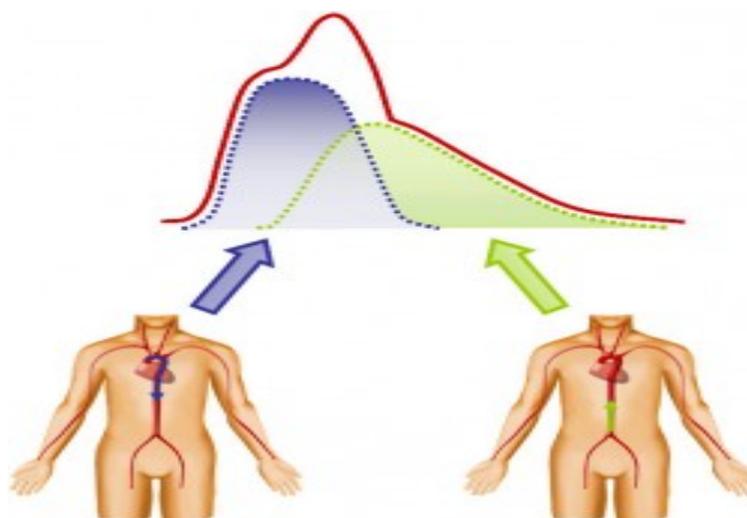


Figure 1.2: The two waves of the observed arterial pulse pressure waveform

The observed pulse pressure wave (red) is a composite of a forward travelling wave generated by left ventricular contraction (blue) and a reflected wave from the periphery (green). *Image adapted from (25)*

Central blood pressures (cBPs) reflect the pressure at the root of the heart and have been more closely linked to CVD than peripheral (brachial) ones (22). Central, but not brachial BP predicts cardiovascular mortality independent of traditional CV risk factors: 'We don't die from the arm' (Pr. D. Chemla). Central pulse pressure (systolic-diastolic BP) has the highest predictive value amongst all BP indices and can be assessed non-invasively via analysis of the pulse pressure waveform (6).

The augmentation index (AIx) is a partly hereditary, indirect index of AS that is independently associated with CVD (26). It is a measure of pulse wave reflections and the resulting impact they have on central pressures and consequently, ventricular afterload as well as the pressure waveform shape and amplitude (27). Non-invasive assessment of the AIx can be carried out using automated, specialised oscillometric devices (28).

Large artery stiffening disrupts wave reflection dynamics. It increases PWV, resulting in a shorter transit time to and from wave reflection sites (29) and thereby, greater amplitudes of reflected waves. This increases the AIx and subsequently, central pulse pressure (18). (Figure 1.3). Stiffening of the central elastic arteries also attenuates the AS gradient, which interferes with the continuous, smooth flow of blood to downstream vessels (30). Fortunately, improvements in AS, central haemodynamics and wave reflection indices reduce CV risk, independent of normalization of traditional CV risk factors (24).

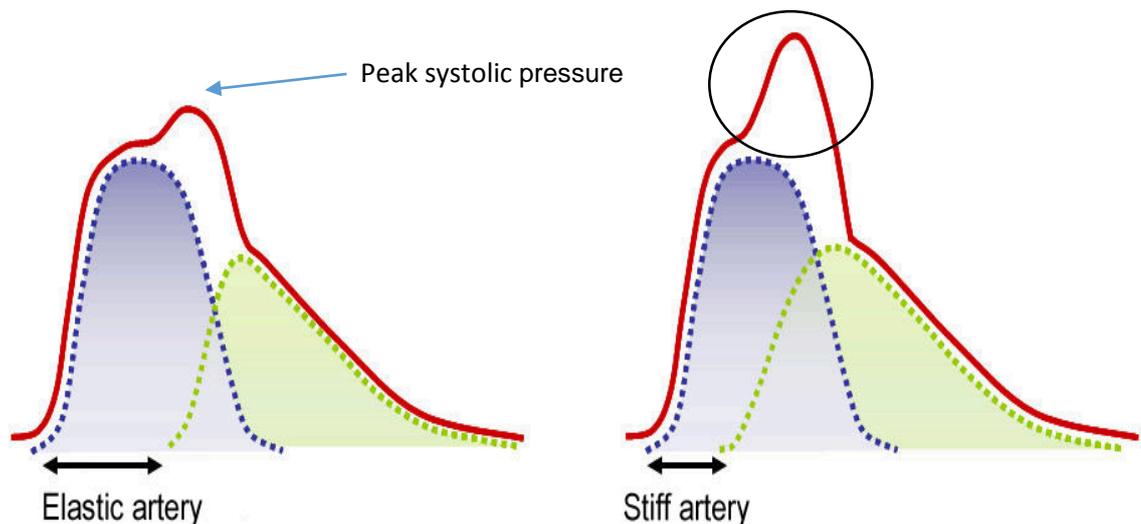


Figure 1.3: Effects of large artery stiffening on central pulse pressure waveform

The picture on the left indicates the pulse pressure waveform when large arteries maintain their elasticity. However, when these arteries stiffen (right), pulse wave velocity decreases so the transit time (arrow) of the incident wave (blue) to and from wave reflection sites decreases. The disrupted wave reflection dynamics (earlier return, greater amplitude) result in an increased augmentation index, central peak systolic pressure (circled) (and therefore, increased central pulse pressure). Image adapted from (25)

Arterial structural modifications and geometrical parameters

Pulse wave velocity, the gold-standard index of AS depends on both intrinsic wall properties in addition to geometrical parameters such as wall thickness and lumen diameter (31). Due to the influence of these remodelling indices on AS, they will be investigated as secondary measures in the current study.

Advanced atherosclerosis is one of the first overt manifestations of CVD whereas structural arterial wall modifications, including intimal thickening, occur over a prolonged subclinical phase (32). Intima-media thickness (IMT), an 'intermediate phenotype for early (local/generalised) atherosclerosis' (33), is a measure of the thickness of the tunica intima and media and is currently the most widely studied and accepted sonographic marker for early atherosclerotic lesions (34). The use of B-mode ultrasonography to measure *in-vivo* common carotid IMT (cIMT) is a reproducible method for determining the degree of atherosclerosis and CV risk (35). The assessment of arterial calibre can provide information regarding both autonomic functions as well as arterial remodelling induced by external stimuli (36). End-diastolic intra-luminal diameter (EDD) is assessed via B-mode ultrasonography and wall:lumen ratio (WLR) is calculated from the IMT and EDD.

Exercise and arterial health indices

Whilst pharmacological therapies are capable of improving arterial health indices, non-pharmacological lifestyle interventions, centred on diet and exercise, have shown considerable success in normalizing these markers and thereby reducing CV risk. The deleterious effects of physical inactivity are concerning given the increasingly sedentary lifestyle being adopted globally (37). Regular physical activity reduces CVD mortality by up to 35% and all-cause mortality by up to 33% (38) in a dose-dependent manner (39). Only 59% of the cardioprotective effects that exercise has consistently been shown to confer can be attributed to favourable alterations in traditional CV RFs (40, 41), with the rest being largely explained by haemodynamic alterations and direct effects on the structure and functions of vascular walls both acutely and in the long-term (42, 43).

Aerobic exercise (AE) of various modes can be effectively used to combat arterial ageing in healthy adults (44). Amongst healthy, sedentary, normotensive young adults, 8 weeks of moderate-intensity AE (cycling) for 30 minutes, 3 times a week reduces carotid artery stiffness (45). Even 4 weeks of moderate-vigorous AE of this volume and frequency can decrease the AIx (46) and central as well as peripheral PWV (47) and simultaneously increase whole-body arterial compliance in a way that is linearly related to changes in VO_{2max} (48). Several cross-sectional studies have observed greater arterial compliance (49) and lower aortic PWV (50-52) and AIx (53) in endurance-trained athletes compared to less active (recreationally-active/sedentary) age-matched counterparts. Regular low-intensity AE also results in a reduction in aortic PWV (54).

Cross-sectional studies have generally reported lower peripheral IMT values in endurance-trained athletes relative to sedentary age-matched counterparts (55, 56). It is uncommon, but not

impossible, to observe a reduction in cIMT in exercise interventions lasting shorter than 3 months (57). Furthermore, AE training has been shown to cause systemic arterial remodelling, that is, structural adaptations in conduit arteries of inactive arterial beds. Eight weeks of moderate to high-intensity AE reduced carotid pulse pressure and wall thickness in healthy, sedentary young adults (58) whilst 8 weeks of high-intensity cycling reduced carotid (in addition to peripheral) wall thickness and WLR (59). Aerobic exercise is also known to increase arterial diameter in a phenomenon known as outward remodelling (37) and 3 months of moderate to high-intensity lower limb AE increased the femoral diameter by 9% in healthy, sedentary middle-aged men (56).

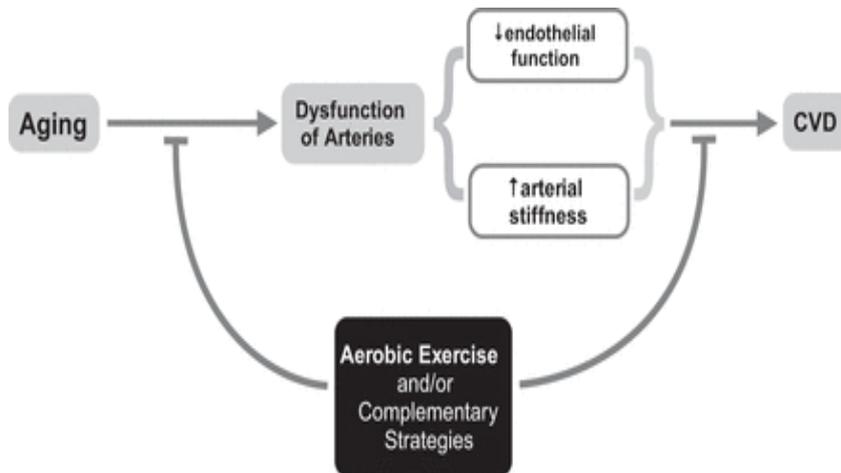


Figure 1.4: Ability of aerobic exercise to attenuate the natural age-associated arterial dysfunction and prevent CVD

This simple illustration depicts the manner in which aerobic exercise can prevent both arterial dysfunction with ageing as well as the development of CVD in the presence of arterial dysfunction. Image adapted from (60)

In contrast to moderate-intensity, continuous AE (MCAE), aerobic interval training (AIT) involves episodic, brief bursts of vigorous exercise, interspersed by low-intensity exercise or complete rest. Rest and work characteristics (ratio, duration, intensity) can be tailored specifically to match the requirements of individuals/groups.

Although sprint interval training (SIT) can increase peripheral arterial compliance (61), exercise sessions based solely on SIT may not be well tolerated by certain (ill/injured/sedentary/unfit) individuals. A more practical, safer option is low volume high-intensity interval training (HIIT), which employs relatively longer work intervals of lower intensity with shorter recovery periods. Three months of merely 20 minutes of HIIT, 3 days a week, can reduce both aortic PWV and the AIx in healthy, sedentary young men (62). There is considerable literature regarding the potent and time-efficient central and peripheral physiological effects of AIE in various healthy/clinical populations (63). These benefits are similar in magnitude if not superior to those conferred by MCAE.

Group fitness classes, which include studio cycling ('spin') are becoming increasingly popular. Group indoor cycling classes are inspired by interval training and offer a combination of HIIT, SIT and AE in a single session and can therefore be classified as AE with concurrent (two types of)

interval training. Furthermore, these classes are self-paced (individuals select their own intensities). Self-paced AIT likely has practical significance because exercise intensities have been controlled such that they have been maintained within a certain range in most previous intervention studies investigating the effects of exercise on arterial health,

While the benefits of AE on AS are well-documented, the effects of resistance exercise (RE) on arterial health are less clear. Resistance exercise triggers different muscular adaptations to AE and is thus associated with different haemodynamic, cardiovascular and muscular responses. Four months of moderate-intensity RE can decrease arterial compliance (64, 65) and only 4 weeks of moderate-intensity RE can increase aortic and peripheral PWV (47) in healthy, sedentary, young adult men. Furthermore, resistance-trained individuals have lower whole-body arterial compliance than sedentary age-matched counterparts (66, 67). Findings regarding the effects of RE and concurrent exercise (AE+RE) are equivocal and further research is needed to draw definite conclusions about their population-specific effects.

1.2 STUDY RATIONALE: WHAT IS KNOWN AND GAPS IN KNOWLEDGE

What is currently known:

- Arterial health indices are modifiable tissue biomarkers of early vascular ageing
- Extensive cross-sectional and controlled, laboratory-based interventions have demonstrated the vasculoprotective effects of both AE and several types of interval training
- Aerobic interval training (AIT) confers similar if not superior health benefits relative to MCAE in a more time-efficient manner; HIIT may be more suitable than SIT for sedentary (exercise beginners) or clinical populations as the former has relatively longer work intervals of lower intensity and shorter rest periods
- There are equivocal results regarding systemic arterial remodelling, especially in conduit arteries
- Persistence of the acute effects of repetitive bouts of exercise can partly explain chronic changes in arterial health with exercise training
- Discrepancies in findings between studies relate primarily to arterial health measurement methods and subject characteristics at baseline
- Mechanisms underlying the benefits of exercise on arterial health warrant further investigation; potential mechanisms relate to training-induced modifications in vascular smooth muscle cell proliferation/hypertrophy, alterations in arterial wall composition; anti-oxidant and anti-inflammatory properties of exercise and changes in endothelial and autonomic function (eg. nitric oxide upregulation)

Gaps in knowledge addressed in the present study

In the past, longitudinal studies investigating the effects of exercise on arterial health have been carried out in laboratory settings where intensities are individually tailored and environmental factors are controlled. However, it is important to determine the feasibility of these training stimuli

in pre-existing community environments where individuals are responsible for monitoring the intensity of their own exercise. In the current study, the self-paced exercise, undertaken in a group training environment outside the laboratory, prevents the need to speculate whether laboratory-based findings have practical application to settings where neither training characteristics nor environmental conditions (eg. humidity, temperature) are consistent, as these factors may influence arterial adaptations to exercise.

Secondly, several studies have documented the beneficial effects of pure AE or concurrent AE and RE on various arterial health indices. Fewer studies have done the same with solely HIIT and SIT, or AE with a single mode of interval exercise. However, no study to our knowledge has investigated the effect of concurrent AE, HIIT and SIT on these indices. In the present study, aerobic interval training refers to a moderate to high-intensity exercise session based primarily on AE of fluctuating moderate intensities, interspersed with regular intervals of HIIT and SIT bouts (thus, the concurrent AE, HIIT and SIT nature in a single exercise session).

There is much debate as to whether specific muscle group training confers systemic benefits (that is, on arterial beds supplying inactive muscles. The current study proposes that systemic arterial adaptations which occur with supposedly isolated muscle training may be due to contractions of secondary musculature not directly involved in the exercise and therefore considered to be inactive.

1.3 PROJECT AIMS AND OBJECTIVES

1. To determine the effects of an 8 week concurrent aerobic interval community-based, self-paced cycling intervention on arterial health indices related to early vascular ageing and remodelling amongst apparently healthy, sedentary young and middle-aged adult males.
2. To describe an average aerobic interval exercise session and study changes in intensities achieved across 8 weeks
3. To investigate the effects of the intervention on:
 - Cardiorespiratory fitness
 - Resting indices of EVA related to stiffness and wave reflections:
 - cfPWV
 - Peripheral Alx (and peripheral Alx standardised to a heart rate of 75 bpm)
 - Central haemodynamics (SBP, DBP and PP)
 - Resting arterial structure and geometry/remodelling parameters
 - Common carotid and common femoral IMT and resistivity index
 - Common carotid end-diastolic diameter
 - Common carotid wall:lumen ratio
 - General anthropometric and clinical measures
4. To explore:
 - Relationships between selected outcome measures
 - Time courses of adaptations

- Changes in CV risk based on Framingham Risk Scores

5. To use the data collected to determine whether community-based group-fitness mixed-intensity concurrent aerobic, HIIT and SIT exercise classes, attended and conducted by choice, would beneficially impact the arterial health and overall CV risk status in healthy, sedentary young-to-middle-aged adult males

NOTES TO READER: the aim is to quantify the impact of the exercise intervention on some traditional CV risk factors accounted for in the Framingham Risk Scoring system. It is outside the scope of this study to estimate or quantify cardiovascular risk reduction.

Furthermore, it is not in the scope of this study to investigate or discuss endothelial function. However, it will be referred to in instances when deemed relevant to explain structural adaptations.

1.4 HYPOTHESES

In this cohort, 8 weeks of thrice weekly moderate-high intensity indoor cycling group-fitness classes will improve tissue biomarkers of early vascular ageing related to AS (AIx, cfPWV, cPP) and cIMT in addition to bringing about adaptive outward arterial remodelling and improving cardiorespiratory fitness and traditional CV risk factors.

1.5 METHODOLOGICAL OUTLINE

All participants were community-dwelling, apparently healthy sedentary males between the ages of 20 and 45 years. Ten participants took part in three 45-minute group-centred indoor (gym-based) cycling classes each week for 8 weeks whilst 5 participants served as controls and refrained from physical activity. Ultrasound-based measures of arterial health were taken at baseline, midway and at the end of the intervention period. Basic anthropometric and arterial stiffness measures were obtained on a weekly basis whilst aerobic capacity and biochemistry were assessed at the start and end of the intervention period. Participants in the intervention group wore heart rate monitors to determine class intensities.

1.6 ASSUMPTIONS

- The sample size was large enough to obtain sufficient power (*a priori* analysis indicated that a sample size of $n=10$ would be adequate)
- Absence of subject bias when completing the International Physical Activity Questionnaire to determine physical activity status prior to the intervention
- There was no major dietary manipulation over the 8 weeks; Physical activity logs were maintained honestly and any moderate to high-intensity activity outside the planned intervention exercise was reported on all occasions
- Participants did not have any underlying undiagnosed chronic illness
- Good reliability and validity of the automated oscillometric device and ultrasound equipment

1.7 DELIMITATIONS

- This study was limited to 20-45 year old non-smoking males who were previously sedentary (<2 hours moderate aerobic exercise per week for the past 6 months) and currently apparently healthy (absence of chronic disease or prescription medication)
- Arterial function was not investigated due to time constraints placed on the investigator and time demands already placed on participants
- To specify results to the specific cohort used in this study, literature pertaining to females, smokers, clinical populations and elderly individuals was considered but neither elaborated upon nor presented due to their unique responsiveness and vascular adaptations which may not apply to healthy individuals
- Arterial health parameters were obtained by ultrasonography or a specialised (validated and reliable) automated oscillometric device. Other techniques (such as invasive methods, applanation tonometry and MRI) were not carried out
- Measurements presented are based on published guidelines and consensus
- The exercise intervention was RPM™, an indoor cycling group-fitness class trademarked by Les Mills International. Substitute engagement in alternative indoor cycling programmes ('spin' classes) were not permitted

1.8 THESIS STRUCTURE

This thesis comprises seven chapters, each regarding a specific aspect of this single interventional study. An extensive literature review will be carried out in chapters 2 (Part A) and 3 (Part B). Chapter 2 concerns the physiology and assessment of arterial health indices. The concepts, relevance, clinical applications and assessment methods of outcome measures in the current study will be discussed. Chapter 3 focuses on the applied physiology and use of exercise as a non-pharmacological therapeutic tool to improve arterial health. In this part of the literature review, findings from key cross-sectional, acute and longitudinal intervention studies investigating relationships between exercise and arterial health indices will be summarised and potential underlying mechanisms pertaining to the vasculoprotective effects of exercise will be presented.

Chapter 4 describes the methods and methodologies used to address the research questions of the present study and chapter 5 is a presentation of the findings.

Chapter 6 is a detailed discussion and interpretation of key findings and relationships between the different outcome measures such that an integrated approach to addressing the topic is adopted. Finally, chapter 7 summarises the contribution to knowledge of the present study and explains the practical implications, significance and limitations of the current study and proposes further work ideas.

CHAPTER 2: THE PHYSIOLOGY, RELEVANCE AND ASSESSMENT OF ARTERIAL HEALTH

2.1 CARDIOVASCULAR DISEASE (CVD)

Cardiovascular disease is a general term used to describe any condition that affects the heart and/or blood vessels. The six main types of CVD are ischaemic heart disease, cerebrovascular disease (stroke), peripheral vascular disease, heart failure, rheumatic heart disease and congenital heart disease (68). Cardiovascular disease has a gradual onset (10) and it is vital to track its progression using simple, reliable and valid techniques (69). This disease is the leading cause of mortality in modern societies (2) and in 2012, the prevalence of CVD was higher than any other health condition in New Zealand, especially in Maori, Pacific and European ethnic groups (70). As the leading cause of death in New Zealand, CVD accounts for approximately 40% of deaths annually (70), many of which are premature and can be prevented.

2.1.1 CARDIOVASCULAR RISK FACTORS (CV RFS) AND RISK PREDICTION

Cardiovascular disease is a multifactorial condition with no single cause. The presence or absence of traditional cardiovascular risk factors (CV RFs) accounts for more than 75% of the risk of developing CVD (3). Several CV RFs have been proposed to help identify individuals who would benefit from preventive measures and to track the effectiveness of prevention programmes. In asymptomatic individuals, risk prediction is currently based on scoring equations which consolidate several CV RFs (71) but most of these scoring system algorithms do not incorporate preclinical atherosclerosis measures, which would be useful to include (72) given that this condition underlies numerous CV events and has a prolonged, progressive course (73). The traditional CV RFs fall into several categories, with primarily genetic, physiological, behavioural and socioeconomic characteristics. Non-modifiable RFs include increasing age, a family history of CVD, ethnicity (African/Asian ancestry) and gender (74). Modifiable physiological/genetic RFs include hypertension, type II diabetes, an abnormal blood lipid profile and left ventricular hypertrophy. Physical inactivity, obesity, smoking/tobacco use, excessive alcohol consumption and certain medications (such as the contraceptive pill and hormone replacement therapy) are amongst the behavioural/lifestyle RFs whilst poverty, social isolation, anxiety and depression are primary examples of socioeconomic RFs (74).

Metabolic syndrome (MS) is a multiplex CV RF (75) and is typically defined by the presence of at least three out of abdominal obesity (increased waist circumference), atherogenic dyslipidaemia (raised triglyceride and low HDL levels), elevated blood pressure (BP), insulin resistance (or glucose intolerance), a proinflammatory state or a prothrombotic state (75). In 2007 in Auckland, the prevalence of MS was Maori 32%, Pacific people 39% and other ethnic groups 16% (76). This emphasises the need for practical lifestyle changes within communities to reduce the numbers of

CV RFs and consequently, risk. Figure 2.1 illustrates the age-associated distribution of the various MS components in adults with this condition.

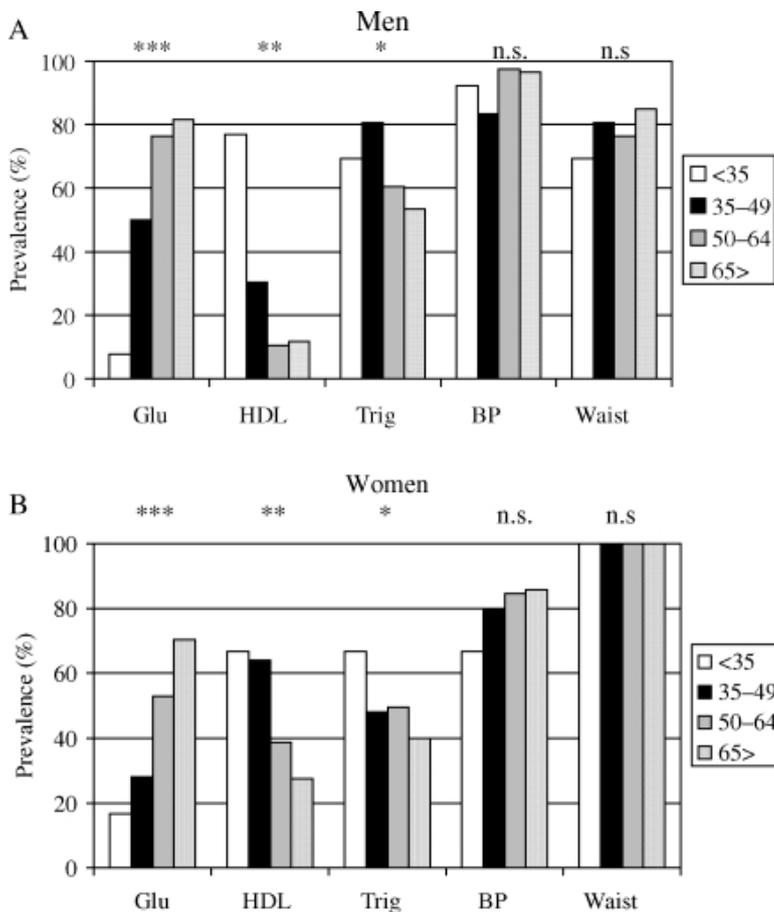


Figure 2.1: The age-associated distribution of risk factors in adults with metabolic syndrome

With increasing age, the general trend is for the metabolic syndrome risk factor profile to deteriorate in both men (A) and women (B), especially in terms of increasing glycaemia and decreasing HDL. *Glu, glucose; HDL, high-density lipoprotein cholesterol; Trig, triglycerides; BP, blood pressure; waist, waist circumference*

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s., not significant. Image adapted from (77)

2.2 THE ARTERIAL SYSTEM AND CARDIOVASCULAR HEALTH

The arterial system is divided into three main anatomic regions. The elastic conduit arteries which lie in close proximity to the heart are composed primarily of elastin and act as a buffering system to dampen down BP fluctuations by distending and storing blood during ventricular systole. Their diastolic recoil prevents pulsatile flow to the microcirculation so that organs such as the brain and kidneys are not exposed to barotrauma-inducing peak systolic pressures. (78). The muscular arteries, located more peripherally and composed primarily of smooth muscle cells and collagen, determine pulse wave reflection dynamics and alter the velocity of blood transmission by

modifying smooth muscle tone. Finally, the arterioles determine peripheral vascular resistance and maintenance of mean arterial pressure (MAP) (78).

Arterial structure and function are modified during the normal vascular ageing process (6) but in some individuals, this process is accelerated (7) and compounded by atherosclerosis-based pathological vascular ageing (8). This is known as premature, or early vascular ageing (EVA) and it increases predisposition to cardiovascular (CV) events (7). Direct damage to arterial walls probably explains the remaining CVD risk which cannot be attributed to traditional CV risk factors (9) and therefore, optimal functioning of arterial walls is necessary to maintain the integrity of the arterial system.

Major changes in elastic arteries occur passively over long time periods whilst changes in muscular arteries and arterioles occur acutely and actively (79, 80). Damage to the large elastic arteries predisposes to CVD (14) with diseases of conduit arteries accounting for the majority of the global heart disease and stroke burden (81). Primary adverse changes to arteries with ageing are increased stiffness, changes in wave reflection patterns and endothelial dysfunction (82). The early evaluation of arterial structure and function when assessing CV RFs is thus important to reduce the age-associated CVD burden (83) as is the implementation of strategies to delay, slow or prevent these changes.

2.2.1 NORMAL ARTERIAL WALL PHYSIOLOGY AND ANATOMY

Optimal functioning of the arterial wall is vital to the integrity of the arterial tree, as evidenced by the potentially fatal effects of arterial wall dysfunction, disease or ageing. The cells and extracellular matrix (ECM) are organised into three distinct concentric cylindrical layers, known as the tunica intima (innermost layer), media (middle layer) and adventitia (outer layer). The proportion of elastic fibres and the thickness of the smooth muscle layer varies between arteries, giving rise to a heterogeneous arterial tree. From the proximal to distal (peripheral) portion of the arterial system, the diameter of the vessels and the proportion of the elastic fibres decrease whilst the amount of smooth muscle increases (13). Due to the non-linear viscoelastic properties as well as the anisotropic and highly adaptive nature of arteries (84, 85), conclusions drawn regarding a specific segment should not be generalised and extrapolated to different parts of the heterogeneous arterial tree (13) unless there is substantial evidence of systemic effects and generalisations are permitted by current standards.

As the demands on the vascular system are dynamic and constantly changing, the cellular components of the wall need to carry out efficient remodelling by altering the quantity, distribution and/or orientation of collagen fibres (17). Equilibrium between the production and degradation of collagen and elastin, the two main arterial wall scaffolding proteins, is paramount to ensure optimal arterial wall function and vascular integrity.

2.3 ARTERIAL HEALTH AND EARLY VASCULAR AGEING

In addition to the traditional CV RFs, programming from intrauterine growth retardation contributes towards CVD and this is known as the 'mismatch hypothesis' (86). Vascular structure and function are partly determined by early programming in life and impaired foetal growth is associated with various changes in arterial health which can eventually cause CVD (86, 87). Some individuals are prone to exhibiting early ageing of arteries (a condition termed early vascular ageing, EVA, or EVA syndrome), the main consequence of which is target organ damage (8). However, there is controversy regarding the definition of EVA (86). Given that most CVD-related events are associated with ageing (the most important CV risk factor), determination of EVA has been proposed as a means to improve CV risk stratification (12). The main characteristics of EVA are arteriosclerosis, endothelial dysfunction, atherosclerosis, altered metabolism and inflammation (Figure 2.2).

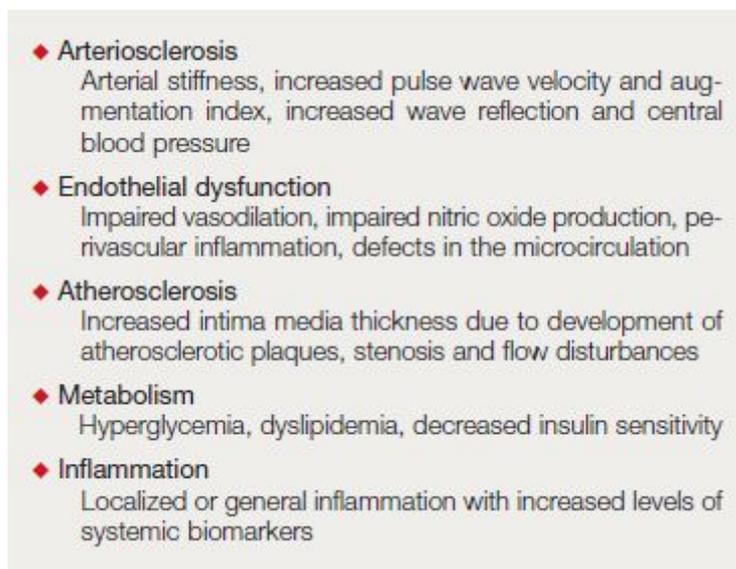


Figure 2.2: Major characteristics of early vascular ageing *Image adapted from (86)*

Early vascular ageing is assessed by the non-invasive measurement of arterial stiffness (and central blood pressure), endothelial function and wall thickness (IMT), all of which are tissue biomarkers and underlie target organ damage (12). Assessing the tissue biomarkers, especially AS, gives a whole picture of risk as opposed to assessing circulating biomarkers (traditional CV RFs), which only provide a snapshot of arterial wall damage (12).

The term arterial health by no means has an established definition and in the present review, is used broadly to describe the degree of EVA, indexed by arterial stiffness, wave reflections, structural modifications and arterial geometry. Arterial geometry and wall elasticity influence the efficiency of the dampening function of the arterial tree as these characteristics determine the amount of energy needed for arterial distension and recoil (31). The relationships between these indices are depicted in Figure 2.3. Arterial stiffness is the primary determinant of left ventricular afterload and it is influenced by both structural and functional aspects (16). Pulse wave velocity depends on both the intrinsic material of the arterial wall as well as structural modifications and

arterial geometry. Geometrical parameters include lumen diameter, cross-sectional area and wall:lumen ratio whilst structural factors include wall thickness and atherosclerosis (Figure 2.3). These properties are also associated with arterial remodelling and will, therefore, be discussed presently. It is important to note that arterial stiffness, structural modifications, pulse wave reflections and vascular geometry are all inter-dependent and act in synergy to determine arterial health.

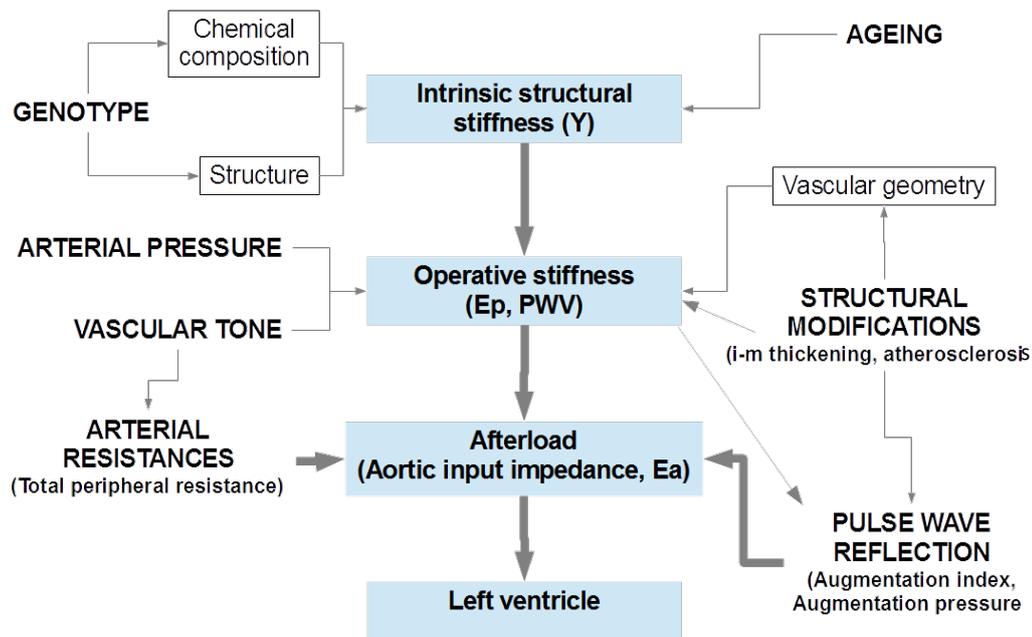


Figure 2.3: Arterial components of left ventricular afterload

This figure depicts the arterial components of left ventricular afterload (blue boxes) as well as their structural and functional determinants. *Y*, Young's elastic modulus; *Ep*; Peterson's elastic modulus; *PWV*, pulse wave velocity; *Intima-media thickness*, *Ea*, effective elastance. Image adapted from (16)

2.3.1 THE CONCEPTS OF COMPLIANCE AND DISTENSIBILITY

During ventricular systole, incompressible blood is pumped out of the heart and must be accommodated via vascular expansion (17). To avoid being ruptured, it is necessary that vessels, especially arteries, are *compliant*. Compliance, a dynamic and fluctuating parameter, describes a volume (or diameter) change in response to a change in blood pressure (ΔP). Distensibility is simply compliance relative to initial volume (or diameter) (18). The distensibility of a specific arterial segment reflects the mechanical stress the wall undergoes during the cardiac cycle (88). Compliance and distensibility describe how stiff the vessel is and in general, distensibility is greater in the proximal portion of the arterial tree (6). Ageing and several pathological conditions affect the interaction between the cellular and structural components of the arterial wall and lead to an increase in arterial wall rigidity (arterial stiffening) (13).

2.4 OVERVIEW OF ARTERIAL STIFFNESS

Arterial stiffness (AS), or arterial wall rigidity, the reciprocal of distensibility (69), has not yet been well defined (28), but it generally refers to the impaired ability of arteries to constrict and expand in response to pressure changes (18). It is a primary determinant of compliance associated with subclinical systemic target organ damage and is a reflection of the integrated, cumulative influence of traditional CV RFs on the arterial tree (12) (Figure 2.4).

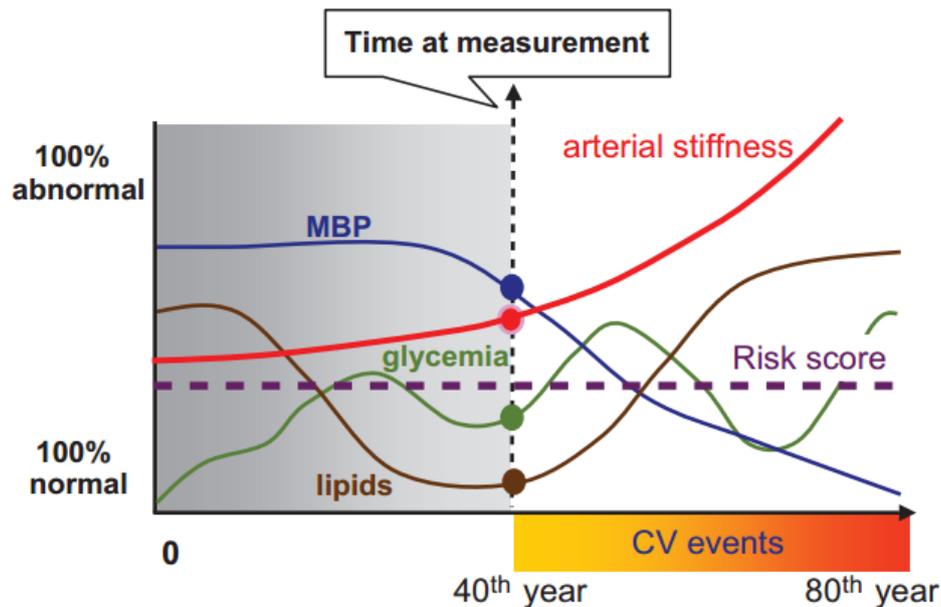


Figure 2.4: Arterial stiffness as a reflection of the integrated and cumulative influence of traditional cardiovascular risk factors on arterial walls

Arterial stiffness increases with ageing whilst mean blood pressure (MBP), glycemia and lipids fluctuate. The latter risk factors may give a constant CV risk prediction score if fluctuations between risk factors occur in opposite directions. Assessing circulating biomarkers only gives a snapshot of arterial wall damage. Image adapted from (12)

Arterial stiffness reflects structural and functional arterial wall properties and describes the change in arterial diameter for a given increase in BP (37). Until the age of approximately 50 years, pulsatile flow to the microcirculation is limited by means of a stiffness gradient along the arterial tree (31). From the proximal to distal segments, there is a progressive increase in stiffness because of an increase in the rigid wall material and a decrease in the cross-sectional area (89). Arterial stiffening, a dynamic process (83), occurs heterogeneously in the arterial tree. Peripheral (muscular) arteries display less stiffening (if any) than central and conduit vessels (elastic arteries) (90-92). As arteries stiffen, the tunica media exhibits a decreased elastin:collagen ratio (93, 94) whilst the intima hypertrophies, a process associated with atherosclerosis (18).

Large artery stiffness, which is partially genetically determined, independently predicts CV risk and has also been linked to several CV RFs and atherosclerosis (95). The concept of AS has

gained increased interest over the past decade due to its association with CVD (including its use as a biomarker for CVD risk and potential vascular disease). An increase in arterial wall rigidity has important implications for arterial and overall CV performance (6, 84, 96-98). Despite the use of various specific AS indices, the pulse pressure wave characteristics associated with central haemodynamics and wave reflections as well as the travel velocity of the pulse wave are integral to the evaluation of AS using non-invasive techniques.

2.4.1 THE PATHOPHYSIOLOGY OF ARTERIAL STIFFENING

The two main arterial wall scaffolding proteins, collagen and elastin, determine the structural integrity of the wall (98). With ageing, the major determinant of arterial stiffening (89), histological changes, (independent of those associated with atherosclerosis) are observed in the walls of elastic arteries. Elastic fibres in the TM of central arteries (92) display fraying, splitting, fragmentation and thinning (99) and the elastic laminae become disoriented (100), probably due to repetitive cyclic stress (101). Histological examination shows disorganised endothelial cells, infiltration of vascular smooth muscle cells (VSMCs), macrophages and mononuclear cells and greater amounts of cytokines and cell adhesion molecules (82), which indicate the presence of atherosclerosis, a 'pathological condition of the intima' (18). Between the ages of 20 to 70 years, the stiff collagen content more than doubles (78). Furthermore, up-regulation of the tissue renin-angiotensin-aldosterone system (RAAS) is associated with tunica intima thickening and arterial wall remodelling (102-104).

Equilibrium between the synthesis and degradation of wall scaffolding material

Several pathological events at the molecular level have been implicated in increased AS by causing an imbalance between the synthesis and degradation of structural proteins of the ECM. Equilibrium must be maintained between elastin and collagen synthesis and degradation to maintain normal arterial distensibility (105). Disruption of elastin and collagen cross-links (106-108) as well as changes in elastin production and repair mechanisms also increase AS (106-108). Catabolic (elastolytic and collagenolytic) matrix metalloproteinases (MMPs) (18), produced by vascular and inflammatory cells (109), fray intimal and medial elastin molecules (110, 111) and uncoil collagen thereby degrading the ECM (98).

Inflammatory changes due to nuclear factor kappa β intracellular signalling lead to production and deposition of excessive abnormal collagen and decreased amounts of normal elastin (93, 112). Non-enzymatic glycation of ECM proteins forms advanced glycation end-products (AGEs) (93) which make collagen stiffer and less prone to turnover by MMPs, resulting in the accumulation of abnormal collagen in arterial walls (113). Advanced glycation end-products also increase the production of reactive oxygen species (ROS), thereby reducing nitric oxide (NO) activity and impairing endothelial cell function (114). Advanced glycation end-products are pro-inflammatory (115, 116) and may contribute to arterial stiffening and atherosclerotic plaque formation (117-119). Finally, increased luminal pressure can stimulate excessive abnormal collagen production (120) and can explain both hypertrophy of the smooth muscle layer as well as the increased

thickness of the intima and media by two to three times between the ages of 20 and 90 years (121, 122).

2.4.2 THE RELEVANCE AND CONSEQUENCES OF INCREASED LARGE ARTERY STIFFNESS

Increased AS can alter ventricular-vascular coupling (refer to Figure 2.3 and Figure 2.5) and increase arterial load, resulting in target organ damage (16). Coronary perfusion is normally diastolic and is not significantly affected by changes in SBP. The raised SBP brought about by greater AS (elastance) increases circumferential arterial wall stress. This causes elastin fragmentation in the TM and also increases local fatigue, predisposing to endothelial dysfunction and the build-up of atherosclerotic plaque (84).

An artery with lower stiffness can absorb energy during cardiac systole and minimise energy loss by ensuring the smooth flow of blood (123). When the central arteries are elastic, the energy of ventricular contraction is stored in their walls during systole as potential energy (PE) which is then dispelled as kinetic energy (KE) during diastole as they recoil (79). However, when these large central arteries stiffen, they are able to store less PE and they cannot convert the KE of ventricular contraction to PE so systolic BP (SBP) rises. Therefore, with increased AS, whilst SBP increases, diastolic BP (DBP) and MAP slightly decrease such that ascending aortic pulse pressure (SBP-DBP) increases (78).

The pulsatile blood flow ejected into the arterial tree during ventricular systole has static and dynamic components, the latter of which depends on pulse wave reflections and elasticity of the central arteries (84). Arterial stiffening, especially that of central vessels, increases the afterload of the left ventricle (6, 84) which then hypertrophies (124). An increase in the pulsatile component of ventricular afterload causes sub-optimal ventricular-vascular coupling (that is, a mismatch between ventricular emptying and pulse wave transmission of the stroke volume). When ejecting blood into a stiffer arterial tree which is less able to accommodate the stroke volume, the heart needs to generate greater end-systolic pressures for a given stroke volume and therefore requires more energy to bring about similar ejection (125) (cardiac ejection efficiency decreases). Developments of left ventricular hypertrophy, atherosclerosis, and coronary artery disease are promoted, increasing the risk of myocardial infarction (MI), and heart failure/arrest (79) (Figure 2.5). Cardiac hypertrophy and fibrosis then ensue even if MAP remains unchanged. The recoil ability of arteries reduces as they stiffen and their reservoir capacity decreases as does DBP. The increased central pulse pressure (PP) and decreased DBP may cause subendocardial ischaemia (126, 127).

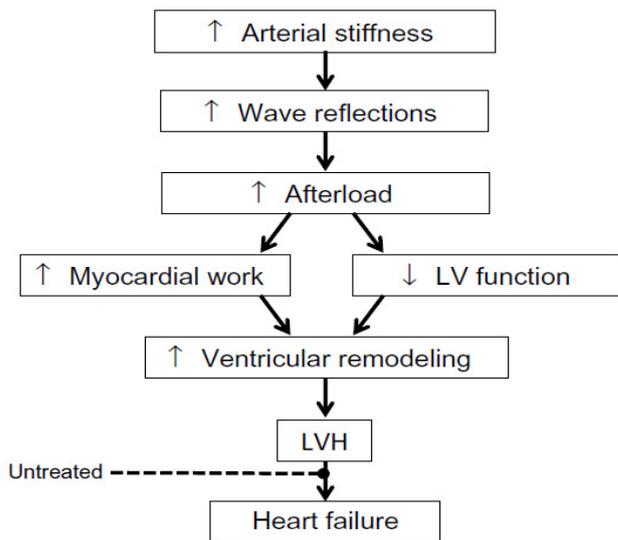


Figure 2.5: The consequences of arterial stiffening

Increased intrinsic arterial stiffness disrupts wave reflection dynamics, thereby increasing the augmentation index and left ventricular afterload, eventually resulting in heart failure if left untreated. *LVH, left ventricular hypertrophy* Image adapted from (1)

2.4.3 CLINICAL APPLICATIONS OF AS ASSESSMENT

Arterial stiffness is the primary tissue biomarker of early vascular ageing (12) and CVD (6, 128), (109-111). It can also be used as a surrogate index of potential vascular disease (23), diabetes mellitus (129), hypercholesterolaemia (130) and end-stage renal disease (ESRD) (91). Greater elastic artery stiffness, which occurs during the natural ageing process, is most likely associated with the age-related increase in CVD morbidity (131-133). The stiffness of elastic arteries independently predicts CV risk (6, 22, 134) and elastic properties of arteries are currently being used for risk stratification amongst various populations (22). Therefore, over the past few decades, the incidence and prevalence of clinical surrogate markers of AS have risen (98). A detailed summary of the common AS indices, as well as their advantages and disadvantages, is provided in Appendix B. Guidelines set in 2007 suggest that pulse wave velocity (PWV), the gold standard measurement of AS, be used as a tool to evaluate subclinical target organ damage (135) as depicted in Figure 2.6.

Despite the results from individual studies showing evidence of the predictive role of AS, no definite quantitative approximation of this role exists (22). The majority of studies carried out have been cross-sectional in nature and although they have shown that AS is a marker of CV risk, its predictive value as an intermediate endpoint has not been shown (6). Nevertheless, it has been foreseen that AS will become an increasingly important part of risk assessment (136). The measurement of AS also serves as an endpoint in both pharmacological and non-pharmacological studies and enables haemodynamic changes and the pathogenesis associated with various clinical conditions to be evaluated.

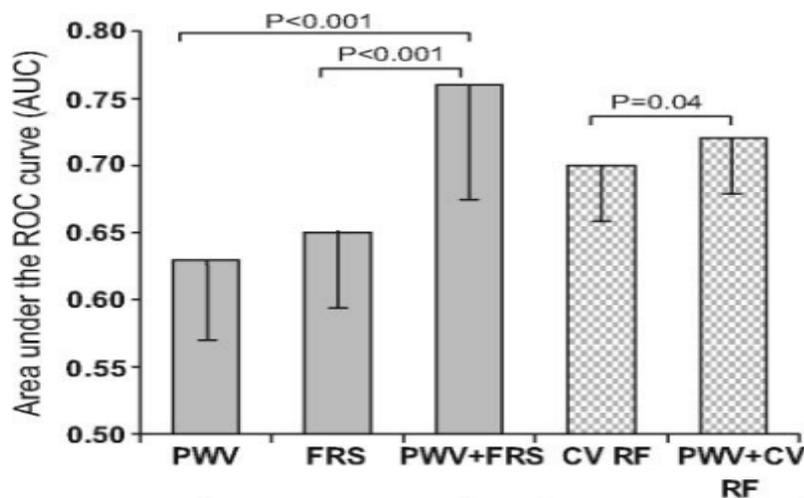


Figure 2.6: Additive predictive value of arterial stiffness and traditional cardiovascular risk factors

In low-to-moderate risk hypertensives with a 5.9-year follow-up, predictive values of PWV and FRS for coronary heart disease were assessed from the area under the curve. PWV and FRS had similar predictive values. When PWV and FRS were combined, the predictive value significantly increased. In elderly participants, PWV significantly improved the predictive value of combined criteria for future CV events. *PWV, pulse wave velocity; FRS, Framingham Risk Score; CV RF, cardiovascular risk factors* Image and legend adapted from (12)

2.5 MONITORING THE STATUS OF ARTERIAL HEALTH

The main CV complications associated with EVA gradually progress on a continuum presenting as subclinical target organ damage (refer to Figure 2.2). Prior to overt clinical manifestation, these events can be identified using validated surrogate markers which are all independent CV RFs. Structural/morphological changes are evaluated by measuring the thickness of the intima-media complex (intima-media thickness, IMT) (13). Non-invasive techniques used to monitor CVD progression primarily assess atherosclerotic status and regional/local/systemic AS. The methods (including the benefits and drawbacks of each) used to determine the degrees of arterial stiffening and remodelling parameters related to atherosclerosis are described in Appendix C.

2.5.1 THE ASSESSMENT OF AS: EVALUATION OF PULSE WAVE VELOCITY, THE PULSE PRESSURE WAVEFORM AND WAVE REFLECTIONS

The main indices of AS other than ultrasound-derived measures of compliance and distensibility are based on central haemodynamics and wave reflections assessed via pulse wave analysis (PWA) and evaluation of pulse wave velocity (PWV), the gold standard measurement of AS. Attention is increasingly being directed toward PWV, central pulse pressure and wave reflections as independent CV risk factors in various populations because they are examples of target organ

damage, which is a mediator between traditional CV RFs and CV events (12). Each AS index can be measured in several different ways using specific equipment and protocols (refer to Appendices B and C) Simple, non-invasive, reproducible, accurate and relatively inexpensive methods are ideal for regional/local/systemic AS evaluation in large-scale studies (83). Unfortunately, normative data is equivocal due to the different assessment methods and populations so values will not be reported here.

Note on Appendices B and C: Appendix B summarises the advantages and disadvantages of the main AS indices and Appendix C summarises the equipment and procedures used to assess the arterial health parameters relevant to the present study. These indices have been compiled after considerable literature reviews. The main points have been covered in references (137), (138), (13), (18), (83, 139) and (15).

2.6 PULSE WAVE VELOCITY (PWV)

The pressure pulse generated by left ventricular ejection propagates along the arterial system with a speed determined by arterial wall elasticity and geometry (140). Therefore, arterial wall elasticity determines pulse wave velocity (PWV), a parameter which describes the speed of blood propagation along the arterial tree (18). Pulse wave velocity is a simple, non-invasive and reproducible method used to assess operative AS (23) and is considered the gold standard surrogate marker for AS (18). Aortic (central) PWV usually tends to increase during the healthy ageing process (30, 141). Aortic PWV is related to mechanical properties of arterial walls (142), wall thickness and luminal diameter (83) and to a lesser extent, characteristics of the blood. Pulse wave propagation and arterial distensibility are inversely related such that the greater the AS, the higher the speed of both the forward and retrograde waves (143).

2.6.1 CLINICAL APPLICATIONS OF PWV

Arterial stiffness measured via PWV independently predicts all-cause and CV mortality and morbidity (18), coronary events and stroke in hypertensives (144), type II diabetics, elderly individuals and in those with ESRD (6). Although PWV is used to evaluate the status of central arteries, there is a poor correlation between PWV and established CV RFs other than age and BP (145). Individuals classified as low-to-intermediate risk using the Framingham Risk Score (FRS) who having no current target organ damage may benefit most from risk assessment using PWV (6). As an EVA tissue biomarker related to *systemic* target organ damage, cfPWV can be validly added to the FRS to improve risk classification and predict CVD-related death and risk in addition to traditional risk factors (12). Age, SBP and gender explain >50% of the variability of aortic PWV with the former two parameters being positively correlated to aortic PWV. Less than 1% of PWV variance is explained by clustering of MS RFs (146). Cecelja et al. suggest that central PWV is a better measure of BP than those currently used in clinical settings (145). A 5m/s

increase in central PWV raises the risk of mortality by an amount equivalent to 10 years of ageing (144).

2.6.2 THE ASSESSMENT OF PWV

As the most commonly used method to determine regional AS, aortic PWV is assessed by recording the time taken for the forward pressure wave to travel between two predetermined points ($PWV = D/\Delta T$) and this can be done in several arterial tree segments (84). This is known as the foot-to-foot velocity method (Figure 2.7) and is obtained from pressure (147), distension (148) or Doppler (149) waveforms obtained transcutaneously. Details of this process are provided in section 4.7.1.1. As the elastic common carotid artery (CCA) and muscular common femoral artery (CFA) are quite superficial, these are the most common set points (18), enabling the calculation of carotid-femoral PWV (cfPWV). Carotid-femoral PWV assessment carried out between the CCA and CFA covers the region over the aorta which exhibits the greatest pathological effects of AS and the highest increase in AS with ageing (30). This region also has the greatest buffering capacity of the arterial system (84, 150-153) and independently predicts outcome in various populations (91, 100, 144, 149, 154-159).

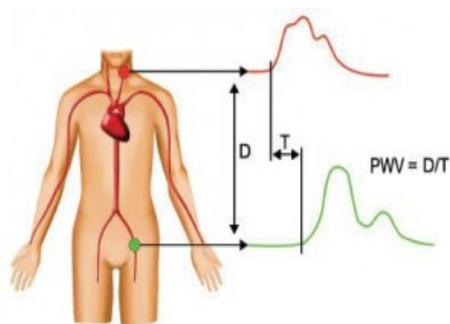


Figure 2.7: Foot-to-foot velocity method used for the determination of PWV

Pulse wave velocity is assessed by recording the time taken for the forward pressure wave to travel between two predetermined points, usually, the common carotid and common femoral arteries. *PWV*, pulse wave velocity; *D*, distance between measurement points; *T*, time taken for forward pressure wave to travel over distance *D*. Image adapted from (25)

2.7 PULSE WAVE ANALYSIS (PWA) AND THE PULSE PRESSURE WAVEFORM

The pulse is described by flow, pressure and dimension (160). Pulse wave analysis typically involves examination of the morphology of the arterial pressure waveform. It is a simple, reliable, inexpensive and valid technique (69). Systemic AS is a reliable reflection of how large central arteries oppose the pulsatile flow of blood ejected by the ventricles (6, 69). Pulse wave analysis is based on the idea of heterogeneity in viscoelastic properties along the arterial tree, that is, the stiffness gradient between the proximal elastic arteries and stiffer distal arteries (6).

The blood ejected during ventricular systole acutely dilates the wall of the aorta, generating a pulse wave (83). This incident (sphygmic) wave travels along the arterial tree at a finite speed, the velocity of which is positively correlated to vascular wall rigidity (84). The pressure wave would attenuate with an exponential decay if the arterial tree was a single viscoelastic tube with no branch points. However, this is not the case and referring to the propagative model of the arterial tree (6), the pressure waves generated during cardiac systole are partially reflected back to the aorta, particularly at sites of impedance mismatch caused by the stiffness gradient, changes in aortic geometry, lumen narrowing and arterial bifurcations (31). In this way, retrograde waves, which travel back towards the aorta, are generated (6, 84). The arterial pressure wave is, therefore, a composite of a forward travelling wave generated by left ventricular ejection and a wave reflected from the periphery (78) and its amplitude and shape thus depend on the phase relationship (overlap) between the two waves. The shape of the waveform changes as it travels along the arterial tree due to the stiffness gradient and varying wave reflection intensities (31). An illustration of the arterial pressure wave is depicted in Figure 2.8. Central BP, or BP at the aortic root, is largely influenced by the time point at which the travelling forward and reflected waves merge as well as the amplitude of the reflected wave (26). The reflected waves depend on the elastic properties of the elastic and muscular arteries, the PWV, and the distance to the main sites of wave reflection (78). Aortic stiffening, which influences the amplitudes of incident waves and the timing of reflected waves, can affect left ventricular anatomy and function. Arterial stiffening influences the shape and amplitude of the pulse pressure waveform directly (as the aorta ejects the stroke volume into a stiffer arterial tree) or indirectly (via an increase in PWV as described in section 2.7.3) (31).

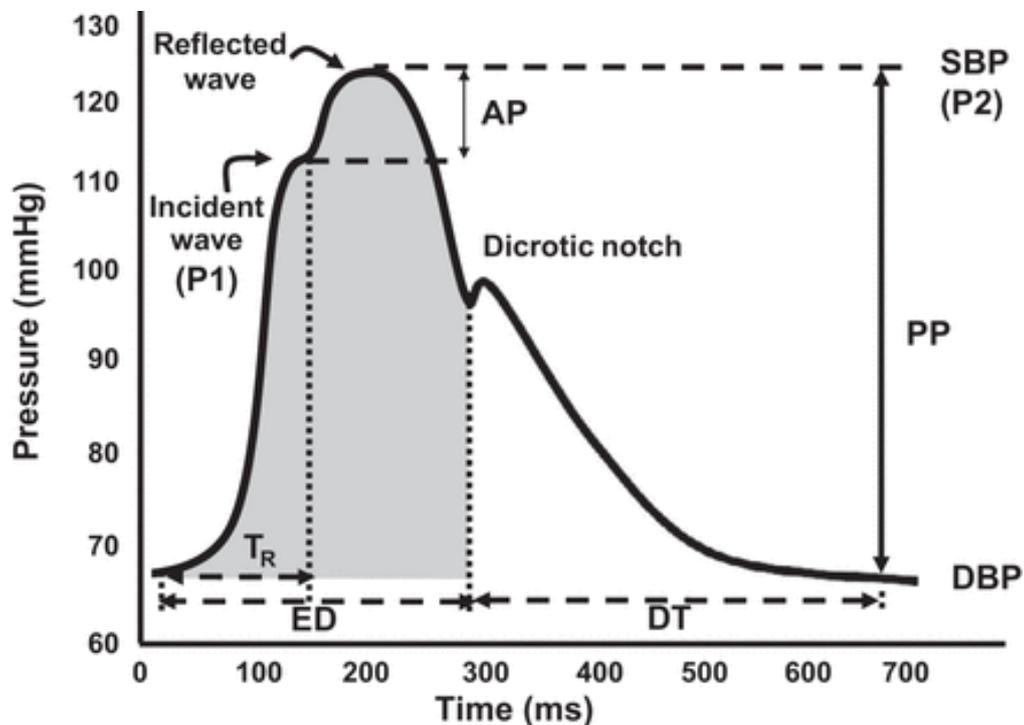


Figure 2.8: Central arterial pulse pressure waveform

As the incident wave travels from the aorta to the periphery, it is reflected back to the heart. T_R is travel time for forward pressure wave from the aorta to travel to reflection sites and

back. The reflected wave augments the pressure of the incident wave (calculated by AP which is P2-P1) such that SBP increases, resulting in widening of the PP. The observed pulse wave is a composite of the incident and reflected waves. The dicrotic notch represents aortic valve closure. *ED, left ventricular ejection duration (grey area); DT, diastolic time interval; SBP, systolic blood pressure; DBP, diastolic blood pressure. AP, augmentation pressure; PP, pulse pressure* Image adapted from (161)

2.7.1 PULSE PRESSURE

Pulse pressure (PP) is a recognised surrogate marker for AS (162). It is modulated by arterial compliance, pulse wave reflections and ventricular ejection (13, 79) and is also affected by factors other than large artery stiffness (wall degeneration and hyperplasia), including heart rate, venous pressure, and cardiac contractility. Increased AS causes widening of the PP (162) because the loss of elasticity of large arteries results in a high SBP and low DBP (23). Considered individually, brachial PP independently predicts CV risk (185-195) better than SBP or DBP alone (79, 163). However, despite this predictive value of peripheral pressures, it is now generally well accepted that the assessment of central arterial function is more useful for predicting vascular health outcomes than traditional peripheral BP measurements as discussed in the following section. A chronically elevated central PP increases the risk of renal failure, stenosis and plaques (164, 165) and plaque rupture (166). It also results in altered remodelling of both intra- and extra-cranial arteries, increased arterial wall thickness, and a greater incidence and severity of lesions of cerebral white matter (167).

2.7.2 CENTRAL HAEMODYNAMICS AND PRESSURE AMPLIFICATION

In healthy, normotensive, young individuals, SBP and PP (the two pulsatile components of BP) increase a lot whilst DBP and MAP decrease slightly as the pulse pressure wave travels peripherally (29). This 'pressure amplification' (Figure 2.9) is brought about by the stiffness gradient and greater wave reflection amplitudes towards the periphery (168) (6). The extent of amplification depends on the distance to wave reflection sites and differences in elastic moduli of involved arteries (84, 169, 170).

The age-associated arterial stiffening is usually confined to central, elastic arteries such that at the age of approximately 55 years, a reversal in the stiffness gradient is generally observed, whereby central arteries are relatively stiffer than peripheral ones (30). Central SBP increases and PP amplification is therefore attenuated with age. As wave reflection sites move distally and wave reflection amplitudes decrease with vascular ageing, there is less dampening of the forward pressure waves whose intensities therefore increase (171), exposing the microcirculation to greater pulsatile flow. This necessitates the need to delay or attenuate these age-related changes.

Due to the amplification phenomenon, sphygmomanometer-derived brachial BP does not always accurately reflect central (aortic) SBP and PP (172) as cBP depends on large artery stiffness and

pulse wave reflections (173). It has been proposed that central BP (cBP) may be more accurate at predicting risk than peripheral (brachial) pressures (22, 174-178) and at predicting CV events, diabetes and all-cause mortality, independent of peripheral BP (22, 178, 179) because the major organs which are the main sites of CV events are directly exposed to the former. In addition, the detrimental effects of hypertension and atherosclerosis do not significantly affect the brachial artery (160) and left ventricle afterload and mass are influenced by the pressure in the ascending aorta rather than that in the brachial artery. Furthermore, although general AS parameters can be obtained from both peripheral and central arterial pressure waveforms, only the latter can provide absolute values of wave reflection characteristics to better predict CV outcomes.

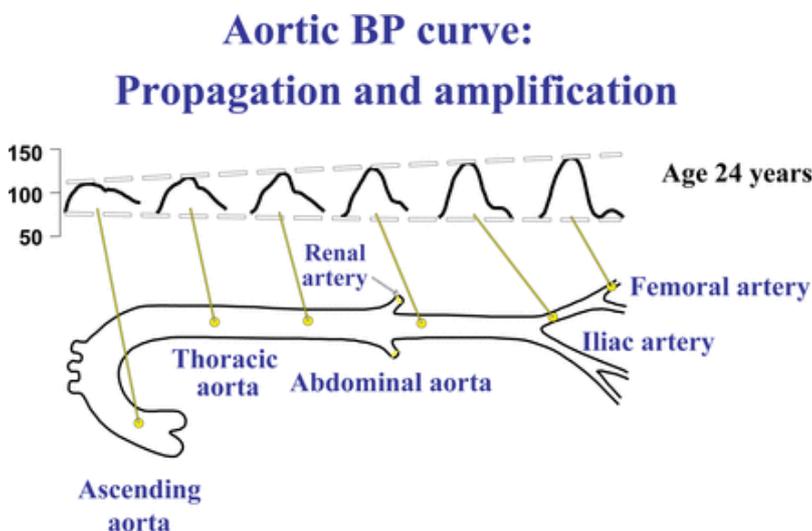


Figure 2.9: Pulse pressure amplification from the aorta to the periphery in a young adult

As systolic blood pressure and pulse pressure increase from the aorta to the periphery (alongside minor changes in mean arterial pressure and diastolic blood pressure), the shape of the blood pressure curve changes as well. *Image adapted from (173)*

2.7.3 THE AUGMENTATION INDEX (AIx)

The augmentation index (AIx) is a partly heritable (180) measure of wave reflections and the resulting impact they have on ascending aortic (central) pressure and consequently, the pressure waveform shape and amplitude. In the peripheral pressure waveform, there is an uninterrupted upstroke during systole followed by a small systolic ‘shoulder’ during the *downstroke*. In the central aorta (refer to Figure 2.8), the initial early pressure rise (P1) is ‘augmented’ by a second pressure rise (P2) during late systole. This brings about a systolic ‘shoulder’ during the *upstroke*. P1 coincides with left ventricular ejection and is the point of the greatest PWV.

Augmentation pressure (AP) is P2-P1 (ie. second systolic peak – first systolic peak) and is brought about due to reflection of the forward wave. This index defines the absolute increase in BP during the early phase of ventricular systole which is brought about by a reflected wave at the aorta (13).

Augmentation pressure is usually expressed as a percentage of central PP (cPP) and is then referred to as the central augmentation index (cAIx) (Figure 2.10). The peripheral augmentation index, (assessed in the present study) is defined as P2/P1.

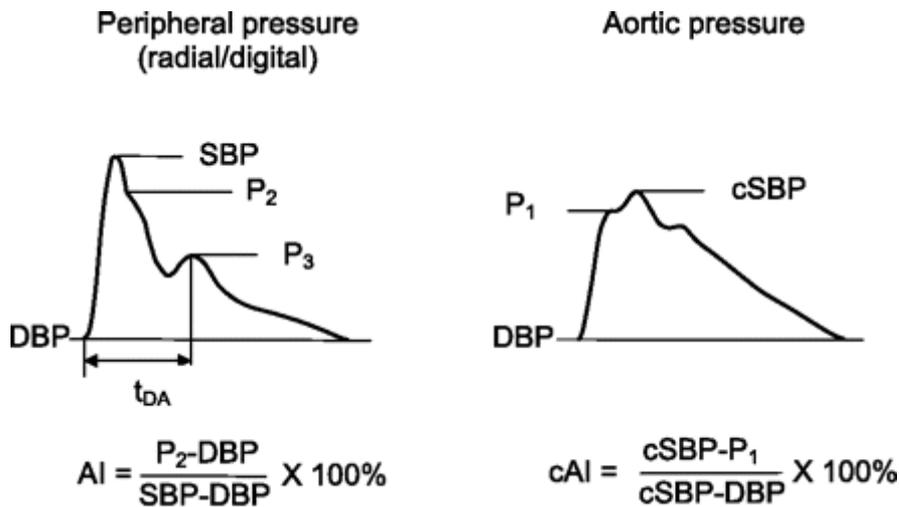


Figure 2.10: The augmentation index

The AIx defines the increased systolic pressure due to the summation of the reflected and incident pressure waves. The shapes of the peripheral (left) and central (right) pulse pressure waveforms are different. *SBP, systolic blood pressure; DBP, diastolic blood pressure; AI, peripheral augmentation index; cAI, central augmentation index; P1, early systolic pressure; P2, late systolic pressure* Image adapted from (181)

The AIx, defined as the ‘supplement of SBP due to the reflected wave’ (89) is the ratio of the amplitude of the late to that of the early systolic peak and represents the proportion of central PP and the extra load on the left ventricle brought about due to wave reflections (182). A stiffer aorta is unable to accommodate a given stroke volume and the speed or amplitude of the reflected wave increases, thereby directly raising central AP (183) (Figure 2.11). It is important to note that despite both being used to assess AS, the AIx and PWV are distinct but inter-dependent measures.

Conversely, central PP can be related to AS due to the effect that increased PWV has on the forward wave (184, 185). When arteries maintain their elasticity and PWV is relatively low, the transit time decreases and reflected waves arrive back at the central aorta during diastole (ie. the incident and reflected waves are out of phase in central arteries) (31), thereby maintaining DBP and coronary perfusion (19). Arterial stiffening increases the speed at which the pulse wave travels (ie. PWV) resulting in a reduced transit time and consequently, earlier wave reflections and retrograde waves which arrive in the central aorta during systole and superimpose on the incident (forward) wave (84). When the reflected wave returns at an earlier point of the cardiac cycle (that is, during ventricular ejection (systole) as opposed to diastole), the two component waves are nearly in phase and systolic pressures are augmented, thereby increasing wave reflections (15). Although the major factor influencing the AIx is the arrival time of the wave

reflection, body height and resting heart rates influence wave reflection patterns and should ideally be corrected for when measuring the AIx (186, 187).

The AIx measures wave reflections and systemic AS (26, 188) and is independently associated with CVD (26). Changes in wave reflection dynamics are also associated with ageing and vascular disease (78). Central AIx and central PP independently predict all-cause mortality and adverse CV events (189-191). Although the AIx is associated with target organ damage, findings regarding the association between the AIx and atherosclerosis are equivocal (192-194), (195, 196).

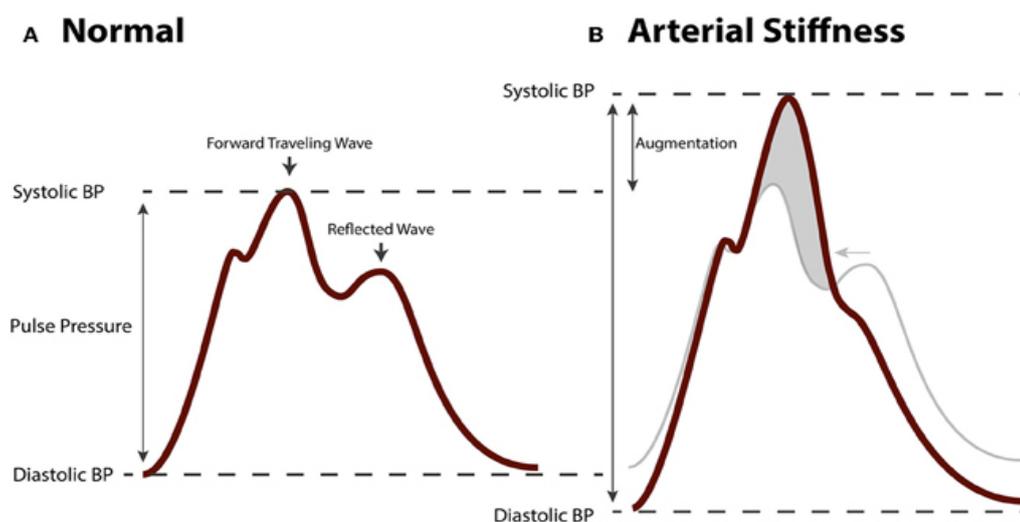


Figure 2.11: Influence of arterial stiffening on wave reflections and central haemodynamics
When arteries maintain their elasticity (A), reflected waves arrive back at the central aorta during diastole. With arterial stiffening, (B), pulse wave velocity increases, resulting in the earlier return of the reflected wave to the aorta and thereby, greater augmentation of the incident wave and finally, increased peak central systolic pressure. *Image adapted from (197)*

2.7.4 NON-INVASIVE ASSESSMENT OF THE AIx AND CENTRAL PRESSURES

Despite the proposal that central arterial BP (cBP) has better prognostic value than peripheral BP, the former is not measured regularly in clinical practice or non-specific research settings. Until recently, this was probably due to the complex methods (such as radial tonometry, refer to Appendix C) required to assess the former. The technical difficulty and error introduced using generalised mathematical transfer functions from peripheral to central waveforms using applanation tonometry to measure cBP has led to the development of devices adapted from standard sphygmomanometers which estimate central aortic pressure, using a physics-based model, from suprasystolic waveforms obtained at the brachial artery when it is occluded (28, 198).

Easily operated automated devices, which either use a microphone to record Korotkoff sounds or use an oscillometric principle (199) have been evaluated, with scientific research providing evidence of their effectiveness. Electronic oscillometric instruments which record BP from the brachial artery have become popular over the past few years (200). They are the main tool for self-measurement of BP (199) and the demand and market for such devices has increased rapidly

over the past 3 decades (201). In oscillometric techniques, arterial pulsation/vibrational changes are detected during cuff inflation and deflation. Pressure pulses in the cuff are brought about due to arterial volume pulses (199), giving rise to a pulse pressure curve (pulse oscillogram). Systolic and diastolic pressures are not obtained directly. They are determined via a microcomputer, from the shape of the envelope curve provided by the pulse oscillogram (202). Maximum oscillation represents mean arterial BP and built-in electronics (algorithms) convert the pressure wave signals into the BP readout according to a device-specific computation.

One such commercial, specialised oscillometric device which has recently been developed to measure peripheral AIx and cBP non-invasively is the BP+ (Uscom, Sydney) (198) (Figure 2.12). The BP+ device exhibits satisfactory accuracy (similar to that of radial tonometry) (203) as indicated by the good correlation between SBP derived from this device and that measured invasively using a catheter during coronary angiography (198). As an operator-independent method, it also exhibits good reproducibility, again, similar to that of radial tonometry, excellent intra-test reliability and acceptable inter-test reliability (203).

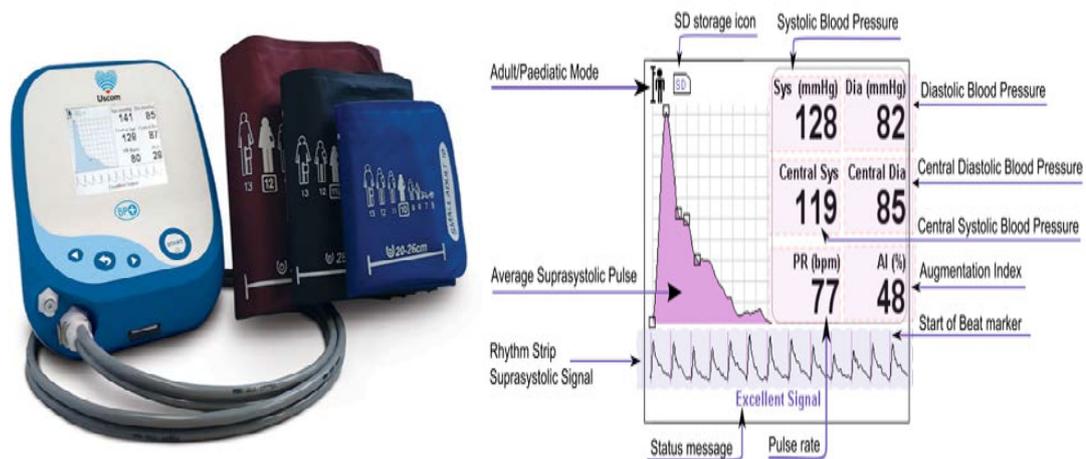


Figure 2.12: The BP+ device used in the present study

A specialised oscillometric device (left) used to assess peripheral augmentation index, peripheral and central blood pressure and heart rate in addition to enabling central pulse pressure wave analysis. The image on the right is an example of the BP+ screen and measures derived from it. Images adapted from (204, 205)

2.8 FLEXIBILITY AND ARTERIAL STIFFNESS

A study carried out in 2009 investigating the relationship between trunk flexibility and peripheral PWV amongst 20-83 year old adults found that flexibility predicts arterial stiffening independently of other components of physical fitness (206). Possible reasons relate to either higher BPs observed in those with poor trunk flexibility, or more optimal equilibrium between collagen and elastin in individuals who have greater flexibility (and better scaffolding protein equilibrium in muscle tissue) (207, 208).

2.9 INTIMA-MEDIA THICKNESS (IMT)

As the name suggests, IMT is a measurement of the combined thickness of the tunica intima and media. Whilst AS indices provide an indication of the functional vulnerability of arteries, IMT is a marker of structural compromise, and as aforementioned, a tissue biomarker of EVA.

Atherosclerosis is an occlusive vascular disease characterised by plaque formation and lumen narrowing (31). The IMT index gives an indication as to the degree, if any, of atherosclerosis, and can be used as its surrogate marker (11) to track changes in lesions over time (209) (Figure 2.13).

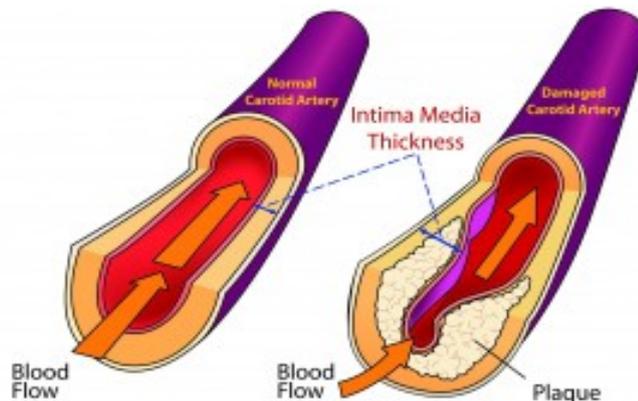


Figure 2.13: Relationship between intima-media thickness and atherosclerosis in the carotid artery

In healthy arteries (left), blood flow is laminar and smooth but in the presence of atherosclerotic plaques (right), intima-media thickness increases resulting in lumen narrowing and turbulent blood flow. Image adapted from (210)

The early detection and quantification of atherosclerosis and its determinants is important because this condition progresses without symptoms and can affect any and many parts of the arterial tree (3). Subclinical alterations in arterial walls precede CV events which in turn reflect underlying atherosclerosis. B-mode ultrasonography is often used to detect early stages of atherosclerosis as it is simple, widely available and has relatively better resolution than other techniques (211). The IMT is used to monitor arterial wall alterations as it is associated with both CV RFs and incident CVD (209, 212-215). Although IMT is influenced by atherosclerosis and its risk factors, its value can also increase due to non-atherosclerotic local stimuli which provoke intimal hyperplasia and hypertrophy (216)

Intima-media thickness assessments are often carried out on the carotid artery (CCA, internal carotid or bifurcation) (121). As atherosclerosis is a diffuse disease, findings from one localised segment can be generalised to other parts of the arterial tree. The IMT is defined as a double line observed on both walls of the CCAs in a longitudinal, echographic image (216). The two parallel lines represent the intima-media and media-adventitia interfaces.

Intima-media thickening of the carotid artery can be due to hypertrophy of either one of or both of the layers. The structural and molecular factors associated with intima-medial thickening are also associated with the development and/or progression of atherosclerosis (11). Carotid IMT also

predicts future CV events independent of age, CV risk and gender (217) and until early adulthood, is the only atherosclerotic index of the carotid arteries. Intimal thickening is observed with increasing age and weight and in those with an elevated BP and/or cholesterol levels and in diabetics and smokers (218). Carotid IMT is associated not only with traditional CV RFs but also with both incident and prevalent CVD (121). It can serve as an endpoint instead of CV mortality and morbidity (219) and for this reason, it is being increasingly used in risk stratification (220). Although intimal thickening is associated with atherosclerosis, it is currently difficult if not impossible to accurately measure the thickness of the intima alone using ultrasound technology. Therefore, when evaluating IMT in any artery, the combined thickness of the intima and media is measured.

2.9.1 THE PHYSIOLOGY AND SPECULATED REASONS BEHIND INTIMA-MEDIA THICKENING

Intima-medial thickening is possibly an adaptive response to alterations in flow, lumen diameter or wall tension because changes in shear stress (the 'dragging frictional force') (19) and thereby lumen diameter, may alter IMT to normalise local tensile stress. When PWV, wave reflection intensities, the AIx, and peak systolic pressures increase, circumferential wall tension increases as well (216). During this compensatory non-atherosclerotic remodelling, the tunica intima undergoes hypertrophy and hyperplasia (216). Thus, until a certain level, increased cIMT can be considered a physical reaction of the vessel wall brought about by local, non-atherosclerotic stimuli (216) and should therefore be considered relative to lumen diameter (219). During intima-media thickening, there is an accumulation of elastin, VSMCs, and proteoglycans but not lipids (221). As a feature of early atherosclerosis, the increased IMT co-localises to areas where atherosclerotic lesions and LDL-C deposition are most likely to occur (222), which is in regions of low wall shear stress (223) (a force inversely related to vessel diameter, age, SBP, BMI, cIMT and atherosclerotic plaque formation).

2.9.2 THE USE OF CAROTID IMT IN CV RISK STRATIFICATION

Normative data for (left and right) cIMT are depicted in Table 2.1. Common carotid IMT values amongst healthy, non-smoking 21-60 year old men and women range from 0.4-0.8mm with no differences between left and right CCA diameters (224). Usually, a common carotid IMT value of greater than 0.9mm is an indicator of target organ damage (225). Atherosclerosis in the CCA is assumed to reflect general atherosclerosis and cIMT measurements obtained via ultrasound are often used as an endpoint to assess the effectiveness of interventions (216) and as quantitative indices of coronary atherosclerosis (226). The use of cIMT to help predict CV events comes secondary to evidence from epidemiological studies showing that it is a good independent predictor of CHD and stroke, the two leading causes of CVD-related death (227). The relative risks for myocardial infarction (MI) and stroke remain significant despite decreasing slightly after adjustment for traditional CV RFs (228). The annual *change* in cIMT also offers prognostic

information (229). One meta-analysis found that after adjustment for gender and age in asymptomatic individuals, an increase in cIMT of only 0.1mm increased the relative risk of MI and stroke by 10-15% and 13-18% respectively (209, 230). Carotid IMT has better predictive power for stroke than any other indices of atherosclerotic risk (231). Although carotid plaque area alone better represents atherosclerosis than cIMT alone (232), a combination of traditional RFs, cIMT and plaques optimally predicts risk (11, 233, 234).

Table 2.1: Normative data of common carotid IMT provided by the European Society of Cardiology

Age	P25	P50	P75
Men <30	0.39	0.43	0.48
Men 31-40	0.42	0.46	0.50
Men 41-50	0.46	0.50	0.57
Men >50	0.46	0.52	0.62
Women <30	0.39	0.40	0.43
Women 31-40	0.42	0.45	0.49
Women 41-50	0.44	0.48	0.53
Women >50	0.50	0.54	0.59

Median (P50), 25th (P25) and 75th (P75) percentile common carotid IMT values for men and women at different age categories are given. The American Society of Echography (ASE) Task force proposes that $IMT \geq 75th$ percentile is considered high and indicative of increased cardiovascular risk. Values from the 25th to the 75th percentile are considered average and indicative of unchanged cardiovascular risk. Values $\leq 25th$ percentile are considered low and indicate lower than the expected cardiovascular risk. Table and legend adapted from (225)

However, there are currently conflicting conclusions regarding the use of cIMT measurement (by adding it to traditional CV RFs) to help improve the prediction of CV events (33, 235-238). In one study, numerous healthy individuals classified as 'high-risk' for stroke, myocardial infarction or death using the FRS were reclassified to the 'low-risk' category when cIMT data was included (33).

Previous studies have used different ultrasound protocols, equipment, scanning sites and arbitrary cut-offs which most likely affect cIMT values. Thus, the validity of comparing inter-study conclusions regarding the ability of cIMT to predict CV risk is questionable (228). The general consensus is that to improve CV risk prediction, cIMT measures should only be carried out on the intermediate risk group (239). These findings show that advanced subclinical atherosclerosis can be identified by measuring cIMT after which CVD risk estimates can be altered, thereby increasing the accuracy of risk classification (240). In addition, as cIMT measurement can be expensive, not freely available and because there are difficulties with standardisation, it is not currently used in

routine risk assessment (11). If approaches used for image acquisition and analysis are standardised, larger databases can be created and inter-study results can be compared more accurately as the power of individual studies would be increased (211).

2.9.3 THE ASSESSMENT OF CAROTID IMT

Carotid IMT is the most widely used non-invasive imaging technique to quantify (subclinical/all stages of) atherosclerotic burden in different segments of the arterial tree (11) and is useful for monitoring change over time (121). Carotid IMT can be evaluated using both invasive methods and external radiology (refer to Appendix C). As an 'intermediate phenotype for early atherosclerosis' (209), *in-vivo* cIMT measurement using B-mode ultrasonography (Figure 2.14) is useful in large-scale studies and in the quantification of atherosclerosis and CVD risk (11). It is a safe, non-invasive, highly sensitive and reproducible, well-validated technique and individuals are not exposed to radiation (241-245). In addition, all stages of atherosclerosis can be imaged using ultrasound technology. Further details are provided in section 4.7.2.1.

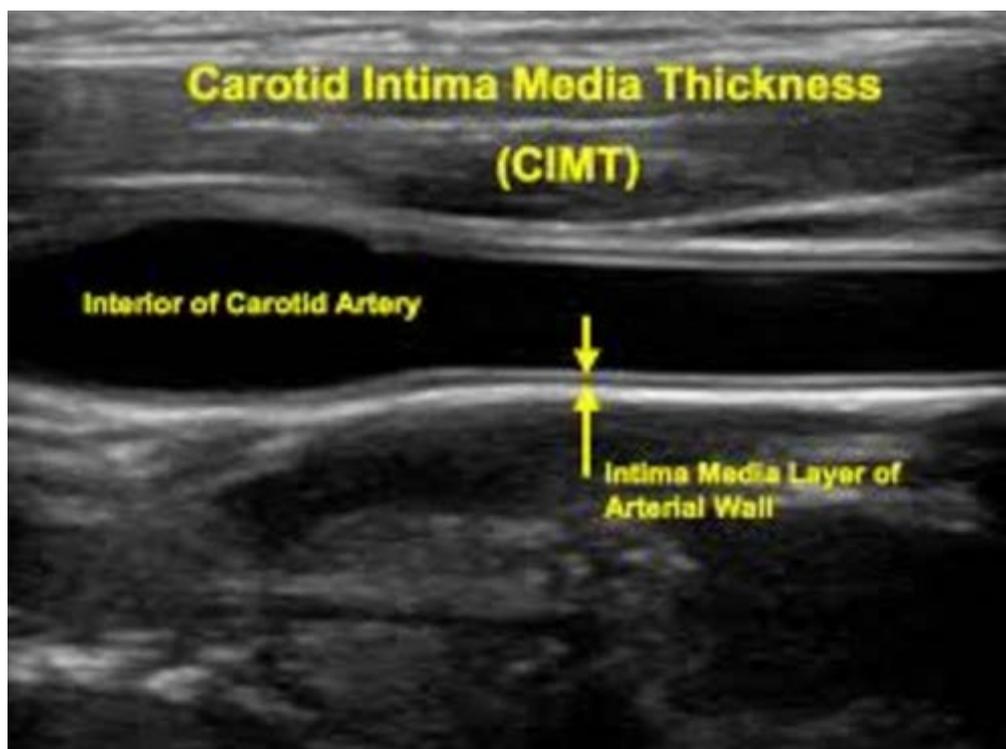


Figure 2.14: Common carotid artery IMT assessment using B-mode ultrasound

B-mode ultrasonography is the most widely used non-invasive method of cIMT assessment. The two echogenic lines, separated by a hypoechoic space, define the IMT.

Image adapted from (246)

Wall thickness in peripheral arteries

Lower limb arteries are prone to atherosclerosis and those in the upper limb experience wall thickening even though they are not usually subject to atherosclerotic plaque formation (57, 247),

implying that arterial wall thickening occurs systemically (248). Several studies have carried out IMT measurements on the common femoral artery (CFA) as it too, is superficial. Differences in the degrees of atherosclerotic lesions are often observed between the CCA and CFA because the latter is exposed to greater hydrostatic pressure and flow variations and more advanced atherosclerosis can be studied in the CFA (226). Increased femoral IMT (fIMT), is associated with traditional CV RFs (249-251), peripheral atherosclerosis indices (252), the FRS (250) and the severity and extent of coronary artery disease (253).

2.10 THE RESISTIVITY INDEX

The haemodynamic RI (also known as the Pourcelot index) is an indirect marker of the severity of atherosclerosis (254) and is an important parameter to be studied when examining the arterial waveform using Doppler ultrasonography. The CCA RI has been shown to be positively correlated to the cIMT, even after correcting for vascular risk factors (216). At a fixed compliance, the renal RI increases with increasing local resistance and the higher the compliance, the more the RI is influenced by local vascular resistance (255). The RI, which increases with natural ageing, is calculated from blood flow velocity curves obtained by pulsed wave Doppler ultrasonography (256). However, very few studies have assessed the common carotid RI (especially in relation to exercise).

2.11 ARTERIAL GEOMETRY AND REMODELLING – ARTERIAL DIAMETER AND ARTERIOGENESIS

Central and peripheral factors determine blood flow to muscles. The large conduit vessels deliver blood to small resistance vessels at high pressure (257). Blood flow to downstream vessels is optimised by arteriogenesis, which describes the enlargement of existing vessels. Arteriogenesis, which involves remodelling of endothelial cells, fibroblasts and VSMCs, occurs because of an increase in wall calibre (diameter) and dimensions as opposed to simply being brought about by wall stretch due to increased luminal pressure or compliance (257).

In hypertension and with ageing, chronically raised intraluminal pressure escalates radial wall stress (258), which, according to the law of Laplace, increases wall mass. However, the case is quite different when luminal pressure surges during episodic bursts such as during exercise. Muscle activity determines the size of the arteries which supply it and large increases in flow through conduit arteries result in their significant enlargement due to greater shear stress on the endothelial surface (259).

Increased flow velocity through a vessel stimulates arteriogenesis even if luminal pressure does not change because the former directly increases shear stress, the main stimulus for arterial enlargement (259). Arterial remodelling is primarily endothelium-dependent and upregulation of

endothelial nitric oxide synthase is the main process permitting arteriogenesis (257) and a secondary, understudied mechanism involved in arteriogenesis, is inflammation (260).

Amongst healthy, non-smoking 21-60 year old men and women, common carotid diameters range from 4.3-7.7mm. There are no differences between left and right common carotid diameter and men tend to have larger diameters (224).

2.11.1 CAROTID WALL: LUMEN RATIO (cWLR)

Conductance is a function of WLR, the calculation of which enables expression of the IMT relative to the intraluminal diameter, thereby indicating the extent of wall remodelling. Inward remodelling, or an increase in the WLR, has been observed in rheumatoid arthritis (261), hypertension (262), type II diabetes (263), obesity (264) and physical deconditioning (37) whereas training has been shown to decrease the WLR, indicating outward remodelling (increased lumen diameter and decreased wall thickness) (57, 265, 266).

2.12 ASSOCIATIONS BETWEEN ARTERIAL STIFFNESS, STRUCTURAL MODIFICATIONS AND TRADITIONAL CV RISK FACTORS

2.12.1 ARTERIAL STIFFNESS AND TRADITIONAL CV RISK FACTORS

Increased central AS is a hallmark of ageing (98) and increases in several CVD-related disease states (267) but it is difficult to conclude whether AS is a marker for potential vascular disease or whether the two are associated (136). However, AS is associated with CV and MS RFs (Figure 2.15) and atherosclerotic disease (6, 84, 90, 96-98, 268). The association between AS and (total and CV) mortality is significant even after adjustment for the traditional RFs (144, 154, 155). Interestingly, the predictive value of AS is greatest in individuals who are at low risk for CV events (154). A review carried out in 2009 confirmed a strong association of increased AS with ageing and elevated BP but only a weak association of increased AS with smoking, a high body mass index (BMI), hyper/dyslipidaemia and diabetes (18, 145). A decrease in insulin sensitivity (increased insulin resistance), a feature of both diabetes and MS, is associated with reduced vascular compliance in all age classes (269-271) and positively correlated to central AS (272, 273). Smoking, excessive alcohol and obesity are associated with increased pulse pressures (274-276). In addition to structural proteins determining the ability of an artery to expand and recoil, luminal pressure also contributes towards AS (18). When arterial pressure is low, elastic fibres support wall stress whilst at higher pressures, stiffer collagen fibres take over (79), causing arterial stiffening in the long term (84, 277) despite no change in wall structure (278). Evidence suggests that genetic factors could influence AS by directly or indirectly affecting the arterial wall (20).

2.12.1.1 ABDOMINAL OBESITY AND VASCULAR HEALTH

Although cIMT is an accepted surrogate marker for subclinical vascular disease which is an early marker of atherosclerosis, dyslipidaemia and lipid abnormalities also play a role in the development of this condition (279). Higher central fat distribution (abdominal obesity), as opposed to total body fatness, is associated with higher AS at 36 years of age (280). Waist circumference (WC) is a traditional marker of abdominal obesity, with BMI and WC being more strongly related to MS than body fat percentage indices (281). Waist circumference is also better than the BMI and body fat percentage indices at predicting dyslipidaemia and carotid atherosclerosis (281). Central obesity can alter arterial wall properties and increase AS, directly resulting in early arterial damage and premature vascular ageing (256). Although the WC is a preferable index to use routinely due to its sensitivity and simplicity (281), an elevated triglyceride level (≥ 2.0 mmol/L) in the presence of increased WC (for sex, height and ethnicity) better indicates excess visceral adiposity (282). Individuals with this 'hypertriglyceridaemic waist phenotype' are at increased CVD risk (283) and can therefore benefit the most from lifestyle changes to reduce their visceral fat and triglyceride levels.

2.12.2 THE ASSOCIATION BETWEEN ATHEROSCLEROSIS AND ARTERIAL STIFFNESS

Atherosclerosis, a chronic, systemic inflammatory disease, is brought about in response to physical injury of the vascular endothelium (284-286). Intimal-medial hypertrophy, typically observed in the common carotid arteries, usually occurs during the early stages of this disease whilst plaque formation, typically observed in the carotid bulbs or internal carotid arteries, occurs later (220). The relationship between plaque formation and AS is not fully understood but generally, larger artery stiffness is increased in the presence of atherosclerosis (18). Arterial stiffness, in addition to IMT, can therefore be used as a marker of the extent of this condition in the aorta (287). A 2009 review stated that in the early stages of atherosclerosis, increased AS may occur independently from the development of this condition (145). However, atherosclerosis involves several events and factors which can influence vascular remodelling and structure but since AS and atherosclerosis usually coexist, it is difficult to establish causation (98). The consequences of increased AS can accelerate the development of atherosclerotic lesions given that AS may either serve as a marker for potential atherosclerotic disease or it may be involved in the process of atherosclerosis directly (136). The risk factors for the development of atherosclerosis are similar to those of CVD (and MS) and include elevated BP, high cholesterol levels, high fasting blood glucose levels, obesity, smoking/tobacco use, physical inactivity and a family history of early heart disease.

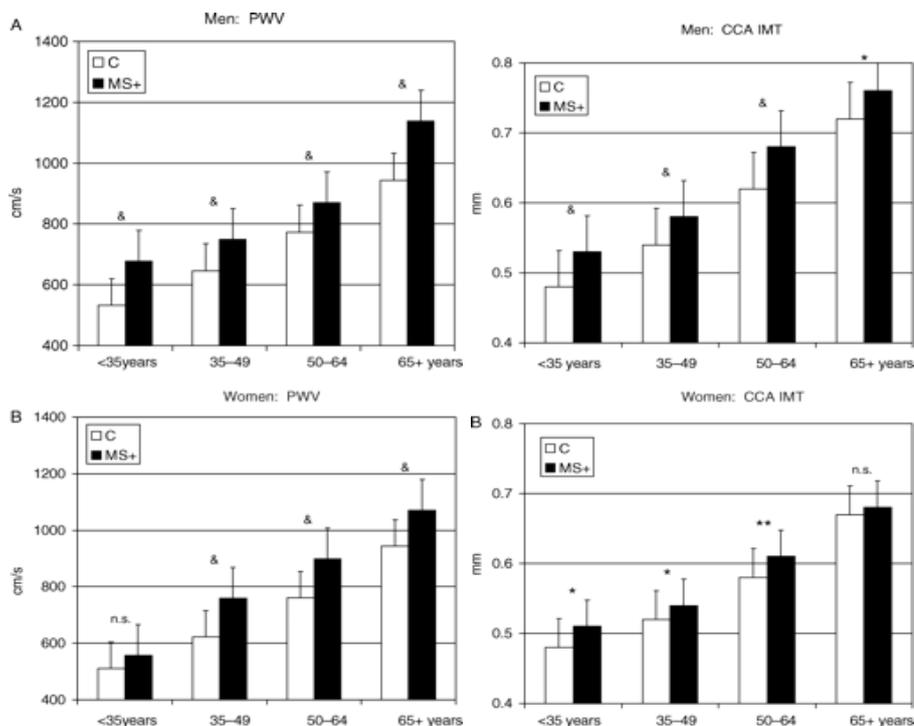


Figure 2.15: Effects of metabolic syndrome on arterial health indices

Gender-specific effects of MS on aortic pulse wave velocity (A and B) and common carotid IMT (C and D) across age groups. There are age-associated increases in aortic PWV and CCA IMT. Individuals with MS have higher (worse) PWV and CCA IMT than healthy individuals of the same age and sex. C, healthy controls; MS+, presence of metabolic syndrome; PWV, pulse wave velocity; CCA IMT, common carotid artery intima –media thickness)

***P < 0.05; **P < 0.01; &P < 0.001 vs. control of the same age group. Image adapted from (77)**

Although increased AS is not always linked atherosclerosis in a particular region, PWV has been associated with coronary atherosclerosis (288, 289). One study reported that PWV decreases in the initial stages of atherosclerosis and then quickly increases during the advancement of atherosclerosis (140). A second study found no significant differences in distensibility between regions with and without plaques (290) and a third study found that PWV is only increased during stages of advanced plaque formation (291). Initial stages of arterial stiffening are determined by non-atherosclerotic mechanisms which involve BP-related mechanical stretch which transfers loading from elastin to the stiffer collagen fibres (145). The correlation between AS and atherosclerosis (140, 292) is probably due to the effects that cyclic stress has on arterial wall thickness (293). Only weak, if any, positive correlations between IMT and PWV have been found (294, 295), which is possibly because an increase in IMT normalises the shear stress brought about by an increase in BP (296). In essential hypertension, there is a sustained increase in BP and this causes hypertrophy of arterial walls which normalises circumferential wall stress (20). This adaptive response only occurs until a certain level after which increased IMT is associated more with atherosclerosis (219). Contrary to what might be expected, studies on individuals with essential hypertension and rat models of hypertension have shown that wall thickening induced by the sustained elevated BP is not associated with increased AS (151, 297-301).

CHAPTER 3:-THERAPEUTIC STRATEGIES AIMED AT OPTIMISING ARTERIAL HEALTH: THE VASCULOPROTECTIVE ROLE OF EXERCISE

The strategies aimed at improving arterial health fall into two broad categories, namely, pharmacologic and non-pharmacological ones.

3.1 GOALS OF PHARMACOLOGICAL THERAPIES

Ideally, pharmacologic therapy should normalise AS completely by reducing BP and bringing about structural changes in the vasculature (302). The aim is to target the events that cause AS and endothelial dysfunction, that is, oxidative stress and inflammation. Both elastic and muscular arteries have been targeted because whilst compliance of the former determines left ventricular afterload, compliance of the latter determines wave reflections (84). Long-term (but not acute) drug administration is capable of changing arterial wall components and their spatial organisation, independent of BP reductions (302). These modifications include an increased elastin:collagen ratio, decreased collagen content, a reduced IMT and lumen diameter and changes in connections between VSMCs and the ECM (80, 303). The fact that lowering local PP and not MAP reduces wall hypertrophy and lumen diameter means that by decreasing AS, pulsatile stress is reduced, bringing about arterial remodelling (80).

On the other hand, acute drug administration causes a BP-independent decrease in the AS of muscular arteries, implying that smooth muscle relaxation affects the proportions of the structural components of arterial walls (84). In normotensive adults with CV RFs, it may take much longer (relative to clinical populations) to improve AS.

3.2 NON-PHARMACOLOGIC THERAPIES USED TO IMPROVE ARTERIAL HEALTH

Non-pharmacological treatments usually revolve around physical activity (exercise) and dietary manipulation, but much is left to be discerned due to small sample sizes in relevant studies to date. Blood pressure-dependent large artery compliance increased in healthy, obese men after weight loss associated with reduced MAP (304). Caloric restriction (CR) improves systemic atherosclerosis RFs, reduces BP and has direct anti-ageing effects on the vasculature (305) by up-regulating endothelial nitric oxide synthase (eNOS), therefore improving nitric oxide bioavailability and endothelial function (306). In addition, CR reduces reactive oxygen species (ROS) production (307), oxidative stress (308, 309) and vascular nuclear factor kappa-B (NF- κ B) activation (310). Whilst long-term CR reduces chronic low-grade inflammation by decreasing circulating TNF- α and C-reactive protein levels, (311), even short-term CR can have anti-inflammatory effects (217). Other studies have demonstrated the beneficial impact of α -linolenic

acid, isoflavones (derived from red clover or soy products) (312), fish oil therapy (313), >300mg garlic powder daily and a reduced sodium intake on arterial compliance and PWV (314).

3.3 PHYSICAL ACTIVITY STATUS AND ARTERIAL HEALTH

The possibility of a future CVD epidemic is mounting given that advancing age is the primary risk factor for CVD and that most individuals with this condition are middle-aged and older (MA/O) adults whose numbers are rapidly increasing (315). Sedentary lifestyles account for a staggering one-third of all deaths (316) which is an additional concern, given the low daily energy expenditure in modern life (317). The effects of physical inactivity and activity on traditional CV RFs are not profound and do not explain the entire magnitude of the impact that physical activity status has on risk (318). Therefore, it has been proposed that physical activity and inactivity can both influence CV risk by affecting vascular structure and function directly (318) (ie. vascular deconditioning and conditioning). However, the effects of exercise and deconditioning on arteries are not simply opposite to one another. Endothelial dysfunction and the stiffening of large elastic arteries are the two primary components of arterial dysfunction, the main intermediary event associated with age-related CVD (82). Despite being impaired in high-risk individuals, vascular structure and function are susceptible and responsive to change.

3.3.1 THE EFFECTS OF PHYSICAL INACTIVITY AND DECONDITIONING ON ARTERIAL STRUCTURE AND FUNCTION

Physical inactivity and low cardiorespiratory fitness (CRF) independently predict atherosclerosis/CVD and all-cause mortality respectively (319, 320). Alterations in haemodynamic stimuli which directly impact blood vessels explain part of the association between physical inactivity and the increased CV risk (319) which cannot be attributed to changes in traditional CV RFs. Accelerated arterial stiffening of conduit arteries occurs with physical inactivity (321, 322), which results in dose-dependent inward arterial remodelling and a pro-atherogenic phenotype (318).

Future CV events are strongly predicted by resting conduit artery diameter (323) and wall thickness (73). Deconditioning rapidly reduces conduit artery diameter (end-diastolic diameter, EDD) in a dose- and time-dependent fashion and this may be due to physical inactivity-induced muscle atrophy and reduced metabolic demands (37). Deconditioning also increases conduit artery wall thickness (IMT) but these changes are probably reversible adaptations as opposed to a reflection of atherosclerotic changes (37). The final result is an increased WLR. In addition, physical inactivity brings about the remodelling of resistance vessels, whose cross-sectional area decreases in a dose- and intensity-dependent way in response to deconditioning, possible due to muscular atrophy (37). The increased resistance is explained primarily by vasoconstrictor (324) as opposed to dilator pathways, the latter being involved in the cardioprotective effects of exercise. Although changes in conduit arteries occur earlier than those in resistance vessels (325), there is no definitive conclusion as to whether structural or functional deconditioning-

induced changes occur first (37). Usually, blood flows through conduit arteries in an oscillatory fashion, with anterograde and retrograde components during systole and diastole respectively (318). Deconditioning results in a pro-atherogenic phenotype by increasing retrograde flow and altering patterns of shear stress (326).

3.4 EXERCISE AND VASCULAR HEALTH

Extensive research has been carried out on the benefits of exercise on the CV system, including its impact on arterial health (41), with evidence coming from both human and animal cross-sectional and longitudinal studies. Only 59% of the cardioprotective effects that exercise has consistently been shown to confer can be attributed to favourable alterations in traditional CV RFs (40). One hypothesis proposes that exercise-induced cardioprotection can also be largely explained by haemodynamic alterations and direct effects on the structure and functions of vascular walls both acutely and in the long-term (318).

3.4.1 EXERCISE MODES CONSIDERED IN ARTERIAL HEALTH RESEARCH

'Endurance' or 'aerobic' exercise (AE) involves repetitive, dynamic contractions of large muscle groups and causes systemic changes in PP and HR. There is evidence from both cross-sectional (Table 3.1 and Table 3.2) and longitudinal (Table 3.4 and Table 3.5) studies that moderate intensity AE can improve AS and wave reflection indices and induce outward arterial modelling in healthy, sedentary individuals. It is therefore effective at combating arterial ageing in healthy, normotensive adults. Figure 3.1 depicts a mechanism by which AE can exert its vasculoprotective effects.

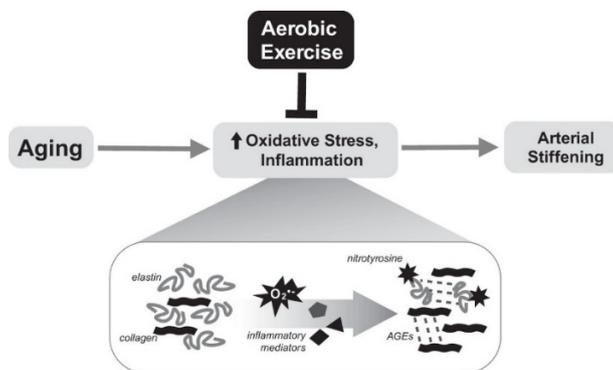


Figure 3.1: Mechanism by which aerobic exercise attenuates age-associated arterial stiffening

Increased oxidative stress and inflammation during the natural ageing process predispose individuals to arterial stiffening by altering the composition of the arterial wall extracellular matrix. Aerobic exercise can reduce this risk as it confers both anti-oxidative and anti-inflammatory effects. Image adapted from (60)

'Resistance' training triggers different muscular adaptations to endurance training and is thus associated with different haemodynamic changes and cardiovascular and muscular benefits (81). However, resistance exercise (RE) is generally thought to have a negative impact on arterial

health indices and wave reflections (67) and depending on the index in question, adding AE to RE either attenuates, cancels out or overrides the effects of RE (65, 327). However, it is also possible that RE triggers greater remodelling than AE (265).

Interval training involves brief, regular bursts of vigorous intensity exercise interspersed by rest or low-intensity exercise. This is in contrast to moderate, continuous exercise at which the intensity is constant, as depicted in Figure 3.2. There is considerable literature regarding the potent and time-efficient central and peripheral physiological effects of interval exercise in various healthy/clinical populations (63). The beauty of interval exercise lies in its diverse nature and the ability to manipulate its work and rest ratios, durations and intensities to suit specific populations or individuals. Two major types of interval exercise based on AE are sprint interval training and high-intensity interval training. Sprint interval training (SIT) is comparable to AE at reducing AS but requires a longer time to do so and may only confer benefits if the baseline AS status is a lot lower than optimal (61). Although SIT can increase peripheral arterial compliance, it may not be well tolerated by certain (ill/injured/sedentary/unfit) individuals. A more practical, safer option is low volume high-intensity interval training (HIIT), which employs relatively longer duration work intervals of lower intensity with shorter recovery periods (328).

The potent and time-efficient central and peripheral physiological effects of HIIT in various healthy/clinical populations [reviewed by Shiraev and Barclay (329)] have been previously demonstrated. Briefly, the health benefits of HIIT usually occur faster than those conferred by high-volume steady state moderate continuous aerobic exercise (MCAE) training. The effects of HIIT on cardiac contractility, left ventricular mass, stroke volume and VO_{2max} are comparable if not superior to those of MCAE. Relative to MCAE, HIIT is superior in terms of improving vascular function (measured by brachial artery FMD) and is usually considered to be more enjoyable which may imply greater adherence to the latter (330). This would therefore be particularly useful amongst previously sedentary individuals who may lack the motivation to begin or stick to a training programme. The health and practical benefits of HIIT have been reviewed elsewhere [(63, 329)]. Three months of merely twenty minutes of HIIT, thrice a week can reduce both central AS and the intensity of wave reflections (the Alx) in healthy, sedentary young men (62). Despite the extensive studies on arterial health indices, few have employed interval training. The approach in the present study was to investigate the effects, on arterial health, of exercise based on a combination of AE, HIIT and SIT concurrently in one session.

3.5 SUMMARY OF STUDIES INVESTIGATING RELATIONSHIPS BETWEEN PHYSICAL ACTIVITY AND ARTERIAL HEALTH

Physical activity reduces primary and secondary vascular events, CVD mortality by 35% and all-cause mortality by 33% (when compared to a sedentary lifestyle (38, 331, 332) and there is an inverse dose-response relationship between physical activity and CVD risk/mortality (39). Key findings from cross-sectional studies regarding the associations between firstly, training status and various AS indices and secondly, training status and remodelling, are summarised in Table 3.1 and Table 3.2 respectively.

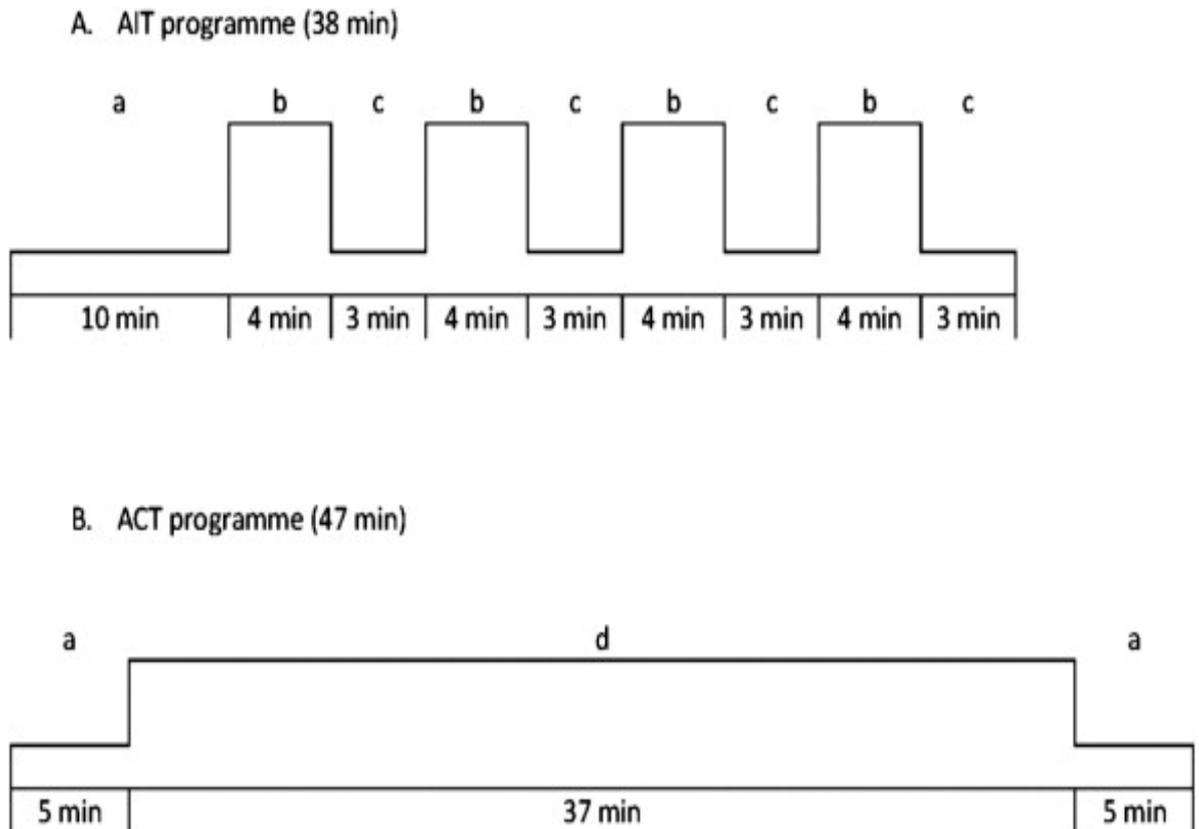


Figure 3.2: Schematic illustrations of aerobic interval training and aerobic continuous training

AIT (A) involves brief periods of vigorous exercise interspersed by low-intensity exercise or rest. During ACT (B), a sustainable intensity (a level between the peaks and troughs of AIT) is maintained for the duration of the entire session. *AIT, aerobic interval training; ACT, aerobic continuous training (moderate continuous aerobic exercise)*

a = 50–60% VO_{2peak} ; 60–70% HR_{peak} ; 11–13 Borg scale, no shortness of breath.

b = 85–90% VO_{2peak} ; 90–95% HR_{peak} ; 15–17 Borg scale, shortness of breath.

c = 50–70% HR_{peak} .

d = at least 60–70% VO_{2peak} ; \geq 65–75% HR_{peak}

Image and legend adapted from (333)

Table 3.1: Findings from cross-sectional studies investigating the relationships between physical activity levels/training status and AS indices

ref (s)	Key findings
(50)	The cfPWV of endurance-trained athletes is, on average, 2m/s lower than sedentary age-matched counterparts
(52)	MA/O men and women performing vigorous AE have lower SBP and aortic PWV than sedentary counterparts and their values are close to that observed in younger sedentary individuals
(54)	Low-intensity regular AE is associated with lower aortic PWV
(334)	Endurance-trained athletes exhibit greater compliance than age-matched sedentary counterparts
(49)	Endurance-trained men had 20-35% greater central arterial compliance than sedentary and/or recreationally active age-matched controls
(335)	Aortic PWV of resistance-trained > sedentary > endurance-trained men; systemic arterial compliance of resistance-trained < sedentary < endurance-trained men
(53)	Endurance-trained athletes have a lower Alx than recreationally active age-matched counterparts
(336)	Amongst 20-22 year old males, aortic PWV endurance-trained < sedentary controls < resistance-trained men; systemic arterial compliance endurance trained > controls > resistance-trained
(51)	Physically active women do not exhibit age-related increases in central AS (aortic PWV & carotid Alx); upper and lower limb PWV is not different between post-menopausal endurance-trained women and sedentary counterparts
(327, 337)	Compliance of peripheral (muscular) arteries do not usually show improvements with AE
(67)	Resistance-trained athletes have lower whole body compliance and higher central and peripheral PP than less active age-matched counterparts
(66)	Resistance-trained individuals have (20%) lower arterial compliance than sedentary counterparts (greater age-related reductions in arterial compliance in resistance-trained than sedentary men)
(338)	Muscular strength and central AS (cfPWV) are inversely associated, independent of cardiorespiratory fitness
(321)	Physical inactivity causes accelerated conduit artery stiffening; daily electrically-induced training of a deconditioned limb increases local conduit artery compliance and improves endothelial function
(322)	Physically inactive men with spinal cord injury have significantly higher aortic PWV values than able-bodied age-matched counterparts
(339)	Amongst adults with hypertension, physical inactivity is positively correlated to PWV and Alx; regular physical activity of all intensities is inversely related to PWV and Alx; physical activity is a significant predictor of Alx and PWV

PWV, pulse wave velocity; PP, pulse pressure; CFA, common femoral artery; cfPWV, carotid-femoral PWV, Alx, augmentation index; AE, aerobic exercise; MA/O, middle aged/older; SBP, systolic blood pressure

Table 3.2: Findings from cross-sectional studies investigating relationships between physical activity levels/ training status and arterial structure

ref(s)	Key findings
(340)	Elite squash players have lower brachial IMT, fIMT and cIMT than less active individuals; no difference in IMT between the preferred and non-preferred limb in squash players despite the larger arterial diameter in the preferred limb
(341)	Elite runners and cyclists have greater arterial diameters systemically, lower systemic arterial IMT and lower carotid and brachial WLRs than recreationally active individuals
(342)	No differences in cIMT between endurance-trained men and sedentary counterparts
(343)	In healthy young adults, VO_{2max} is negatively correlated to cIMT
(344)	In 50-60 year old men, VO_{2max} is inversely related with carotid bifurcation IMT but not with cIMT
(345)	In older men, VO_{2max} is inversely related to atherosclerotic markers (IMT and roughness)
(334)	Endurance-trained athletes exhibit greater common femoral artery lumen diameter than age-matched sedentary counterparts
(346)	Endurance/recreational exercise does <i>not</i> beneficially affect early carotid atherosclerotic markers
(347)	Physical inactivity is positively associated with cIMT in middle-aged adults
(348)	Physical activity predicts cIMT in middle-aged men but not women
(334)	Leisure-time physical activity is not associated with reduced cIMT in males
(349, 350)	Amongst young adult borderline hypertensives, physical activity is positively related to cIMT
(56)	Femoral diameter was 6% greater and fIMT and femoral wall:lumen ratio were 16-21% lower in endurance-trained individuals than in sedentary counterparts but there were no intergroup differences in cIMT
(55)	fIMT was 20-27% (men) and 45% (women) lower in endurance trained/moderately active adults compared to sedentary age-matched counterparts The natural age-associated increase in fIMT was 33% (men) and 15% (women) smaller in endurance-trained than sedentary age-matched peers AE has a larger effect on muscular compared to elastic arteries

AE, aerobic exercise; IMT, intima-media thickness; cIMT, common carotid intima-media thickness; fIMT, femoral intima-media thickness

3.6 THE ACUTE EFFECTS OF EXERCISE ON ARTERIAL STIFFNESS AND STRUCTURE

Although it was generally believed that elastic properties of arteries change over long time periods, studies are now showing that arterial compliance can change over shorter periods and even acutely. Table 3.3 summarises the major studies investigating the acute effects of various forms of exercise on AS, wave reflection indices and structural/geometrical parameters. The acute decrease in (central and peripheral) AS brought about by either moderate (351) or maximal (352) AE has been attributed to changes in vasomotor tone as opposed to changes in arterial wall properties (123). A decrease in the Alx after a single bout of MCAE (353) or HIIT (354) is due to increased intrinsic arterial compliance (353) (indexed by Young's elastic modulus, refer to Figure 2.3). Acute AE can also cause vasodilation of peripheral muscular arteries and thereby, reduce the intensity of wave reflections from the lower body in a way that ventricular-vascular coupling is improved (181) (refer to Figure 2.3). The magnitude of these changes is positively correlated to exercise intensity and persist for up to an hour post-exercise (181). One study compared the acute effects of HIIT and MCAE on the Alx@75 and found a reduction of relatively greater magnitude and longer duration after the former, implying that HIIT improves pulse wave reflection dynamics to a greater extent than MCAE (354).

Acute decelerations in PWV following maximal AE (355) may be a result of rapid alterations in vasomotor tone (endothelium-dependent NO-induced relaxation of vascular smooth muscle) (356) due to increased shear stress brought about by hyperaemia-induced increased blood flow (351, 357). In the exercised limb, PWV decreases 2 minutes after low-intensity AE, possibly to counter the effects of the increase in BP (123). However, high-intensity exercise may be required to increase NO production in non-exercised limbs (358). The decrease in total peripheral resistance (TPR) may also contribute to the increase in whole-body arterial compliance after 30 minutes of moderate-intensity AE (351).

After sedentary, healthy young to middle-aged adults engaged in a single bout of maximal treadmill exercise, PWV in the exercising lower limb ('second-order system') showed a biphasic response by decreasing from 3 mins to 10 mins post-exercise and then increasing until 60 mins post-exercise at which point it was 10% below baseline (352) (Figure 3.3B). The duration of the increased aortic PWV may depend on the exact exercise intensity and duration (355). Conversely, upper limb ('first-order system') PWV decreased continuously for 1 hour post-exercise to a level that was 10% below baseline at 60 mins (Figure 3.3A). The authors ruled out potential influences of changes in post-exercise HR, BP and blood viscosity on these findings which they therefore explained using the two possible and opposing consequences of vasodilation as follows: arterial wall distensibility is related to its radius which depends on both vascular tone and elastic properties of the wall. Increased arterial diameter during vasodilation stretches structural components of the wall and decreases distensibility (thereby increasing PWV). On the other hand, vasodilation can attenuate the influence that vasomotor tone has on AS and therefore it can

increase distensibility. (352). The credibility and relevance of both these occurrences in association to distensibility warrants further research to better explain findings.

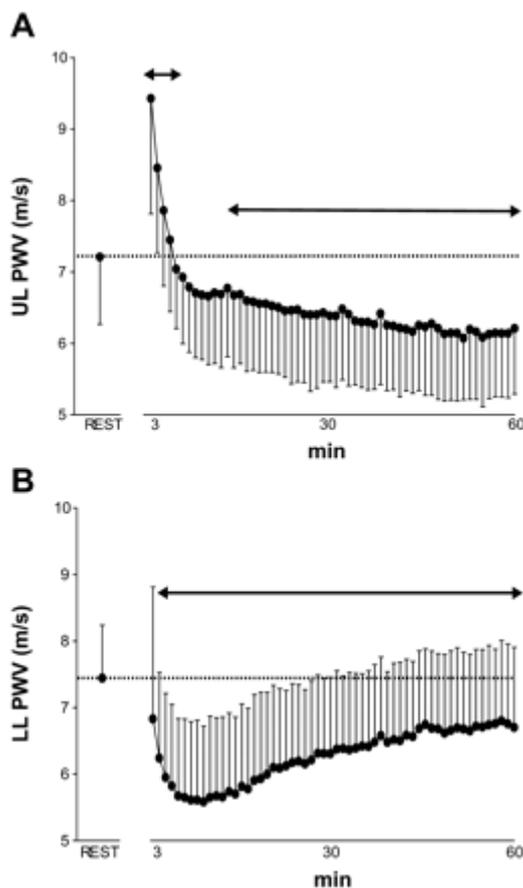


Figure 3.3: Acute responses of PWV in upper and lower limbs following maximal treadmill exercise in healthy adults

Values are means \pm SD; n=25. PWV in the upper limb (UL, A) gradually decreased over the hour post-exercise whilst PWV in the lower limb (LL, B) showed a biphasic response by decreasing until 3 mins post-exercise and then increasing until 60 mins post-exercise. At one hour post-exercise, PWV in both limbs was 10% below baseline. Horizontal arrowed bars indicate individual data points significantly different from the 10-min averaged pre-exercise data ($p < 0.05$). *Image adapted from (352)*

An acute rise in aortic PWV after AE would not be due to increases in PP (6), MAP (passive stretch) (351) or HR (359), factors which all affect PWV, but rather, due to increased vasomotor tone brought about by increased ET-1 and catecholamine levels (360). These levels are higher at baseline in resistance-trained individuals and therefore do not increase as much in these individuals compared to those who are sedentary or endurance-trained (361). Before and after a 6 month AE programme, BP but not the AIx significantly reduced acutely after a bout of MCAE (353). Greater acute reductions pre-training were observed in those with higher BP and AIx at baseline and greater acute reductions post-training were observed in those whose baseline reductions post-exercise were greater. This finding implies that chronic, regular AE can alter the acute effects of exercise on arterial health and the magnitude of change may be related to baseline TPR.

Shear forces increase anterograde flow which explains how exercise positively modifies endothelial function acutely. Retrograde (backward) shear, however, may attenuate these benefits (362). When these effects of increased shear stress occur repeatedly during acute exercise bouts, they provide a stimulus for vasodilator pathways and vascular adaptation to long-term exercise training (81). Regional factors other than NO (eg. prostacyclin, endothelium-derived hyperpolarizing factor and interstitial metabolites such as lactate, adenosine, phosphate and H⁺) may also play a role in the acute effects of AE on AS (123).

On the other hand, the pressor response during RE can alter the load-bearing properties of collagen and elastin (363) to overcome the acute, repetitive increases in BP associated with RE (65, 66). This increases left ventricular afterload, thereby contributing to its hypertrophy (364), and coupled with increased SNA and vasomotor tone, this is associated with reduced arterial compliance (64). Furthermore, RE increases the formation of cross-links and AGEs in arterial walls (365). However, vasodilation of the vasa vasorum supplying the aorta might explain the acute decrease in aortic PWV following exercise in resistance-trained athletes (360).

3.6.1 SUMMARY: THE ACUTE EFFECTS OF EXERCISE ON ARTERIAL HEALTH

Altered stiffness and wave-reflection indices after exercise are influenced primarily by vasomotor tone and shear forces. Acute decreases in PWV and the Alx following exercise are attributed to vasodilation, which counters the effects of vasoconstriction on AS. Acute increases in PWV and the Alx following maximal AE are usually due to arteriogenesis-induced wall stretch and decreased distensibility as well as increased catecholamine release during exercise. Beneficial effects usually occur in a dose-dependent manner (with high-intensity exercise eliciting greater NO release) and the effects can persist for up to an hour post-exercise. Increased SNA to overcome the pressor response elicited during RE may explain the acute decrease in arterial compliance following this type of exercise. The magnitude of changes generally depends on baseline values. Acute effects of exercise on arterial health may explain long-term adaptations whilst chronic exercise training may alter the acute effects of exercise on arteries.

Table 3.3 Studies investigating the acute effects of exercise on arterial health indices (continued on next page)

refs	Subject characteristics	Exercise characteristics			Main findings
		Intensity	Duration	Type	
(123)	Apparently healthy, non-smoking sedentary/recreationally active young adult (22-28 years) males (n=9)	Low (30W)	5 mins	single-leg cycling	↓ PWV in exercised limb only at 2 mins post-exercise (p<0.05); increased nitric oxide production is not a major factor in this decreased regional AS
(351)	Apparently healthy, sedentary, non-smoking 24±6 year old males (n=12)	Mod (65%VO _{2max})	30 mins	cycling	↑ whole body arterial compliance (66±26%) and carotid pressure until 30 mins post-exercise (p=0.04) then ↓ back to baseline at one hour post-exercise; ↓ aortic and leg PWV by 4±2% (p=0.04) and 10±4% (p=0.01) respectively at 30 mins post-exercise; ↔ MAP but ↓ cSBP (p=0.03); ↑cardiac output (p=0.007) & HR (p=0.001) but ↓total peripheral resistance(p=0.01)
(355)	Sedentary (n=15), endurance-trained (n=19) and resistance-trained (n=18) 20-45 year old adults	Mod (65%HR _{max})	30 mins	cycling	↑ central PWV at 3 mins post-exercise then ↓ back to baseline at 15 mins post-exercise in sedentary controls (from 7.4±2.2 to 9.1±3.1m/s, p=0.02) and endurance-trained athletes (from 7.7±2.2 to 10.6±4.2ms, p=0.01); insignificant decrease in PWV at 30 mins post-exercise in resistance-trained athletes; ↔ post-exercise peripheral PWV in all participants
(366)	Sedentary controls, endurance athletes and resistance athletes	Mod (60%HR _{max})	30 mins	cycling	↔ central & peripheral post-exercise PWV in resistance athletes and controls; post-exercise central PWV ↑ in endurance athletes (from 8.0±2.0m/s to 10.5±4m/s at 3 mins post-exercise, p=0.027) but returned to resting values by 15 mins post-exercise
(352)	31±6 year old apparently healthy, sedentary adults (n=50)	Maximal	symptom-limited	running	↓ upper limb PWV until 60 mins post-exercise; ↓ then ↑ lower limb PWV to baseline at 60 mins post-exercise; post-exercise upper and lower limb PWV were ~10% less than respective baseline values
(335)	22±0.5 year old sedentary (n=15) and resistance-trained (n=15) males	Start at 30W with 50W increment after every 2 mins	VO _{2peak} test	cycling	10 mins after maximal AE, ↓ peripheral PWV in all subjects (p<0.05), ↔ central PWV, ↔ intensity of central pressure wave reflections (AIx) in resistance-trained and sedentary counterparts at 10 mins post-exercise but decreased thereafter until 30 mins post-exercise (p<0.05) (arterial reactivity to acute stressors is not impaired in resistance-trained individuals)

Table 3.3 continued: Studies investigating the acute effects of exercise on AS indices (continued on next page)

refs	Subject characteristics	Exercise characteristics			Main findings
		Intensity	Duration	Type	
(367)	20-40 year old endurance-trained males (n=11)	Maximal (treadmill) or submaximal (4000m field run)	symptom-limited maximal test or time-to-4000m completion	running	<p>Maximal: at 5 mins post-exercise, central AIx@75 was greater than baseline (p<0.01) and then ↓ such that at 45 mins post-exercise, it was less than baseline (p<0.05); central and peripheral SBP, PP and MAP (but not DBP) were greater at 5 mins post-exercise than baseline (p<0.01)</p> <p>Both maximal and submaximal exercise, systolic AI (AIx) was less than pre-exercise levels at 5, until 45 mins post-exercise (p<0.05); Diastolic AIx was lower than pre-exercise levels at 5 mins post-exercise (p<0.05 for submaximal and p<0.01 for maximal) but progressively increased and was greater than pre-exercise levels at 45 mins post-exercise (p<0.05 for submaximal and p<0.01 for maximal)</p> <p>Submaximal: central and peripheral blood pressures were greater at 5 mins post-exercise than baseline (p<0.01)</p>
(368)	44-70 year old (middle-aged and older) adults with (n=50) and without (n=50) coronary artery disease	Graded-exercise test	symptom-limited	running	↓ peripheral PWV after exercise test in individuals with (from 1.527±245cm/s to -132±155cm/s, p<0.001) and without (from 1.439±202cm/s to -77±93cm/s, p<0.001) coronary artery disease
(369)	31±1 year old healthy males (n=12)	Low-vig (50-80%HR _{max})	≥ 16 mins (4 mins at each intensity)	cycling	During exercise, ↑ peripheral PP and PP amplification during exercise (p<0.001), independent of exercise intensity; ↑ aortic PWV during exercise (p<0.001); aortic PWV α MAP (r=0.54, p<0.001)
(370)	Apparently healthy, South Asian and Caucasian adults (n=69)	Bruce Protocol	Voluntary exhaustion	running	↑ AP, PP, MAP and AIx after exercise in both groups (p<0.05); Similar effects between the two ethnic groups (p>0.05); ↔PWV; exercise capacity inversely related to AS (p<0.05)

Table 3.3 continued: Studies investigating the acute effects of exercise on AS indices (continued on next page)

refs	Subject characteristics	Exercise characteristics			Main findings
		Intensity	Duration	Type	
(353)	67±6 year old sedentary adults with no history of medication for CVD (n=99)	Progressive	30 mins	dancing, hopping, before and after 6 months of AE	Pre- and post-training: acute ↓ BP but ↔ Alx ΔAI & $\Delta AIx@75$ were inversely correlated to pre-training, post-exercise values ($r=-0.47$, $p<0.001$ and $r=-0.54$, $p<0.001$ for Alx and Alx@75 respectively); $\Delta SBP \propto \Delta AIx_{acute}$ ($r=0.24$, $p=0.015$)
(354)	18-35 year old healthy, community-dwelling males (n=21)	Mod continuous 80% HR (± 5 heartbeats) of individual anaerobic threshold; HIIT: 10 min WU at 70% HR _{max} 4x4 min at 90-95% HR _{max} with 3 min active recovery at 70% HR _{max} 3 min CD at 70% HR	Duration computed so expected oxygen uptake was equal to that of HIIT Approx. 40 mins	Treadmill; 1% incline Treadmill; 1% incline	MCAE: ↔ Alx until 24h post-exercise; ↑ Alx@75 immediately after exercise but ↔ at 24h post-exercise HIIT: progressive ↓ in Alx until 24h post-exercise and sig. between-group difference by 35 mins post-exercise; ↑↑ Alx@75 immediately after exercise but ↓↓ at 24h post-exercise There was differences in Alx between MCAE and HIIT at 35 mins ($p=0.045$) and 50 mins ($p=0.008$) post-exercise There were differences in Alx@75 between MCAE and HIIT at 5 mins ($p<0.001$), 20 mins ($p<0.001$) and 35 mins ($p=0.009$) post-exercise

Table 3.3 continued: Studies investigating the acute effects of exercise on AS indices

refs	Subject characteristics	Exercise characteristics			Main findings
		Intensity	Duration	Type	
(360)	20.1±1.2 year old healthy males (n=9)	Wingate test: single or multiple	n/s	SIT (cycling) - Single: 2 min WU at 40W followed by 30s 'all-out' effort against a resistance equal to 0.075 kg (kgbodymass) ⁻¹ Multiple: 2 min WU at 40W followed by 4 Wingate tests with recoveries of 4.5 mins at low cadence (<50rpm) at 40W	Similar findings after single or multiple Wingate tests: central PWV ↑ until 20 mins post-exercise (p<0.05); peripheral PWV ↓ until 45 mins post-exercise (p<0.05); trend towards ↓ SFA distensibility at 2 mins post-exercise (p=0.06); ↑ HR throughout recovery period (p<0.05)
(371)	27±1 year old apparently healthy, non-smoking sedentary/recreationally active adults (n=16)	75% 1RM	n/s	RE WU of 8-12 reps of 9 exercises at 50% 1RM followed by 9 exercises, reps to exhaustion (seated row, seated chest press, leg press, seated overhead press, seated biceps curl, leg extension, seated leg curl, standing triceps extension, and seated calf raise)	↓ central arterial compliance + ↑ β stiffness index at 30 mins post-exercise (p<0.01) and values returned to baseline by 60 mins post-exercise; ↑ carotid but not brachial pressure immediately after exercise (p<0.01)
(361)	20-29 year old healthy, non-smoking males (n=13)	60% 1RM	n/s	RE: 8 exercises; 15 reps, 2 sets (bench press, squat, lat pull down, biceps curl, leg extension, leg curl, upright row, triceps extension)	↑ cfPWV, Alx and HR at 20 mins post-exercise (p<0.05); ↔ pBP, cBP, PP
(372)	Apparently healthy 20-40 year old non-smoking, sedentary males (n=20)	70% 1RM	n/s	RE: 4 sets, 10 reps, 6 exercises; Lower-body (LRE), upper-body (URE) or whole-body (WRE)	Cardio-ankle vascular index (CAVI) (-0.93) and brachial-ankle (peripheral) PWV (-2.08m/s) were a lot lower in LRE than URE immediately post-exercise (p<0.05); Compared to WRE, LRE caused a decrease and URE caused an increase in AS across all time points post-exercise

PWV, pulse wave velocity; cfPWV, carotid-femoral pulse wave velocity; PP, pulse pressure, pBP and cBP, peripheral and central blood pressure; HR, heart rate; Alx, augmentation index; AE, aerobic exercise; RE, resistance exercise; MCAE, moderate continuous aerobic exercise; HIIT, high-intensity interval training; SIT, sprint interval training; CFA, common femoral artery; SFA, superficial femoral artery; AS, arterial stiffness; AP, augmentation pressure; MAP, mean arterial pressure; 1RM, 1 repetition maximum; n/s, not specified; WU, warm up; CD, cool down

3.7 HABITUAL EXERCISE AND ARTERIAL STIFFNESS

Interventional studies investigating the effects of short-to-long term (≥ 4 weeks) exercise training programmes on arterial health indices provide insight into the optimal exercise characteristics to beneficially impact arterial health and CV outcome. Arterial stiffness, via its effect on myocardial work capacity, can influence exercise capacity and as shown in Figure 3.4, lower central AS is associated with higher cardiorespiratory fitness (373).

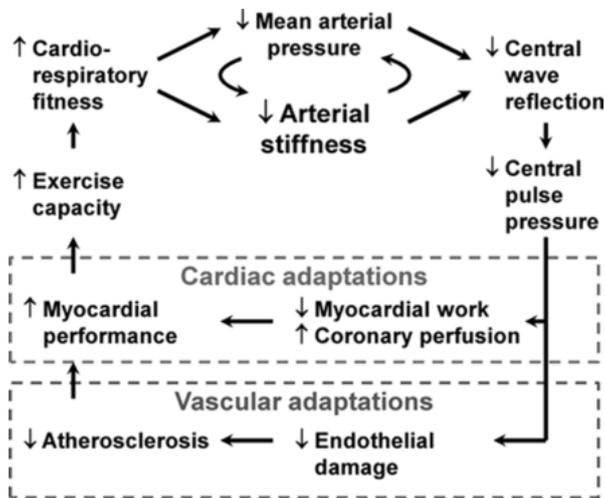


Figure 3.4: Relationship between cardiorespiratory fitness, arterial stiffness and myocardial work capacity

Increased cardiorespiratory fitness is associated with reduced mean arterial pressure and arterial stiffness which lowers wave reflections (augmentation index) and central pulse pressure (cPP). Lower cPP, in turn, confers cardiac and vascular benefits which ultimately increase myocardial performance, the key determinant of cardiorespiratory fitness. Image and legend adapted from (373)

As summarised in Table 3.1, endurance-trained individuals have lower resting AS than resistance-trained (336), sedentary (50) and less active (53) counterparts. Age-associated increases in AS are attenuated or even reversed with both pure AE (374, 375) and concurrent strength-endurance training (49, 376). Aerobic exercise should be a ‘first-line’ strategy in both the treatment and prevention of the progressive decline in arterial function (377) in order to help reduce CVD burden. Several types of AE such as cycling, swimming, running and rowing protect against the age-associated large artery stiffening.

Findings regarding the effects on peripheral arteries in healthy adults and in certain clinical populations are equivocal. Moderate-intensity AE performed for 30 mins/day, 3 times/week reduces resting central AS in healthy, sedentary individuals in as little as one week (48) and if continued for longer, in pre-hypertensive MA/O individuals as well (378). However, this specific prescription of AE may reduce AS only in central but not peripheral arteries (14, 51). Conversely, a recent meta-analysis found that AE improves peripheral more than central PWV and that greater reductions are observed in those with higher PWV measures at baseline and with interventions lasting more than 10 weeks (379).

Aortic PWV and the AIx increase with ageing, independent of the status of other CV RFs or overt CVD (51, 141, 380) but amongst healthy MA/O individuals, AE generally improves PWV (Figure 3.5) and the AIx (377). Individuals with CV RFs may not benefit from the effects of AE on PWV and the AIx unless the exercise is adapted to meet the improvement requirements of the specific RF(s).

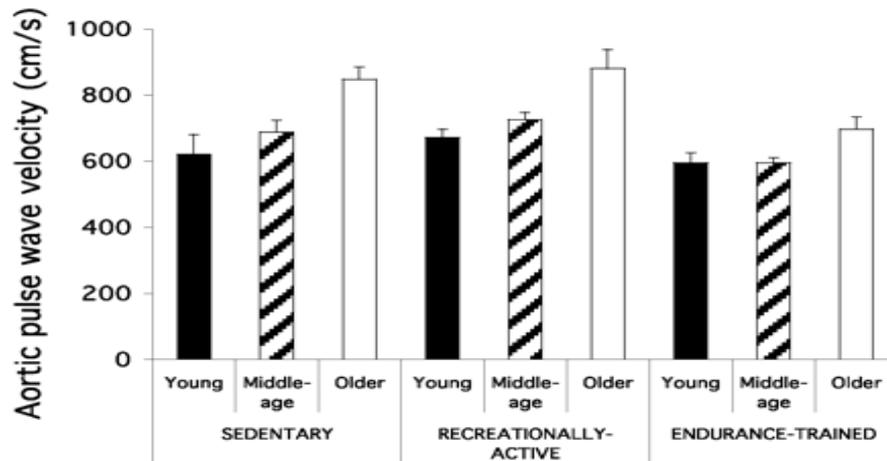


Figure 3.5: The effects of ageing and habitual aerobic exercise on aortic pulse wave velocity

There is a natural age-associated increase in aortic PWV but this rise can be attenuated with endurance training. Recreational activity, which may involve resistance training, may not confer the same benefits as endurance training. Image adapted from (381)

On the other hand, chronic regular RE alone is thought to decrease arterial compliance in healthy populations (64, 66, 67) and resistance-trained men have greater central (64) and peripheral (67) AS compared to endurance-trained and sedentary age-matched counterparts (Table 3.1). The key intervention studies and findings regarding the effects of various forms of exercise on compliance, PWV and the AIx are summarised in Table 3.4.

Interventions employing medium-to-long-term (approximately greater than 6-8 weeks) aerobic interval sessions are lacking. Although higher intensity exercise confers vasculoprotective effects and positively impacts AS and wave reflections (48, 382), more specific longitudinal studies would be useful to ascertain whether the impact of HIIT and SIT on AS is beneficial, or in fact, detrimental, if a pressor response, similar to that of RE, is elicited.

3.7.1 MECHANISMS UNDERLYING THE EFFECTS OF EXERCISE ON ARTERIAL STIFFNESS

The β stiffness index (a measure of arterial compliance, (refer to Appendix B) is inversely related to VO_{2max} . The proposed reason is that those with increased AS experience greater PP during maximal exercise which would decrease coronary perfusion and myocardial capacity and limit cardiac output (383). This reduces exercise capacity and fitness which would further increase AS,

thereby setting up a vicious cycle (Figure 3.4). Carotid artery compliance decreases during healthy ageing, possibly due to hypertrophy of VSMCs, replacement of functional cells with connective tissue and increased cross-linking of collagen (42). Aerobic exercise, especially of a vigorous nature, can mitigate ageing-associated large artery stiffening in healthy individuals (48, 49, 374). Habitual exercise is thought to remodel arteries in a way that wall stress, BP and atherosclerotic thrombus formation risks are reduced (81). Since the Alx is inversely related to both BP and TPR (384), higher-intensity exercise, which causes greater shear stress and NO release (relative to low-intensity exercise), results in a larger drop in the Alx and TPR (385). This positive dose-response relationship between exercise intensity and improvements in the Alx (386) imply that high-intensity exercise may actually be more beneficial than low-intensity exercise for cardiovascular health (387).

Although carotid and aortic compliance improved in most intervention studies employing AE, peripheral (muscular) arteries do not usually show these improvements (refer to Table 3.4). Since central arteries have relatively more elastin in their walls than peripheral arteries do, the processes that underlie age-associated arterial stiffening (elastin fragmentation, abnormal collagen deposits) may occur more rapidly in the former (14). Thus, AE-induced benefits on AS in central arteries may also occur to a greater extent and more rapidly than in peripheral arteries (14), but the latter may be because the common femoral artery, a relatively stiff artery with not much potential for adaptability, has been investigated in most of these studies. As the (aortic and peripheral) Alx partially depends on PWV (353) (refer to Figure 2.3), AE-induced long-term changes in the former are likely due to reductions in the latter. When arterial compliance increases due to internal factors, PWV and the intensity of reflected waves decrease, which would eventually reduce augmentation of central peak systolic pressures (and central PP) (24).

The dilation of elastic arteries during ageing is accompanied by increased wall tension and changes in gene expression which result in vascular remodelling, oxidative stress and a pro-atherogenic phenotype in the arterial walls (306). The mechanisms by which regular AE improves arterial function are largely unknown and still under investigation but existing evidence suggests that reductions in oxidative stress (including decreased fibrosis and AGEs), inhibition of chronic vascular inflammation, promotion of mitochondrial health, biogenesis, antioxidant systems and NO bioavailability may explain most of the benefits (306, 377). The shear stress created by AE plays a pivotal role in mediating the benefits to vascular function in both humans (388) and animals (389).

Exercise-associated increases in PP and haemodynamic modifications in peripheral arteries also trigger vascular structural (arterial wall) and functional (vasomotor tone) adaptations, even in beds far away from the site of the working muscles (81). Endurance training does not reduce AS in pre-hypertensive and elderly individuals unless the training duration is prolonged or SBP drops significantly (378, 390). In this population, the impaired baroreflex predisposes their arterial walls to greater stress during exercise and therefore, smooth muscle content in the walls may increase as a compensatory mechanism which in turn restricts compliance (378).

Many studies providing mechanistic insights into AE and arterial health have been carried out on animals due to the inaccessible, central nature of the large arteries in humans (377). Peripheral arteries cannot be used as surrogates as they do not significantly stiffen with ageing. Increased large artery stiffness with ageing has been attributed primarily to altered expression, bioactivity and/or configuration of the structural proteins in their walls, especially in the tunica media (391). Collagen and elastin contents increase and decrease respectively with ageing in sedentary animals but these changes were less obvious when animals swam for only 4-5 months (392). However, findings from experimental studies carried out on animals show no association between AE-related reductions in AS and modifications of structural proteins (392). In the carotid arteries of sedentary animals, there is increased calcification, increased levels of MMP-2 and the pro-fibrotic cytokine transforming growth factor- β 1 (TGF- β 1) as well as decreased levels of lysyl oxidase, a pro-synthetic elastin enzyme (393). Voluntary wheel running for only 10-14 weeks reversed these changes in old animals possibly by reducing oxidative stress, collagen I deposition, TGF- β 1 levels, calcification and AGE formation. In addition, constant, cyclic transient deformation of vessels could alter collagen/elastin ratios and reduce the cross-linking of connective tissue which increases AS (377).

Chronic RE likely decreases arterial compliance in healthy individuals and may contribute to greater cardiac afterload, with highly resistance-trained athletes exhibiting greater large AS and greater peripheral and central PPs compared to less active age-mates (67). This may be an adaptive response to protect the aorta from too much expansion during acute lifts and this would limit aerobic, but not lifting capacity. However, when prescribed correctly, the CV (and other) benefits of RE outweigh the adverse impact it may have on AS (64). The mechanisms underlying the increased AS with RE are still largely unexplored but the current hypotheses relate to the cumulative effects of single bouts of RE and the effects that acute sessions have on BP (64). Concurrent AE and RE exercise does not usually improve AS measures significantly (386) but Cook et al. reported greater carotid compliance in rowers (who engage in AE and RE) than sedentary counterparts, indicating that AE may override the detrimental effects of RE on AS (327).

3.7.2 SUMMARY: EXERCISE AND ARTERIAL STIFFNESS

Cross-sectional studies generally report lower PWV and AIx indices in endurance-trained athletes than less active/resistance-trained counterparts. Furthermore, these indices are usually inversely related to cardiorespiratory fitness. Interventional studies employing low, moderate and high-intensity MCAE as well as HIIT have shown improved arterial compliance, stiffness and wave reflection indices even within one month. Aerobic exercise can mitigate ageing-associated large artery stiffening in healthy individuals. The mechanisms underlying this are not fully understood but are likely related to the anti-oxidative and anti-inflammatory effects of AE as well as increased NO bioavailability. Resistance exercise most probably has detrimental effects on arterial health indices related to stiffness.

Table 3.4: The effects of exercise interventions on arterial stiffness indices (continued on next page)

refs	Cohort	Intervention characteristics					Main findings
		Duration	Frequency (Days/week)	Intensity	Time (mins/day)	Type	
(48)	18-32 year old sedentary normotensive, healthy non-smoking men (n=13)	4 weeks	3	vig (75% max load)	30	AE (cycling)	↑ VO _{2max} by 5.1ml/kg/min (p<0.01); ↓ SBP by 8.4mmHg (p<0.01); ↑ in systemic arterial compliance (SAC) by 0.26 units (p<0.01); ↓ aortic arch stiffness by 1.03 units (p<0.05); linear relationship between the ↑ in SAC and ↑ in VO _{2max}
(382)	60±3 years old apparently healthy, sedentary adults (n=7; 2 men)	3 months	4-5	vig (65-70% HR _{max})	30-45	AE (walking/jogging)	↑ central (carotid) arterial compliance (p<0.05)
(394)	30-57 year old sedentary/recreationally active, pre-hypertensive, overweight or obese adults (n=35)	6 weeks	≥3	mod (50-60% VO _{2peak}) or vig (80-90% VO _{2peak})	Either 50 (mod) or 33 (vig)	AE (cycling/running)	In intervention group: ↔ cBP, pAIx, central AIx; ↑ VO _{2peak} from 27.0±5.1 to 28.8±5.8ml/kg/min (p=0.0001); ↓ SBP and DBP (p<0.005)
(49)	53±2 year old healthy, sedentary men (n=20)	3 months	Progressive (3-4 to 4-6 days/wk)	progressive mod-vig (60-75% HR _{max})	Progressive (25-30mins to 40-45 mins)	AE (walking)	↑ carotid artery compliance by 30% (to levels of age-matched endurance-trained men) (p<0.01); ↓ β-stiffness index by 20% (p<0.01); ↔ VO _{2max}
(353)	67±6 year old healthy, sedentary adults (n=99; 12 men)	6 months	2	mild-mod (HR of 110-112bpm)	30	AE (dancing/hopping)	↓ pAIx by 3% (p<0.01); (ΔAIx and ΔAIx@75 were negatively correlated to baseline AIx and AIx@75 respectively (r=-0.40, p<0.01); ↓BP (p<0.01)
(14)	Sedentary middle-aged (31-64yr) normotensive, non-smoking men	4 months	3-4	mod (60-70%HRR)	45	AE (walking/jogging)	↓ aortic (central) PWV from 9.3 to 8.7m/s; ↔ peripheral (femoral) PWV; ↑ carotid but not femoral arterial compliance; ↑ femoral but not carotid EDD; ↑ VO _{2max} (p<0.05)

Table 3.4 continued: The effects of various exercise intervention on arterial stiffness indices (continued on next page)

refs	Cohort	Intervention characteristics					Main findings
		Duration	Frequency (Days/week)	Intensity	Time (mins/day)	Type	
(395)	32-59 year old healthy, sedentary women (n=35)	3 months	2	60%1RM (RE) or (60-70%VO _{2max} (AE)	approx. 30	RE: 3 sets, 10 reps, 6 exercises (leg curl, leg press, hip adduction, hip flexion, vertical press and sit-ups) or AE: cycling	↓ cfPWV after AE; ↔ peripheral and aortic PWV after RE; ↔BP after RE and AE
(46)	18-25 year old healthy, normotensive, non-smoking men (n=30)	4 weeks	3	mod (65%VO _{2max})	30	AE (cycling)	↓ pAIx; by 6% (p<0.05); ↔cfPWV; ↑ VO _{2max} by 8% (p<0.05)
(47)	48.2±1.3 year old sedentary, pre-hypertensive adults (n=30; 20 men)	4 weeks	3	mod (65%VO _{2peak} or 65%10RM)	AE: 30; RE: 45-50	RE (3 sets, 10 reps of leg press, chest press, leg extension, lat pulldown, leg curls, shoulder press, bicep curl, tricep press, abdominal crunch) or AE (running)	↑ central PWV (11±0.9–12.7±0.9m/s, p=0.0001) and peripheral PWV (11.5±0.8 - 12.5±0.7m/s, p=0.013) after RE; ↓ central PWV (12.1±0.8–11.1±0.8m/s) and peripheral PWV (2.6±0.8 vs post 11.6±0.7m/s)after AE; (ΔPWV=1m/s in all cases)
(45)	20-23 year old healthy adults (n=25; 18 men)	8 weeks	2-3	mod (60%VO _{2peak})	30	AE (cycling)	↓ carotid artery stiffness (p<0.05)
(65)	19-23 year old healthy, sedentary men (n=39)	4 months	3	80% 1RM + and 60%HR _{max} for AE+RE group; 50%1RM for RE group	75	AE (cycling) +RE (3 sets, 8-12 exercises)	↔ central (carotid) and peripheral (femoral) arterial compliance
					45	RE (3 sets, 14-16 exercises, 2 min rest between exercises): leg extension, seated chest press, leg curls, lateral row, squat, sit-ups	↓ central (Carotid) arterial compliance by 20% (p<0.01); ↔ peripheral (femoral) arterial compliance

Table 3.4 continued: The effects of various exercise intervention on arterial stiffness indices (continued on next page)

refs	Cohort	Intervention characteristics					Main findings
		Duration	Frequency (Days/week)	Intensity	Time (mins/day)	Type	
(64)	20-38 year old healthy adult men (n=28)	4 months	3	80%1RM	approx. 45	RE: 3 sets, 12 reps, 8-12 exercises (leg extension, seated chest press, leg curls, lateral row, squat, and sit-ups)	↓ central (carotid) arterial compliance by 19% (p<0.05); ↑ β stiffness index by 21% (p<0.01)
(62)	24.9±4.3 year old healthy, sedentary men (n=38)	3 months	3	vig (80-90%HR _{max})	20	HIIT (cycling) - 5-min warm-up, 20 min of 8-s sprint and a 12-s recovery, and a 5-min cool-down	↓ cfPWV (p=0.013); ↓central AIx@75 by 3.5% (p=0.024); ↑VO _{2max} by 5ml/kg/min (p<0.01)
(396)	Active/sedentary but not well-trained 18-25 and 50-64 year old women (n=29)	8 weeks	3	80% 1RM	6 exercises	RE: 3 sets, ≥8 reps, 6 exercises (leg extension, leg curl, leg press, lat pull-down, shoulder press, chest press)	↔ cfPWV; changes in cfPWV were positively related to initial cfPWV (r=-0.602, p=0.001)
(61)	23.3±2.8 year old healthy, untrained adults (n=20; 5 men)	6 weeks	5 (AE) or 3 (SIT)	mod AE (65%VO _{2peak}) or SIT	40-60	AE (cycling) or SIT (6×30s "all-out" Wingate tests separated by 4.5 mins recovery)	↑ popliteal artery distensibility (p<0.05); ↔ carotid artery distensibility (p=0.29)
(397)	18-35 year old normotensive and pre-hypertensive novice exercisers (n=58; 30 men)	8 weeks	3	mod-vig AE (65-85%HR _{max}) or 60%1RM	60	Interval AE (treadmill walking/running) or RE (2 sets, 8-12 reps, 7 exercises: leg extension, leg curl, leg press, lat pull down, chest press, overhead press, biceps curl)	RE: ↓ AIx (by 7.5% ± 2.8%, p<0.05), AIx@75 (by 8.0% ± 3.2%, p<0.05), carotid-radial PWV (by 1.02±0.32m/s, p<0.05), femoral-distal PWV (by 1.04±0.31m/s, p<0.05) AE: ↓ AIx (by 8.1% ± 3.2%, p<0.05), AIx@75 (by 9.2% ± 3.8%, p<0.05), carotid-radial PWV (by 0.92±0.36m/s, p<0.05), femoral-distal PWV (by 1.34±0.33m/s, p<0.05) Time-control group: ↔ all parameters
(161)	35-50 year old sedentary, healthy and metabolic syndrome adults (n=43; 15 men)	8 weeks	3	n/s	60	AE (running/cycling/elliptical machines)	In healthy-exercise group: ↓ cfPWV from 6.6±1.8 to 5.6±1.6m/s (p<0.05); ↓AIx from 21±5% to 18±5% (p<0.05); ↑ VO _{2peak} by 23.8 ± 1.6 to 26.3 ± 1.6ml/kg/min

Table 3.4 continued: The effects of various exercise intervention on arterial stiffness indices

refs	Cohort	Intervention characteristics					Main findings
		Duration	Frequency (Days/week)	Intensity	Time (mins/day)	Type	
(398)	25±4 year old healthy, sedentary/lightly active males (n=14)	6 days	Daily	mod (65%VO _{2peak})	120	AE	↓ central PWV (from 5.9±0.8 to 5.4±0.8m/s, p<0.05) and peripheral PWV (from 9.7±0.8 to 8.9±1.3m/s, p<0.05); ↔ Alx@75, VO _{2peak} , vascular conductance & heart rate variability
(333)	25.0±4.4 year old females with familial hypertension (n=44)	4 months	3	60-70%VO _{2max} (MCAE) or 80-90%VO _{2max} (AIT)	40 mins +5 mins warm-up and 15 mins calisthenics	AIT (2 min walking alternating with 1 min of running at 80-90% VO _{2max}) or MCAE (walking/running)	↑ CRF (15% with AIE and 8% with MCAE, p<0.05); non-significant ↓ in cfPWV with both MCAE and AIE (p=0.06) (AIE effects were superior in both cases, p<0.01)
(399)	52±2 year old sedentary, healthy adults (n=37; 11 men)	13 weeks	2 (AE+RE) or 3 (stretching & RE)	mod AE (60-75% HRR) and 70%1RM	30-45	RE (1 set, 8-12 reps, 10 exercises - seated chest press, horizontal leg press, shoulder press, abdominal crunches, seated hamstring curls, seated row, seated calf raises, low back extension, tricep curls, bicep dumbbell curls) or RE+AE (RE+walking) or stretching (3 reps, held for 20 s at end range)	↔ cfPWV and carotid compliance with both RE and RE+AE; ↑ carotid compliance by 23% with stretching

AIT, aerobic interval training; MCAE, moderate continuous aerobic exercise; pAlx, peripheral augmentation index; cBP, central blood pressure; RE, resistance exercise; AE, aerobic exercise; HIIT, high-intensity interval training; SIT, sprint interval training; CRF, cardiorespiratory fitness; EDD, end-diastolic diameter; cfPWV, carotid-femoral pulse wave velocity; HR, heart rate; HRR, heart rate reserve

3.8 STRUCTURAL ADAPTATIONS OF ARTERIES TO EXERCISE - IMT

NOTE: Investigation into autonomic control, endothelial function and shear-stress patterns was out of the scope of the present research but will be introduced in relation to structural remodelling of arteries with exercise training.

An increase in carotid IMT (cIMT) is an accepted surrogate marker of pre-clinical atherosclerosis. Between the ages of 20 and 90 years, IMT doubles in healthy, sedentary adults and this may increase baseline interactions between CV RFs and eventually lead to clinical CVD (82). Half of the cardioprotective effects of exercise are due to its effects on vessel walls, in particular, changes in the wall thickness of the carotid and peripheral arteries (42, 43).

Conclusions drawn about associations between physical activity and cIMT depend on the method used to assess the former (objective/subjective) (9) and should therefore be carefully interpreted. In general, cross-sectional studies using specific questionnaires to gauge physical activity levels have found cIMT and physical activity to be negatively correlated (400, 401) with sex and age as modulators (402). There is also an inverse dose-dependent relationship between physical activity levels and AE intensity and the 3-year rise in cIMT (401, 403) possibly because higher intensity exercise confers a greater stimulus for arterial remodelling (9). Furthermore, lower fitness levels are independently correlated with higher cIMT values (343-345) and impaired fitness is the strongest predictor of a 4-year increase in cIMT (404). Table 3.2 summarises the key findings regarding the association between IMT and physical fitness/activity.

Findings regarding the effects of training on IMT are equivocal (Table 3.5). In general, interventions less than three months long and those employing low and moderate intensity AE may not bring about systemic reductions in IMT (9). Moreover, it is possible that RE reduces IMT to a greater extent than AE does because arterial walls are exposed to greater remodelling-inducing wall stress in the former (265). However, in healthy young males, vigorous cycling has been shown to bring about systemic arterial structural adaptations (57, 59) as shown in Figure 3.6 and Figure 3.7. These results are supported by a cross-sectional study demonstrating no significant difference in brachial artery IMT between the dominant and non-dominant limbs of elite squash players (340) (Figure 3.8B).

The effects of exercise training on IMT in those with overt CVD or CV RFs may be different to the effects observed in healthy individuals. Nevertheless, amongst hypertensive adult males, fitness and carotid atherosclerosis (cIMT>1.2mm) were inversely related (405) and greater physical activity was associated with a lower 6.5-year increase in cIMT (406). Arterial wall remodelling occurs over many months or years whilst IMT changes may occur over even longer time periods (9). With exercise training, initial changes are functional whilst structural adaptations occur later (407). Furthermore, peripheral arteries have greater internal plasticity than central arteries and the ability to adapt faster than the latter (9).

3.8.1 MECHANISMS UNDERLYING THE EFFECTS OF EXERCISE ON IMT

As is the case with AS and exercise, the relationship between physical activity and IMT is not entirely due to the effects on traditional CV RFs (9). Plaque formation has been linked to low mean shear rates (408) and oscillatory shear (409). In the latter case, retrograde shear stress gives rise to pro-atherogenic vascular endothelial cell phenotypes (410). Systemic and not localised shear stress is important when it comes to arterial wall remodelling (326). Given that BP fluctuates during the cardiac cycle and stretches arterial walls, chronic BP elevations produce similar endothelial cell phenotypes as do low and oscillatory shear patterns (389). Chronic increases in (local) distending pressures can eventually result in increased cIMT (411). Exercise also increases arterial pressure and arterial wall stretch but in the long-term, is associated with lower IMT (anti-atherogenic changes in arterial walls) (326), giving rise to the *speculation* that anti-and pro-atherogenic gene regulation depends on whether the pressure stimulus is evoked by chronic increases in pressure or by the cyclic, transient and episodic increases that occur during exercise (326, 389).

Exercise training can decrease IMT by altering vasomotor tone possibly by decreasing resting SNA (81), thereby preventing smooth muscle hypertrophy brought about by chronically elevated SNA (412). However, it is unknown whether a correlation exists between exercise-induced changes in SNA and IMT. Surprisingly, two studies reported positive relations between physical activity and cIMT in young adult borderline hypertensives (349). The authors suggested that this could be due to autoregulation to counteract the exercise-induced increases in wall stress or due to repetitive RAAS activation. They speculated that in a similar way to the exercise-induced left ventricular hypertrophy, mechanisms underlying the increased cIMT in athletes could relate to medial thickening. Oxidative stress can cause endothelial dysfunction and atherosclerosis as well as arterial wall thickening (413, 414) whilst inflammation is associated with atherosclerotic plaque formation and stability. Aerobic exercise may confer vasculoprotection by its anti-oxidative and anti-inflammatory effects (refer to Figure 3.1).

3.8.2 SUMMARY: EXERCISE AND IMT

Chronic, moderate to high-intensity AE can reduce IMT in the long-term in healthy, asymptomatic individuals as well as in those with CVD or CV RFs, with greater effects being observed in peripheral than central arteries as the latter may require a longer and more intense exercise stimulus. Findings regarding the systemic effects of localised exercise on IMT are equivocal. The main mechanisms underlying the impact that exercise has on IMT are related to local (shear stress patterns) and systemic (arterial pressure) haemodynamic factors as well as non-haemodynamic factors like vascular tone, SNA, oxidative stress and inflammation. Further research is required to determine the best exercise prescription to maximise the benefits on arterial wall thickness because different exercise modes spur unique and diverse haemodynamic and shear stress patterns, leading to benefits of differing magnitudes in arteries.

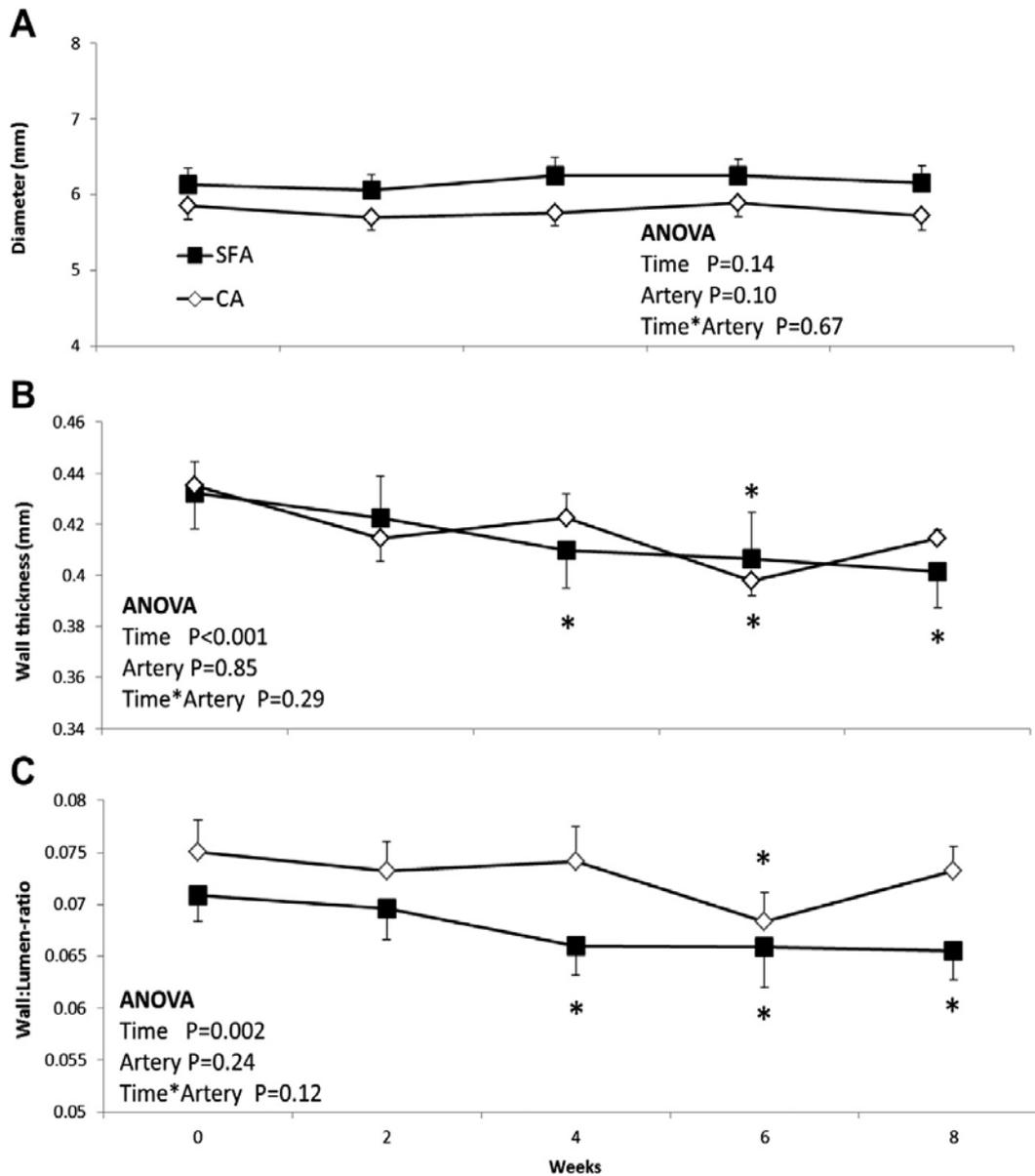


Figure 3.6: Systemic arterial structural adaptations in healthy young males in response to 8 weeks of vigorous (80%HR_{max}) cycling

Superficial femoral (SFA, solid squares) and carotid artery (CA, open diamonds) diameter (A, in mm), wall thickness (B, in mm), and wall-to-lumen ratio (C) before (week 0), during (week 2, 4, 6) and after 8 weeks of lower-limb exercise training in healthy young men (n = 9). P-values refer to a 2-way repeated measures ANOVA examining whether the changes in both arteries differ across the 8-week period of training.

*Post-hoc significantly different from baseline at P < 0.05. Image and legend adapted from (59)

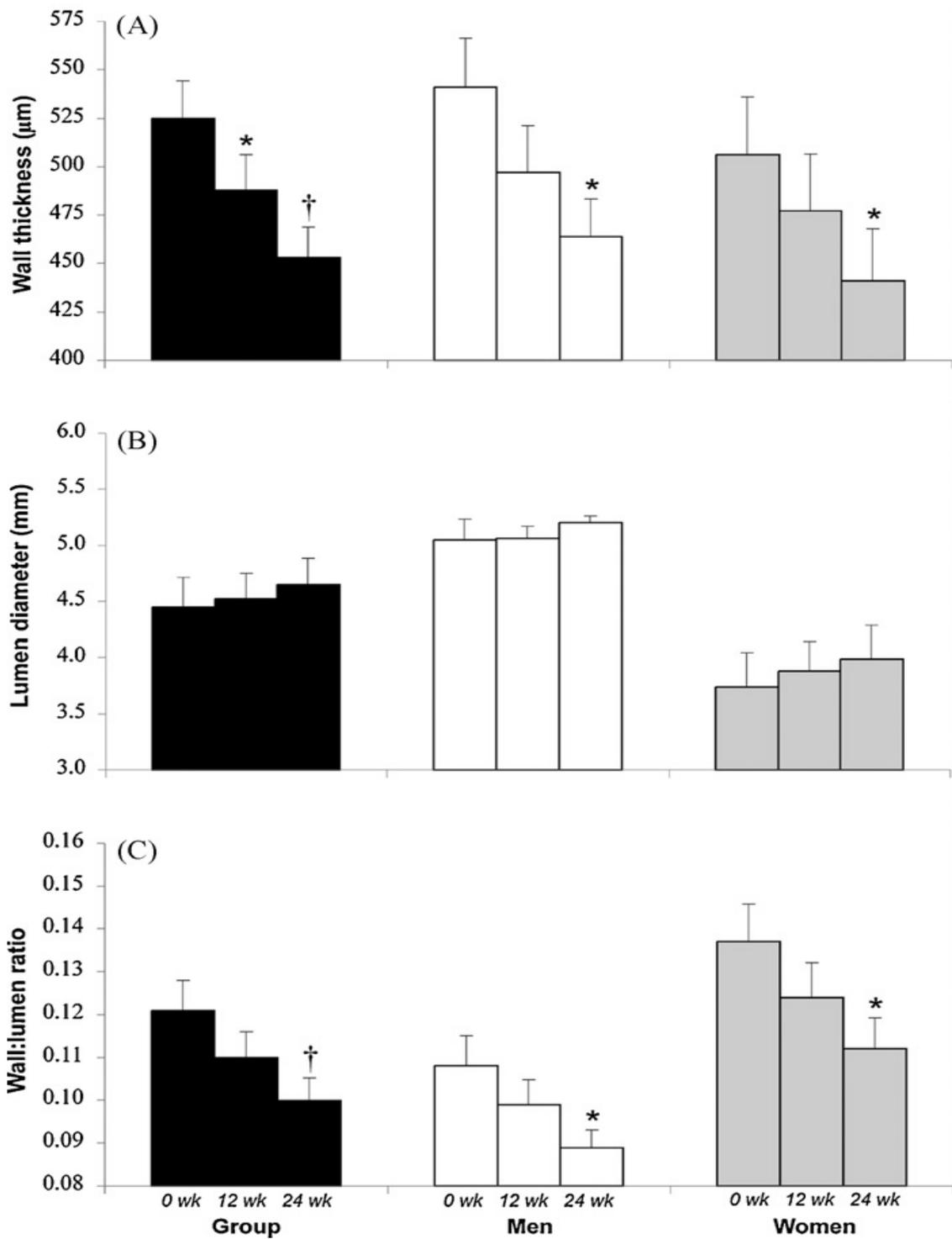


Figure 3.7: Upper body systemic arterial structural adaptations after lower-body aerobic training

Brachial artery wall thickness (A), lumen diameter (B), and wall:lumen ratio (C) at baseline, 12 and 24wk of aerobic exercise training (walking/cycling) (n = 11). Data is presented for the entire group (n = 11, black bars), men (n = 6, white bars) and women (n = 5, grey bars).

*Significant from week 0 at P < 0.05, † P < 0.01. Image and legend adapted from (57)

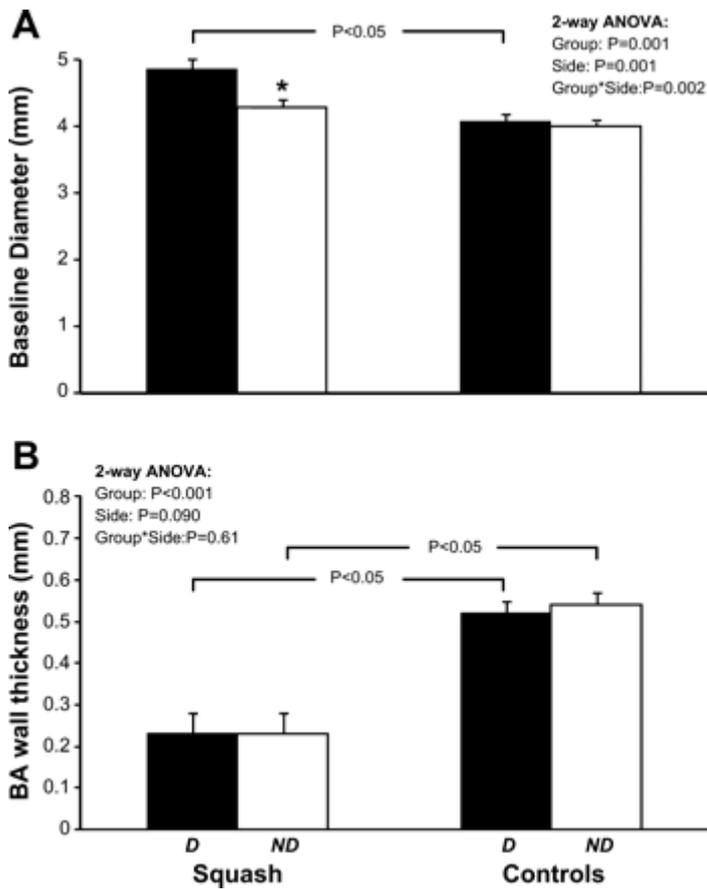


Figure 3.8: Comparisons between brachial artery wall thickness and baseline diameter in dominant and non-dominant limbs in elite squash players and controls

A. Brachial artery diameters are greater in elite squash players than controls and in the former population, the dominant arm brachial artery diameter is significantly greater than that of the non-dominant arm.

B. Brachial artery wall thickness is significantly greater in controls than elite squash players but there are no differences in brachial artery wall thickness between dominant and non-dominant arms in the former population.

**significant difference between dominant and non-dominant arm*

BA, brachial artery; D, dominant arm; ND, non-dominant arm

Image adapted from (340)

Table 3.5 Summary of the main intervention studies investigating the effects of exercise training on arterial structure

refs	Cohort	Intervention characteristics					Main findings
		Duration	Frequency (Days/week)	Intensity	Time (mins/day)	Type	
(57)	58-60 year old healthy, sedentary adults (n=16; 8 men)	6 months	3	mild	30	AE	↓ IMT in upper and lower limb conduit arteries (p<0.01); ↓ WLR in brachial (p<0.01) and popliteal (p<0.05) arteries
(342)	54±2 year old healthy, sedentary men (n=18)	3 months	progressive to max of 6	mild-vig (60-75% HR _{max})	progressive to max of 45	AE (walking/jogging)	↔ cIMT, cWLR and carotid systolic BP
(415)	70±3 year old normotensive, non-smoking, healthy sedentary men (n=8)	8 weeks	3	mod-vig	30	AE	↑ compliance and diameter of arteries in trained limbs only (p<0.05); ↔IMT
(416)	53.4±6.2 year old healthy(n=26; 5 men)	6 months	3	progressive mild to mod	progressive 20-40	AE	↓cIMT by 6.4% (p<0.05); ↔ BP
(59)	Healthy, young adult males (n=14)	8 weeks	3	vig (80%HR _{max})	30	AE (cycling)	↓ superficial femoral and common carotid IMT (p<0.002 and p<0.001 respectively); ↓ superficial femoral and common carotid WLR; ↔ femoral and carotid resting diameter (p=0.14)
(417)	20.0±2.8 year old healthy adults (n=21; 11 men)	8 weeks training + 4 weeks detraining	3	80% 1RM	n/s	RE: leg exercises - 3 sets, 10 reps, 6 exercises (squats, Bulgarian split squats, knee extensions, leg press, lunges, static wall sit)	Training ↑ superficial femoral and carotid artery diameters by 27% and 13% respectively (p<0.001); detraining ↓ femoral and carotid diameters (46% and 10% respectively, p<0.001)
(265)	55-68 year old untrained middle-aged adults with chronic heart failure (n=36; 32 men)	12 weeks	3	AE: progressive 50-70%VO _{2peak} RE: progressive 50-70%1RM	approx. 45	AE (cycling+walking) or RE (3 sets, 9 exercises with 2:1 work:rest ratio)	↓ brachial IMT (from 475±10 to 443±13µm, p<0.01) and WLR (from 0.121±0.004 to 0.107±0.004, p<0.01) with RE only; ↑ brachial diameter with both AE (5%) and RE (6%) (p<0.01)
(56)	51 ± 2 year old sedentary men	3 months	5-7	mod-vig (65-80%HR _{max})	40-50	AE (walking/jogging)	↑ femoral diameter by 9% (from 8.82±0.18 to 9.60±0.20 mm, p<0.001); ↓ femoral IMT (from 0.65±0.05 to 0.56±0.05 mm) and WLR by 15-20% (p<0.001); changes in femoral structure and atherosclerotic risk factors were unrelated
(161)	35-50 year old sedentary, healthy and metabolic syndrome adults (n=43; 15 men)	8 weeks	3	n/s	60	AE (running/cycling/elliptical machines)	↔ cIMT, cCSA, cWLR
(64)	20-38 year old healthy, sedentary men (n=28)	4 months	3	80%1RM	n/s	RE: 3 sets, 12 reps, 8-12 exercises (leg extension, seated chest press, leg curls, lateral row, squat, and sit-ups)	↔ cIMT and carotid diameter
(61)	23.3±2.8 year old healthy, untrained adults (n=20; 5 men)	6 weeks	5 (AE) or 3 (SIT)	mod AE (65%VO _{2peak}) or SIT	40-60	AE (cycling) or SIT (6×30s "all-out" Wingate tests separated by 4.5 mins recovery)	↔ carotid and popliteal diameter and IMT

IMT, intima-media thickness; cIMT, carotid intima-media thickness; WLR, wall:lumen ratio; cWLR, carotid wall:lumen ratio; cCSA, carotid cross-sectional area; AE, aerobic exercise; RE, resistance exercise; SIT, sprint interval training; BP, blood pressure; 1RM, 1 repetition maximum; n/s, not specified; yrs, years

3.9 STRUCTURAL ADAPTATIONS OF ARTERIES TO EXERCISE - ARTERIOGENESIS

Arterial remodelling responses to physical conditioning are shown in Figures 3.6-3.9.

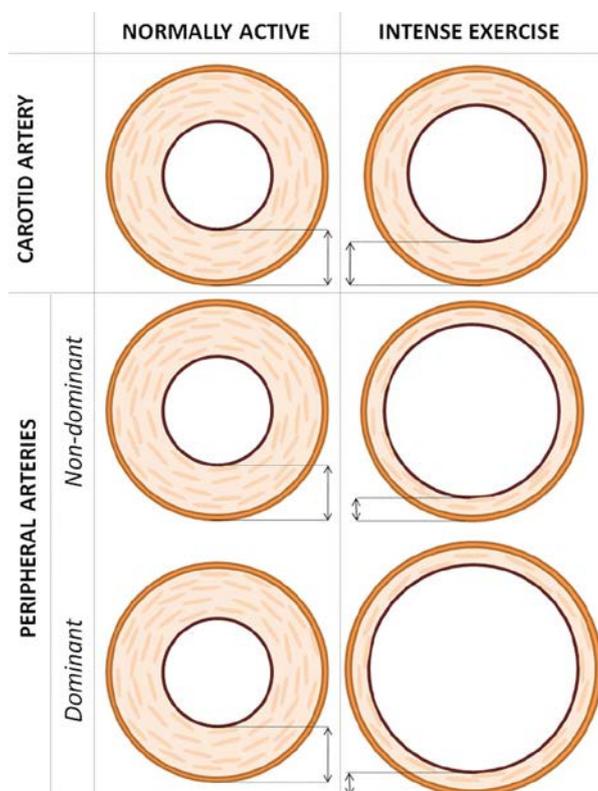


Figure 3.9: Brachial and carotid artery diameters and wall thickness in chronic recreational (normal) versus intense exercise

In normally active individuals, diameters and wall thickness in dominant and non-dominant arms are similar. With intense exercise (elite squash players), carotid wall thickness is reduced whilst diameter is increased. Furthermore, dominant arm brachial artery diameter is greater than that of the non-dominant arm. Effects of exercise on wall thickness are greater in peripheral arteries than the carotid artery. Image adapted from (9)

Arterial remodelling, or an increase in arterial cross-sectional area, occurs in response to physical conditioning (318). Cross-sectional studies have reported greater resting conduit vessel diameters in athletes compared to sedentary and less active counterparts (56, 334, 341, 407). Exercise training increases the ability of resistance vessels to dilate, independent of changes in vasoconstrictor tone (418). It was traditionally thought that localised training of small muscle groups increases diameters of resistance arteries systemically (419) whereas changes in resting diameters of conduit arteries occur in trained limbs only (56), suggesting that endothelium-dependent arterial remodelling in these arteries is brought about by localised changes in shear stress as opposed to systemic changes in MAP (420). There is little evidence suggesting systemic conduit arteriogenesis with interventional exercise, possibly due to the minimal enlargement needed for flow capacity to increase. [Resistance = (blood viscosity × vessel length)/radius⁴].

Nevertheless, both moderate and high-intensity dynamic (but not static) AE can decrease peripheral AS by increasing arterial diameter (421) and some forms of RE can cause remodelling of both resistance and conduit vessels (refer to Table 3.5 for a summary of these findings). Eight weeks of combined AE and RE reduced brachial and superficial femoral but not carotid artery wall thickness and WLR in type II diabetics (266) whereas 12 and 24 weeks of AE decreased wall thickness and WLR in the conduit brachial and popliteal arteries (57) (Figure 3.7B and C) and 8 weeks of lower limb training reduced superficial femoral and common carotid WLR (59) (Figure 3.6C).

3.9.1 PROPOSED MECHANISMS UNDERLYING EXERCISE-INDUCED ARTERIAL REMODELLING

Upregulation of eNOS activity due to the increased shear stress brought about by exercise is associated with vessel enlargement. Repetitive increases in shear stress due to acute bouts of exercise causes endothelium-dependent remodelling (422), enabling the arteries to contain the increased cardiac output. Furthermore, the exercise-induced inflammatory response, especially during intense and/or novel activity, can contribute towards arteriogenesis (260).

Longitudinal training studies have provided evidence that AE influences various vasoconstrictor pathways in healthy humans. Habitual exercise improves heart rate variability (HRV), a measure of autonomic balance and a predictor of CV mortality (423) and reduces vasoconstrictor tone by decreasing muscle SNA. Repeated bouts of exercise may alter input to brainstem regions regulating sympathetic output and vasoconstriction (424, 425). Aerobic exercise can suppress the tonic vasoconstriction observed with ageing in sedentary MA/O adults (426). Endothelin-1 (ET-1) is a potent vasoconstrictor derived from the vascular endothelium and its expression increases during sedentary, healthy ageing in men (427). In healthy middle-aged and older adults, 3 months of vigorous AE suppresses plasma levels of ET-1 whilst increasing arterial compliance (382). One meta-analysis reported that long-term AE increases vagal input to the circulatory system and therefore lowers resting heart rate (428). However, longer, high-intensity exercise would be required to observe significant bradycardia, especially amongst elderly individuals (429). During ventricular systole, distension of the carotid artery or aortic arch causes firing of afferent nerves which send signals to the brain stem to inhibit sympathetic outflow and enhance vagal tone to the heart. Stiff vessels result in less afferent firing. Studies investigating the acute effects of dynamic large muscle-group exercise on arterial remodelling are lacking (most studies have employed static small muscle-group exercise). However, carotid diameter increases during a graded cycling test and immediately constricts upon exercise completion, and these changes are associated with alterations in baroreceptor firing (430). However, there is also evidence that amongst healthy participants, SNA and basal vasoconstrictor tone do not change (431) and may even increase (432) with moderate-intensity AE. The fact that basal blood flow remains unchanged whether vasoconstrictor tone stays the same or increases with AE implies that modifications in sympathetic tone may cancel out the positive effects of exercise on vasodilatory

capacity and arterial remodelling (81). Whilst athletes have larger arteries than less active counterparts, the arterial diameter of the former may not be greater because of increased SNA (vasoconstrictor tone) to maintain BP (432).

3.10 A NOVEL HYPOTHESIS

Recent work shows that AE may reduce CV risk by directly protecting arteries from potentially harmful factors. Endurance training lowers the burden of traditional CV RFs (such as BP, plasma lipids, blood glucose, body mass, and composition) and non-conventional CV RFs (377, 391). However, these influences only account for approximately 50-60% of the ability of AE to reduce CV risk (40). An emerging theory is that regular AE may confer increased intrinsic *resistance* against the adverse effects of existing factors (391, 433) and this resistance decreases with sedentary ageing. For example, habitual AE protects against the effects of atherogenic phenotypes and the effects of raised blood glucose levels. (433, 434). Therefore, regular AE protects arteries from circulating stressors as they age. Western diets impair endothelial function by increasing oxidative stress associated with excessive superoxide production. Voluntary wheel running in old mice prevents the exacerbation in oxidative stress and resulting decrease in NO bioavailability and can improve overall mitochondrial homeostasis and arterial function (435).

This evidence supports the use of regular AE as a first-line strategy for preventing and treating arterial ageing to reduce CVD risk. Healthy adults engaging in habitual AE demonstrate lower AS (than sedentary age-mates) and preserved endothelial function. The beneficial effect of AE on arterial health is attributed to how it reduces vascular oxidative stress and inflammation to favourably modify traditional CV RFs and to provide resistance against existing adverse factors to which ageing arteries are chronically exposed.

3.11 GROUP-FITNESS CLASSES

Self-paced exercise, where individuals select their own intensities promotes motivation, enjoyment and adherence (436) and this likely has important consequences for community practice. Group fitness classes, which include studio cycling ('spin') are becoming increasingly popular and can reduce CV risk factors in healthy adults (437). Group indoor cycling classes are inspired by interval training and offer a combination of HIIT, SIT and moderate intensity AE within a single session. They are one of the few fitness classes that are mainly aerobic in nature but incorporate several intervals of high-intensity peaks. This type of group studio cycling carried out 3 times a week for 8 weeks has recently been shown to improve cardiometabolic health in overweight, sedentary individuals (438). The current study employs tri-weekly indoor group cycling classes as the intervention.

3.12 SUMMARY: EXERCISE AND ARTERIAL HEALTH

When studying the effects of exercise on blood vessels (irrespective of the study design), it is important to carefully consider both the cohort (health status, gender, age, baseline fitness) and

exercise characteristics (mode, volume, frequency, intensity, whole-body/local, peripheral muscle group exercise). Short-term exercise training or even a single exercise bout can alter arterial health indices acutely and the unique changes depend largely on the exercise characteristics and training status of the individual. It has been proposed that the acute changes may compound each other to bring about the long-term benefits of exercise (particularly endurance exercise) on arterial health and wave reflections. Habitual exercise, especially AE, has consistently been shown to improve arterial health aspects such as intrinsic arterial wall stiffness, wave reflections, central haemodynamics, wall thickness and calibre. High-intensity interval training is particularly suitable for those who lack time to exercise and for who SIT may not be suitable. Resistance training usually has adverse effects of arterial health measures but its overall benefits, (coupled with specific low-moderate intensity, individualised prescription), means that it should not be eliminated from a training programme.

3.13. CONCLUSION

The need to monitor arterial health is vital given that arterial disorders account for a staggering proportion of cardiovascular events. Deteriorations in arterial health are associated with the natural ageing process and can occur prematurely. In order to combat this early vascular ageing, it would be ideal to monitor tissue as opposed to circulating biomarkers as the former are more associated with target organ damage. To this effect, the most relevant arterial health indices pertain to stiffness, wall thickness, wave reflections, central haemodynamics and endothelial function. However, other structural and geometrical parameters such as arterial diameter should not be omitted from evaluations due to the interdependence between and synergistic effects of all the arterial health aspects. Despite the continuous development of novel equipment to assess these indices, there is an urgent need to standardise assessment protocols to avoid discrepancies in normative data and intervention study results.

Physical inactivity is associated with arterial health deterioration whilst cross-sectional and longitudinal studies have consistently demonstrated the vasculoprotection conferred by exercise of various modalities and intensities amongst both healthy and clinical populations. Exercise can prevent, attenuate and reverse (premature and natural) vascular ageing but it is important to consider exercise prescription. Generally, aerobic, aerobic interval and concurrent aerobic-resistance exercise have beneficial effects on arterial health whilst resistance exercise may worsen it. Although exercise intensity may be positively correlated to the magnitude and extent of benefits, there remains much controversy as to whether systemic improvements occur and whether peripheral or central artery health improves more.

Following on from previous studies reporting the benefits of aerobic and aerobic interval exercise on arterial health, the purpose of this study was to determine whether a community-based, self-paced aerobic interval training intervention utilising both SIT and HIIT components, would confer similar vasculoprotection as previously observed in controlled laboratory environments amongst healthy, sedentary adult males.

CHAPTER 4: PROJECT METHODOLOGY AND EQUIPMENT

4.1 EXPERIMENTAL DESIGN AND COHORT DESCRIPTION

A between-subjects, repeated-measures intervention study was adopted. Ten participants underwent an 8 week high-intensity aerobic interval programme, requiring attendance to three 45 minute indoor cycling group-fitness classes each week whilst 5 participants served as controls and maintained their sedentary and routine lifestyles for the 8 week period. A range of fitness and health related measures were taken pre, mid- and post-intervention as well as on a weekly basis for all participants. Participants were apparently healthy 20-45 year old community-dwelling, sedentary, non-smoking males. Although the participants were healthy, it was necessary that for at least 6 months prior to commencement, they lead relatively sedentary lifestyles, as defined specifically in the present study. However, they needed to be healthy and fit enough to carry out the fitness test and engage in the exercise intervention (if allocated to that group). It was also necessary that they resided in Auckland and would be available for the entire intervention and testing period. Table 4.1 is a summary of participant and intervention characteristics.

4.2 DEFINITIONS USED IN THE CURRENT STUDY

‘Sedentary lifestyle’: determined via administration of the International Physical Activity Questionnaire (IPAQ) – long form (Appendix D), prior to recruitment

- Had been accumulating, on average <2 hours of moderately intense physical activity per week for ≥ 6 months prior to the commencement of the study (not accumulating ≥ 30 minutes moderate physical activity on most days of the week at the time of recruitment)
- Not participating in a regular exercise programme at the time of recruitment

Individuals accumulating less exercise than 120 minutes per week would likely be ‘unhealthy’ and considered ‘high-risk’ of future CV events and it may have been unsafe for them to exercise and harder to recruit. On the other hand, individuals classified as ‘sedentary’ according to ACSM guidelines (accumulating <150 minutes of moderate-intensity aerobic exercise or <75 minutes of vigorous exercise per week) may have been misclassified due to inaccurate recall, misinterpretation of questions or dishonesty when completing the IPAQ. This volume of exercise (<2 hours of moderately intense physical activity per week) was considered an appropriate compromise in the definition of sedentariness.

Moderate intensity physical activity was defined as an intensity comfortably sustainable for between approximately 30-60 minutes, or physical activity at 40%-60% of maximal oxygen uptake. Vigorous intensity physical activity was defined as physical activity which can last for only up to 30 minutes, or physical activity at greater than 60% of maximal oxygen uptake (439). The term ‘apparently healthy’ referred to the absence of (a history of/current) chronic illnesses and/or injury and no long-term use of prescription medication.

Table 4.1: Summary of subject and intervention characteristics

Subject characteristics				Intervention characteristics				
Age (mean \pm SD)	Gender	Health status	Physical activity status	Duration	Session duration	Frequency	Average intensity	Type
31.8 \pm 6.1 years	Males	apparently healthy (no chronic illness or prescription medication); non-smoking; BMI \leq 32	sedentary for at least 6 months prior to commencing the intervention	8 weeks	45 minutes	3x/week	Moderate to high; 74-87% (75.9 \pm 7.2%) of HR _{peak} ; TL* of 315-405	Community-based, self-paced indoor cycling group-fitness class of mixed intensity; primarily aerobic, interspersed with regular bursts of high-intensity interval and sprint interval training

*TL = session duration \times RPE

HR_{peak}, *maximum individually-determined peak heart rate*; TL, *training load*

4.3 EXCLUSION CRITERIA: as indicated in the advert and re-assessed during the initial session

- Females
- Individuals <20 years or >45 years old
- Those who were not classified as sedentary (according to IPAQ responses)
- A history of or current chronic illnesses: in particular, cardiovascular-related disease (eg. coronary artery disease), cerebrovascular disease (eg. stroke) or kidney disease (or on dialysis), morbid obesity, diabetes type I or II, thyroid disease, dyslipidaemia, known hypercholesterolaemia and/or stage 2 hypertension or worse (BP \geq 160/100mmHg)
- Long-term medication use for cardiovascular-related, metabolic, hormonal or neurological chronic illnesses
- Current injury or illness which may have hindered participation and their ability to exercise
- Long-term tobacco use or smoking
- Regular antioxidant supplementation
- Individuals who were unable to communicate fluently in written and spoken English
- Individuals who indicated that they may not be available throughout the intervention period or may not be able to attend weekly exercise/assessment sessions
- Personal, cultural or religious sensitivities regarding any of the assessment procedures

4.3.1 Justification of choice of cohort

Sedentary people were ideal participants as any significant effects on the parameters of interest could be more definitively attributed to the intervention. Sedentary people are relatively more willing (than active counterparts) to refrain from physical activity outside the formal exercise sessions and therefore potentially confounding activities would be easier to control in the former population. (For justification of the definition of 'sedentary' used in the present study, please refer to Appendix E).

Females were excluded due to the quantitative effects that the circamensal rhythm may have on both maximal and submaximal exercise performance (440) and physiological responses/adaptations to exercise such as the heart rate response (441). In addition, 17- β estradiol is associated with increased vasodilation (442) and local blood flow (443) which could potentially influence arterial geometry and structural remodelling. Cyclic fluctuations in the levels of this hormone would have important implications when measuring Alx on a weekly basis and to a lesser extent, the monthly ultrasound-derived measures.

The 20-45 year age range was appropriate because in healthy adults, free of CVD, ageing progressively increases arterial stiffness and wave reflection indices and the effects of progressive atherosclerotic build-up start to become more evident. Individuals >45 years were excluded to eliminate age as a confounder. Younger people were excluded from this study so that findings could be specific for young to middle-aged adults. (Teenagers, who would probably have

4.5 QUESTIONNAIRES AND ANTHROPOMETRIC, CLINICAL, FITNESS AND PSYCHOLOGICAL MEASURES

Assessments were carried out either weekly or at PRE (baseline), MID (mid-intervention) or POST (post-intervention). For standardisation purposes, all PRE, MID and POST measures were carried out between 8:30am and 11:30am.

Questionnaires

During the baseline assessment session, participants were required to provide written consent and complete a PAR-Q (Appendix F) and food-frequency questionnaire (used in the EPIC-Norfolk study).

Height

Measured PRE and POST using a standard built-in stadiometer (Veeder-root, Elizabethtown, NC, USA).

Waist circumference

Measured weekly using a standard tape measure (AccuFitness MyoTape, CO, USA) at the level of the naval, and not overlying any layers of clothing. Trousers/shorts were loosened prior to measurement. Weekly intra-subject but not inter-subject measurement times were kept fairly constant (PRE, MID and POST measurements were all between 9am and 10am).

Body mass

Measured weekly using a standard set of weighing scales. Scales varied between individuals depending on the group and which gym they did the classes at but each individual used the same set of scales each time. PRE, MID and POST measurements were all between 9am and 10am using the same weighing scales (Weightec NZ Ltd., Australia).

Trunk flexibility test (Image in Appendix G)

Carried out at PRE, MID and POST using the modified sit-and-reach test. Participants were made to sit on a mat with their legs extended. A tape measure was taped to the mat at the 15 inch (38cm) mark, with the 'zero' closest to the individual. The feet were lined up so they were just in contact with the tape and the heels were 10-12 inches apart. The subject was asked to reach as far forward as they could (without flexing their knees) with one palm on top of the other and slide their fingertips along the tape measure to a point where they could hold it for at least 1 second (to avoid jerky movements and momentum). The best result from the 3 attempts was recorded.

Blood lipid profile and fasting blood glucose (FBG)

These were assessed at PRE and POST after a 9-11 hour overnight fast using a simple finger-prick test. Sterile, single-use lancets (ACCU-CHEK Safe-T-Pro Plus, Mannheim Germany) were used to prick the side of the right index or middle fingertip and blood was drawn into 40 μ L capillary tubes. Total cholesterol (TC), HDL-cholesterol (HDL-C), total triglycerides (TGs), the TC:HDL ratio and LDL-cholesterol (LDL-C) levels were assessed using the CardioChek® P•A Point-of-Care Test System and PTS Lipid Panels® Test Strips (Polymer Tech Systems Inc., IN, USA). Fasting blood glucose (FBG) was assessed using the FreeStyle Optium Xceed blood glucose metre and FreeStyle Optium glucose strips (Oxon, UK).

Cardiorespiratory fitness test

A graded incremental exercise test to exhaustion was performed on an electronically-braked cycle ergometer (Lode Sports Excalibur®, Groningen, Netherlands) to determine VO_{2peak} at PRE and POST. After a brief warm-up, participants started pedalling at 25W for 1 minute, with a self-selected cadence between 65 and 85 rpm. After the first minute, the load was increased by 15W every minute until the point of volitional fatigue. Expired gases were analysed using a calibrated metabolic cart (TrueOne 2400, ParvoMedics, Sandy, UT, USA). VO_{2peak} was determined from breath-by-breath samples averaged over 15s. The average of 3 HR measures during the last 15 seconds of each stage and the RPE at the end of each stage (minute) were recorded.

Cardiovascular risk assessment

At PRE and POST, the Framingham Risk Score was calculated, along with the estimated Framingham vascular age (444), to establish the general 10-year CV risk. This score was based on age, sex, pSBP, smoking, diabetes, treatment for hypertension, HDL-C and TC.

4.6 MEASURES OF ARTERIAL HEALTH DERIVED USING AN AUTOMATED OSCILLOMETRIC DEVICE

This device measured peripheral and central BP (pBP and cBP respectively), HR and the peripheral AIx. Resting cBP, pBP, HR and AIx measures were determined weekly in a quiet, temperature-controlled room, using an automated vascular monitor (Uscom R6.5B, BP+, Sydney, Australia) and algorithm (version MR1517). The BP+ device has an in-built brachial BP monitor. First, brachial BP is measured oscillometrically. Within 3s of cuff deflation, the cuff re-inflates and holds the pressure for 10 seconds at 30mmHg above the measured SBP. During this period, a suprasystolic waveform is obtained, from which the cBP is derived from the relationship between aortic oscillatory pressure and total oscillatory pressure under the cuff. During the procedure, participants were made to sit quietly on a chair in an upright position, feet flat on the floor, elbow resting on the table with the palm prone. For standardisation purposes, an appropriately sized cuff was positioned over the left upper arm. Placement of the cuff over clothing was avoided as was rolling up of sleeves which could have caused proximal arterial occlusion. A total of 3

measures were taken each time, with a one minute rest between each measure. The first was taken after the subject had sat quietly for 2-3 minutes. The average of the 3 results each week were used for analysis. The weekly measures for those in the intervention group were taken approximately 10-15 minutes prior to the last exercise session of the week. All data collection using the BP+ device was conducted at least 24 hours after an exercise session to avoid the immediate effects of a single bout of exercise. Weekly intra-subject but not inter-subject measurement times were kept constant.

Note on BP and PP measures: Although central pulse pressure is the index most relevant to AS, peripheral BP is more widely understood, acknowledged and measured. Therefore, results presented will be those for *peripheral* SBP and DBP and *central* PP.

4.7 ULTRASOUND ASSESSMENTS

The ultrasound system (GE Healthcare Vivid S6 Cardiovascular Ultrasound System, WI, USA) used for carotid and femoral examinations was equipped with 2D imaging, colour and spectral Doppler, an ECG monitor and a high-frequency vascular transducer as recommended. A 12MHz linear array transducer was used to acquire high-resolution images. Since most of the indices assessed in the present study may vary quite considerably across a single cardiac cycle, standardisation of the frame relative to the cardiac cycle was necessary. The ultrasound integrated with the ECG facilitated the assessment of these parameters according to the cardiac cycle (R wave).

Ultrasonic assessments were carried out at PRE, MID and POST, between 9 and 11am (at least 12 hours after an exercise session to avoid the immediate effects of a single bout of exercise). Both inter- and intra-subject measurement times were kept constant due to diurnal variation in cfPWV. Participants were requested to arrive in a fasted state and avoid caffeine and high-fat meals for at least 12 hours before testing. (Although avoiding the above for only 3 hours are required for US assessments, the blood tests needed to be carried out in the fasted state). Once the (3 lead) ECG was attached, the subject rested lying down in the supine position for 10 minutes in a quiet, dimly-lit, temperature-controlled (24°C) room. Individuals were requested to avoid falling asleep during the assessments.

4.7.1 RESTING CAROTID-FEMORAL PULSE WAVE VELOCITY (cfPWV)

Doppler guided by 2D ultrasound, with simultaneous ECG gating was used to evaluate cfPWV as previously described (83) (Figure 4.3). As the elastic common carotid artery (CCA) and muscular common femoral artery (CFA) are quite superficial, these are the most common set points (18), enabling the calculation of cfPWV, which covers the region over the aorta which exhibits the greatest pathological effects of AS and the highest increase in AS with ageing (30).

4.7.1.1 THE EVALUATION OF cfPWV

As shown in Figure 4.2, the distance (D) from the carotid point to the femoral point is divided by the transit time (Δt), which is the time of travel of the foot of the wave over distance D (83). The foot of the wave is the end of diastole, just before the steep rise of the wave begins.

Doppler guided by 2D ultrasound is a reliable and reproducible alternative to other mechanical methods used to measure cfPWV (23) and has been used in several population-based studies (52, 100, 149, 445-447). The assumption underlying the Doppler method is that the flow wave of the spectral Doppler corresponds to the actual pulse wave (23).

Aortic PWV (cfPWV) is calculated using the formula:

$PWV = D \text{ (meters)} / \Delta t \text{ (seconds)}$ where

D represents the distance from the carotid point to the femoral point and

Δt is the transit time, which is the time of travel of the foot of the pressure wave over distance D

Figure 4.2: Calculation of carotid-femoral pulse wave velocity

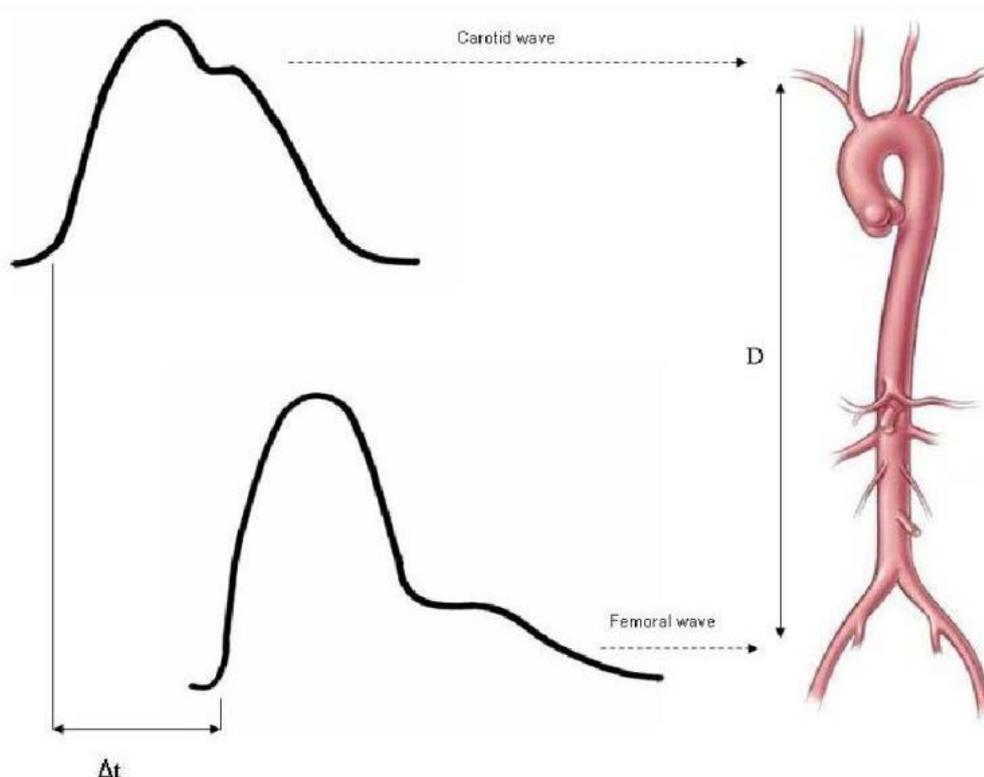


Figure 4.3: The determination of carotid-femoral pulse wave velocity

Transit time is estimated by the foot-to-foot method. The foot of the wave is defined at the end of diastole, when the steep rise of the waveform begins. The transit time is the time delay between the arrival of the foot of the pulse wave (the systolic upstroke, just before the steep rise of the wave begins) at the common carotid and common femoral artery. *Image adapted from (23)*

In this method, waves are obtained successively at the common femoral and common carotid arteries (CFA and CCA respectively). Once the probe had been positioned 1.5-2cm proximal to the carotid bifurcation, wave Doppler flow and ECG were simultaneously identified and a recording was taken over 2-3 cardiac cycles (1 respiratory cycle; 5-6 breaths). After the above step was repeated for the unilateral femoral artery, the transit time was measured by subtracting the time delay of the foot of the wave (top of the R-wave on the ECG equivalent to end-diastolic diameter) to the CCA from the time delay between the R-wave and CFA. Only invasive methods would give an accurate value for the distance between the CCA and CFA. A consensus published by van Bortel et al. in 2011 suggested that 80% of the direct carotid-femoral distance (CCA-CFA \times 0.8) would provide the most accurate estimate of the actual distance (139). Therefore, in the present study, 80% of the measured surface carotid-femoral distance was used for the purpose of PWV calculation. For each recording, the sample volume (gate) was manually adjusted to encompass two-thirds of the vessel lumen and an insonation angle (angle between the ultrasound beam and longitudinal vessel axis) of 50-60° was maintained.

4.7.2 RESTING INTIMA-MEDIA THICKNESS (IMT)

4.7.2.1 RESTING COMMON CAROTID ARTERY INTIMA-MEDIA THICKNESS (cIMT) ASSESSMENT

Carotid IMT as measured by ultrasound is defined as the combined thickness of the intima and media because current technology is not sensitive enough to measure only intimal thickness (11). Two echogenic lines, shown to be the lumen-intima and media-adventitia interface, separated by a hypoechoic space, are observed on the far wall of the common carotid artery (CCA) (35, 448, 449). The distance between the two echogenic lines was not significantly different from the IMT measured under both gross and microscopic examination (448).

B-mode images of the CCAs were obtained bilaterally using Doppler ultrasound and the 12MHz linear array transducer. The CCAs were imaged in the longitudinal plane using the preferred lateral probe approach (single insonation angle). The head position was standardised as much as possible, with the neck extended to an angle of 45-50° and excessive pressure on the CCA with the probe was avoided. Images were acquired 2cm proximal to the carotid bifurcation and IMT measurements were carried out over a 1cm straight arterial segment, using automated edge-detection software. Intima-media thickness was defined as the viewable distance between the two parallel lines corresponding to the lumen-intima and the media-adventitia interfaces. As cIMT may vary between 5-10% during a single heartbeat, IMT measurements were standardised to the R-wave of the ECG which represents the end-diastolic moment (also the moment of the thickest cIMT). Gain settings, focus depth (30-40mm) and frame rates (>15-25Hz) were adjusted manually to optimise edge detection and avoid slice thickness artefacts (11).

Carotid IMT data was collected for both the near and far walls of the right and left CCAs. For each wall assessed, averages of 6 mean, 6 minimum and 6 maximum values were used in data

analysis. Therefore, for each wall of each artery, single values for the mean of means, mean of minima and mean of maxima IMTs were calculated. (Readings were only taken from images in which arteries were horizontal and in which the carotid bifurcation could be clearly identified).

Justification of protocol used for carotid artery IMT assessment

A distal segment of the CCA was assessed in the present study because it has been previously shown that imaging of the near and far walls of the CCA from multiple angles is superior to measuring the carotid bifurcation and internal carotid artery, and that the former is more reproducible and nearly always complete (450). A combination of all segments was unnecessary in the present study as the aim was not to assess plaque formation in the carotid artery. The IMPROVE study found that for both mean and maximum cIMT measures at the CCA, bifurcation and internal carotid artery, cIMT is positively correlated to CV risk (239). The IMPROVE study concluded that when relating increased cIMT to increased CV risk, CCA measures are just as useful as more elaborate measures. However, for risk classification, the more elaborate cIMT measures are more accurate than cIMT measured only at the CCA. Common carotid IMT values are more strongly correlated to adiposity measures than are IMT measurement at other sites of the carotid tree (281). In light of all the previous literature, the mean of means of the right and left cIMT were reported as recommended by the 2008 ASE Consensus statement (11). Although both near and far walls were imaged, only results of the far walls will be presented because only far wall cIMT values are accurate reflections of wall thickness whereas near wall IMT values are merely approximations (450) (usually underestimations of true wall thickness (451)).

4.7.2.2 RESTING COMMON FEMORAL ARTERY INTIMA-MEDIA THICKNESS (fIMT) ASSESSMENT

Femoral IMT was determined from longitudinal 2D B-mode images using a high-resolution 12MHz linear array transducer. Images were acquired 2cm proximal to the CFA bifurcation and IMT measurements were carried out over a distance of 1.5cm using automated edge-detection software. All IMT measurements were standardised to the R-wave of the ECG.

Right and left fIMT data were collected for the far walls only. For each wall, averages of 6 mean, 6 minimum and 6 maximum values were used in data analysis. Therefore, for the far wall of each artery, single values for the mean of means, mean of minima and mean of maxima IMTs were obtained (but only the far wall results will be presented). For each vessel examined, Peak systolic velocity (PSV), End diastolic velocity (EDV) and the RI $[(PSV-EDV)/PSV]$ were automatically calculated for each cardiac cycle. The average of 6 values for the RI was used in data analysis.

4.7.3 RESTING COMMON CAROTID ARTERY END-DIASTOLIC INTRA-LUMINAL DIAMETER (cEDD)

The right and left resting (baseline) cEDDs were measured using grey scale imaging. The boundaries were defined as the visible lumen-intima interfaces of the near and far walls, identified using the automatic edge-detection software. The EDD corresponds to the R wave on the ECG and was measured approximately 1-2cm proximal to the carotid bifurcation. Although the R wave on the ECG represents end diastole, it is also the point of minimum diameter. However, the point of maximum diameter (after the T wave, before the start of the following P wave) is difficult to standardise and therefore, measured diameters in this study were synchronised to the R-wave peaks on the ECG. The averages of 6 measures were used for the right and left carotid diameters each. *NB: Resting common femoral diameters were not assessed as it was not possible to clearly discern the necessary interfaces in the majority of images.*

4.7.4 CALCULATIONS OF RESTING COMMON CAROTID ARTERY WALL:LUMEN RATIO (cWLR)

cWLR was calculated as:

$$2 \times \text{cIMT/lumen diameter in diastole} \quad (452)$$

4.8 STRUCTURE OF STUDY

4.8.1 RECRUITMENT AND GROUP ALLOCATION

Ethical approval for this study was granted from the Auckland University of Technology Ethics Committee (Appendix A). Interested individuals who responded to the advertisement flyer (Appendix H) were sent an information sheet (Appendix I) and were required to complete the IPAQ to confirm their sedentary status. After providing written consent to participate (Appendix J), participants were allocated to either the intervention group ($n=10$) or the control group ($n=5$). Both groups were requested to maintain consistent lifestyles throughout the intervention period, in particular, maintain their normal diets but avoid antioxidant supplements for the 8 weeks. They were also required to keep a physical activity log over the 8 weeks (Appendix K). In the log, they were required to write down any activity they carried out, the duration and perceived intensity, RPE (according to the modified Borg scale). From these logs, average exertion scores for each activity were calculated as: *Exertion score (no units) = activity duration (mins) \times RPE*

The sum of the individual exertion scores during a single day gave an indication as to daily exertion scores. Physical activity logs were collected once a week and were to be completed on any three days of the week (including one weekend day). In this way, it was confirmed that the average weekly inter-group physical activity loads were fairly similar and that only the intervention-based exercise would explain any arterial health adaptations.

4.8.2 CONTROL GROUP

The control group was simply asked to maintain and record their normal physical activity routine and diet for 8 weeks. Assessments were carried out as depicted in Figure 4.1.

4.8.3 INTERVENTION GROUP AND NATURE OF EXERCISE INTERVENTION

Individuals in the intervention group attended 3 supervised indoor cycling group-fitness classes per week for 8 weeks at one of two gyms in Auckland. Les Mills International Limited (LMI) has registered the trademark 'RPM' (Raw Power in Motion) for the popular indoor (studio) cycling group-centred activity known as 'spinning'. LMI describes RPM™ as a moderate-to-high intensity interval programme with aerobic endurance, anaerobic and sprint components with the aim of building muscular endurance, power, strength and speed. In the present intervention, 'regular bursts of HIIT and SIT' implies that after approximately a 10 minute AE warm-up, these bouts alternated such that the structure of the workout was: **warm-up, AE, HIIT, SIT, HIIT, SIT, HIIT, cool-down (each section approximately 5 mins). Each HIIT and SIT bout, has, on average, three 'peaks' interspersed with active (mild/moderate) cycling.**

Appendix L is an official outline provided by LMI describing the structure of a typical RPM™ class. Each session (class) lasts for 45-50 minutes (including a ~5 min warm-up and ~5 min cool-down) and instructions given during class are pre-choreographed to specific music. It is a self-paced class where individuals can choose their own level of intensity by means of a dial on the bike. However, it is possible that the quality and quantity of content (commentary, instruction and feedback) provided by the class instructor could serve as a source of extrinsic motivation, thereby influencing self-selected intensities. For the purpose of the present study, it was assumed that direction provided by instructors were similar across all classes. The temperature and humidity of the cycling studio are unknown and were not controlled.

The first week of the intervention was used for participants to familiarise themselves with the nature of an RPM™ class. The sessions were carried out at any time of day but participants were asked to separate each class by at least 36 hours. The intervention group participants, like the controls, were requested to abstain from any other type of novel physical activity involving major muscle groups for the duration of the intervention (as aforementioned, the physical activity logs ensured that participants did not engage in significant quantities of physical activity outside the intervention-based exercise).

Before the intervention commenced, it was decided that participants would only be included in the analysis if they completed all 24 classes within a period of 9 weeks, with at least one class carried out each week. (An extra week leeway was permitted on account of the community-based setting and subsequent acknowledgement of practical aspects based on self-regulated attendance). Assessments were carried out as depicted in Figure 4.1 with at least 24 hours between a training session and any assessment session (to eliminate the effects of an acute exercise bout). Weekly assessments were carried out approximately 10-15 mins before the last

class of every week so intra-individual but not inter-individual assessment times were maintained throughout the intervention period.

Obtaining RPM™ objective and subjective intensities

Heart rate during the exercise classes was monitored using the Polar Team² system (Polar Electro Oy, Professorintie 5, Kempele, Finland). Data was collected on a weekly basis and averaged out to obtain OBJECTIVE information regarding the exercise intensity which was gauged on an individual basis as a percentage of the maximum HR (%HR_{peak}) attained during the VO_{2peak} test. In addition, participants were required to rate the intensity of each session according to the modified Borg scale (Appendix L) so that session RPE (also known as training load) could be calculated to assess the SUBJECTIVE intensity:

*Training load (no units) = duration of RPM class (ie. 45 mins) × RPE**

The RPE is one of the most popular measures used to assess internal TL (453). It allows individuals to monitor their effort both during classes and retrospectively and has previously been shown to correlate with HR during steady-state exercise and HIIT (454).

Calculating the session RPE, that is, combining the RPE with the session duration, provides a better quantification of the internal TL and the validity and reliability of this method have been previously shown (455).

Gauging RPM™ intensities over the whole class

The raw data of the Polar Team² system observed on the screen is a graph of HR as a % of individually-determined HR_{peak} (an example is provided in Appendix M). Average and maximal %HR_{peak} values throughout a single session are obtained directly and the amount of time spent in each training zone are also clearly illustrated. When the order and duration of bouts of the different training components are known, average and maximal %HR_{peak} values from the corresponding section of the graph can be calculated. As not all durations are identical, these values are approximations but nevertheless, they can be used to gauge the intensities of different components of the session.

Summary of exercise nature

- i) It was a supervised group-centred, self-paced, indoor cycling programme, undertaken in a pre-existing, community-based setting (gymnasium)
- ii) It was a moderate-to-high intensity aerobic interval session with SIT and HIIT components and specific foci on aerobic endurance, anaerobic power and strength.

* Participants were required to provide the RPE of the session **as a whole**, including all the highs and lows.

4.9 STATISTICAL ANALYSIS

All data was included as no participant dropped out and all measures were satisfactorily obtained. The sample size calculation for cfPWV, the main outcome measure, assumed a power of 90% and an α -error probability of 0.05. Based on this a priori analysis, a sample size of at least 10 participants in the intervention group was required to detect a clinically significant effect of the exercise intervention on this index of AS.

All statistical analyses were performed with the statistical package SPSS version 22 (SPSS, Chicago, IL). Descriptive statistics are presented as mean \pm standard deviation. All comparisons were based on a 95% confidence limit with $p < 0.05$ considered statistically significant. Effect sizes were calculated using partial eta squared ($p\eta^2$) with values of 0.1, 0.3, and above 0.5 considered to be a small, medium, and large effects respectively. Data collected was thoroughly checked for errors and outliers and normality was evaluated by the Shapiro-Wilk test. Both Mauchly's tests of sphericity and Levene's homogeneity of variance tests were considered for ANOVAs.

One-way between-group analyses of variance (ANOVAs) with Bonferroni adjustments and Tukey's post-hoc tests were performed to determine the impact of the exercise intervention on outcome measures at pre-, mid- and post-intervention (PRE, MID and POST respectively).

One-way repeated measures ANOVAs were conducted separately for the control and intervention groups to examine the results of the intervention.

Mixed between-within participants ANOVAs were conducted to determine main and interaction effects. After inspection the distribution of data points of preliminary scatterplots to ensure no violations of the assumptions of linearity, outliers and homoscedasticity (variability in scores for one variable similar at all values of the second variable), multiple univariate correlations and regressions were performed to determine relationships between absolute values as well as percentage changes in selected physiological variables. Where necessary, partial correlations were conducted to explore relationships between outcome measures whilst controlling for age and blood pressures.

Significant correlations with cfPWV, $Alx@75$, cPP and cIMT were used in stepwise multiple linear regression analyses to identify independent determinants of these indices.

For outcome measures that exhibited both between-subject differences at POST and linear relationships between PRE and POST values, ANCOVAs were performed (provided ANCOVA assumptions were not violated), to determine whether significant inter-group relationships existed at POST, with PRE values as covariates.

CHAPTER 5: RESULTS

Due to the extensive data analysis and for ease of understanding, the results presented below have been systematically organised into four sections based on the research questions as follows:

5.1 Intervention compliance, training intensity and daily training loads

5.2 Between- and within-group effects

- i) Comparisons between the two groups on all outcome measures across the three time points
- ii) Time course of adaptations

5.3 Exploration of relationships between outcome measures:

- i) Correlations between CRF and arterial health, anthropometric and clinical measures
- ii) Comparisons of absolute values and percentage changes of outcome measures:

Where percent changes in values are presented, the term 'improvement' depicts an impact in the beneficial direction. Therefore, even when 'improvements' for some measures signify that absolute values decreased, negative percentage values are not considered.

- iii) Relationships between bilateral ultrasound-derived measures of AS and atherosclerosis across all time points

5.4 Focus on intervention group

- i) In-depth exploration of metabolic syndrome risk factors together with calculated CVD risk and vascular age at pre- and post-intervention
- ii) Summary of the main adaptations in the intervention group

5.1 INTERVENTION COMPLIANCE, TRAINING INTENSITY AND DAILY TRAINING LOADS

Adherence: There was 100% compliance in terms of class participation and completion (ie. 10 participants carried out all 24 RPM™ classes). However, despite the request to avoid classes on consecutive days, this was not always possible due to subject inconvenience.

Objective intervention intensity: Table 5.1 provides a breakdown of intensities throughout an entire RPM™ class. Figure 5.1 is an example of HR data collected for a typical RPM™ class. The average HR intensity during an RPM™ class ranged from 74-87% ($81.3 \pm 7.2\%$) of the individually-determined HR_{peak} . The average maximum intensity during a class was $93.6 \pm 7\%$ (range: 88-104%) HR_{peak} . Figure 5.2 depicts weekly HR averages during RPM™ classes over the intervention period. Despite the weekly fluctuations, the general trend was for the average % HR_{peak} to gradually and slowly increase such that the average HR_{peak} in the eighth week was 10% greater than that in the first week ($p < 0.05$). Appendix M provides an example of raw HR data collected over an entire RPM™ session for the same individual in weeks 1 and 8 whilst Appendix

N provides a snapshot of the average weekly intensity data for each subject individually for all 8 weeks.

Subjective intervention intensity: The average RPE for all 10 participants over all 24 classes was 8.5 (out of 10 on the Borg scale). The session RPEs (training loads, TLs)* ranged from 315-405 (7-9 on the Borg scale).* RPM™ training loads and HR intensities were not associated (ie. subjective and objective data did not significantly concur).

Daily exertion scores excluding RPM™ classes:** The physical activity logs ensured that participants did not engage in significant quantities of physical activity outside the intervention-based exercise (RPM™ classes). Table 5.2 summarises the average number of hours that (all 15) participants spent doing different physical activities (excluding RPM™ classes) and activities of daily living. The table includes the average session RPE for each activity and the calculated exertion score (the habitual activity equivalent of training load). The intervention and control groups spent, on average, 7 hours sleeping, 12.5 hours sitting, 1.5 hours standing, 1 hour walking and 1 hour doing 'other' light activities (eg. household chores, gardening). The average exertion score range over a whole day was 210-780 (compared to a training load of 315-405 for a single 45 minute RPM™ session). Habitual (non-RPM™) activity between the intervention and control participants did not significantly differ over the intervention period ($p>0.05$). Furthermore, average daily exertion scores did not significantly change across the 8 weeks in either group. In the intervention group, there was no significant difference in extra-intervention training loads on RPM™ and non-RPM™ days. It should be mentioned that on occasion, participants in both groups engaged in physical activities of moderate intensities (eg. playing with kids, a round of golf, slightly harder domestic chores, planned walking, short game of basketball). These activities were extremely rare (assuming they were honestly reported) because participants had been informed at the start of the study that it was required that they did not engage in intense physical activities more than twice over the 8 weeks.

**Training load = subjective RPE during session x session duration*

***Habitual activity exertion score = subjective RPE of activity x duration of activity (mins)*

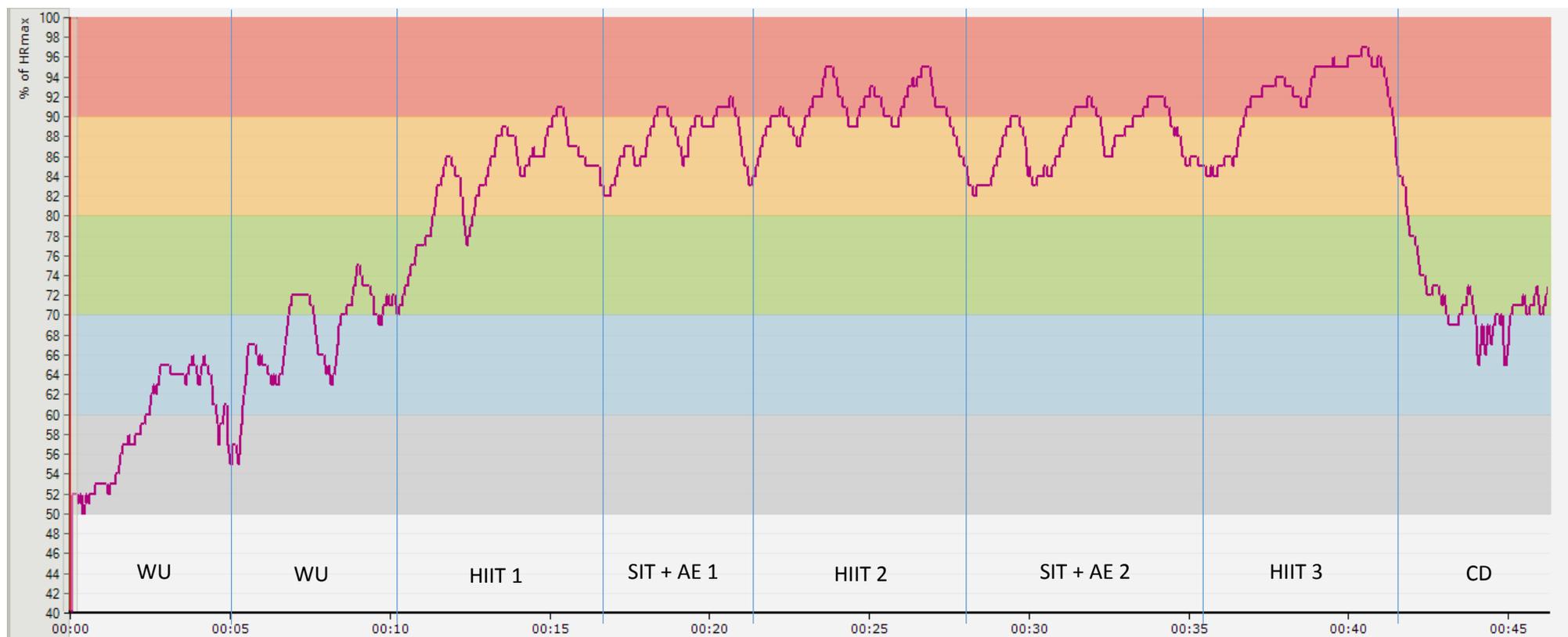


Figure 5.1: Example of raw HR data collected during a typical RPM™ session

This image is an example of typical heart rate data collected using the Polar Team² unit over a 45 minute RPM™ class. Heart rate is presented as %HR_{peak} determined from the baseline VO_{2peak} test. Different training zones are depicted by shades of horizontal bars. Each RPM™ release has different music of different lengths. Outlined in this image, are approximate start and end-points of each track (8 tracks in total, see Appendix L). This individual spent nearly three-quarters of the class in the 80-95%HR_{peak} zone. *WU, warm-up; HIIT, high-intensity interval training; SIT, sprint interval training; AE, aerobic exercise; CD, cool-down*

Table 5.1: Breakdown of class intensity fluctuations

TRACK NUMBER (MEDIAN DURATION)	EXTRA NOTES	AVERAGE %HR_{peak}	PEAK %HR_{peak}
1 (4:47)	Warm-up (only sitting)	56 ± 3	63 ± 5
2 (5:23)	Enter aerobic zone; Explore positions, speeds & loads	70 ± 4	77 ± 3
3 (6:13)	HIIT 1: Strength + power	88 ± 4	93 ± 6
4 (6:08)	Aerobic endurance + SIT	73 ± 5	82 ± 4
5 (5:54)	HIIT 2: Strength + power	89 ± 7	98 ± 3
6 (5:58)	Aerobic endurance + SIT	79 ± 4	79 ± 5
7 (6:32)	HIIT 3: Strength +power	87 ± 8	95 ± 8
8 (3:39)	Recovery+cool down	69 ± 4	77 ± 5
Approx 45 mins		52-97	57-105

Values are presented as means ± SD. In an average class, there are two bouts of SIT and three of HIIT. During the SIT bouts, average intensities ranged from 71-84%HR_{peak} whilst in the HIIT periods, intensities were higher, ranging from 85-98%HR_{peak}. During the second and third HIIT bouts, individuals came close to (or even surpassed) their HR_{peak}.

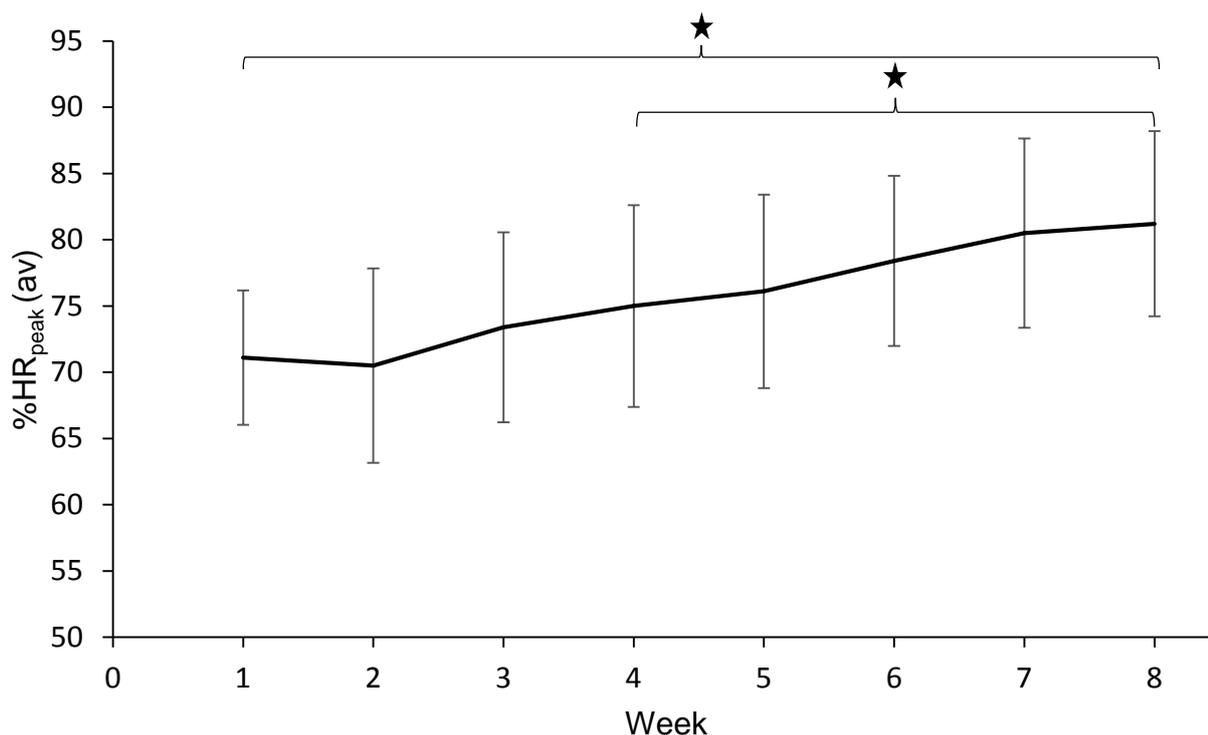


Figure 5.2: Average weekly intensity of exercise sessions across the intervention period

This graph illustrates the mean (and standard deviation bars) %HR_{peaks} averaged over all 10 participants in the intervention group, across the 8 weeks.

★ p<0.05

Table 5.2 Average daily exertion score of all participants on non-RPM™ days

Activity	Duration in hours (mins)	Subjective RPE	Exertion score*
Sleeping	6-8 (360-480)	0	0
Sitting	11-14 (660-840)	0	0
Standing	1-2 (60-120)	1-2	60-240
Walking (<10 mins at a time)	0.5-1.5 (30-90)	2-4	60-360
Other (light activities)**	0.5-1.5(30-90)	3-4	90-180
Average daily range		1.4	210-780

*Exertion score = subjective RPE of activity × duration of activity (mins)

**Other (light activities) included walking at a slow pace for >10 minutes at a time

Participants spent approximately half (52%) of the day sitting, 29% sleeping, 6% standing and only a total of 8% of the day walking or engaging in light physical activity. The range of the average daily exertion score was quite large.

5.2: BETWEEN AND WITHIN-GROUP EFFECTS

Baseline characteristics

Descriptive characteristics, anthropometric measurements, metabolic syndrome (MS) risk factors, cardiorespiratory fitness, flexibility and physical functioning quality of life measures of the intervention and control groups at baseline are presented in Table 5.3. There were no significant differences between groups in any of these parameters at baseline (PRE). There were no significant between-group differences in height ($1.78 \pm 0.07\text{m}$ and $1.83 \pm 0.14\text{m}$, intervention and control groups respectively).

Basic anthropometric measures

There were no differences in any of the anthropometric measures across the three time points in the control group (Table 5.3) and no between-group difference in BMI at any point. In the intervention group only, BMI improved by $1.2 \pm 1.1\%$ ($F=11.220$, $p=0.009$, $p\text{Eta}^2=0.56$) and WC improved by $8.4 \pm 5.4\%$ ($F=25.003$, $p=0.001$, $p\text{Eta}^2=0.74$) from PRE to POST, at which point there was a significant between-group difference ($p<0.05$). Despite no between-group difference at any time point, body mass decreased by 1kg in the intervention group only ($p<0.05$).

Fitness measures

Neither $\text{VO}_{2\text{peak}}$ nor trunk flexibility changed over the 8 weeks in the control group. In the intervention group, $\text{VO}_{2\text{peak}}$ improved by $15.1 \pm 8.3\%$ from PRE to POST ($F=31.403$, $p<0.001$, $p\text{Eta}^2=0.78$), at which point, it was significantly greater than the $\text{VO}_{2\text{peak}}$ of the controls ($F=7.865$, $p=0.034$). The $p\text{Eta}^2$ was 0.3, indicating a large inter-group difference in $\text{VO}_{2\text{peak}}$ at POST. In the intervention group only, trunk flexibility also significantly improved from PRE to POST ($p=0.001$) (Table 5.3) but there were no significant between-group differences.

Resting heart rate (HR) and Peripheral Blood pressure (pBP)

Resting HR did not significantly change in the control group over the 8 weeks. In the intervention group, there was an $8 \pm 6\%$ reduction in HR from PRE to POST ($F=13.774$, $p=0.005$, $p\text{Eta}^2=0.61$) but the 9bpm between-group difference at POST marginally failed to reach statistical significance ($p=0.051$) (Table 5.3). The MAP, peripheral SBP or DBP did not significantly change across the three time points in either of the groups. In the intervention group, the small improvements in MAP ($3.2 \pm 9.5\%$), SBP ($0.9 \pm 4.5\%$) and DBP ($1.3 \pm 4.6\%$) from PRE to POST did not reach statistical significance ($p>0.05$). There were no significant between-group differences in peripheral SBP, DBP or PP at any time point.

Biochemistry

Glucose and lipid markers did not significantly differ at PRE and POST intervals in the control group. In the intervention group, POST TC was 19.5% lower than PRE TC ($F=12.654$, $p=0.006$,

$p\text{Eta}^2=0.58$) and POST LDL-C was 17% lower than PRE LDL-C ($F=7.961$, $p=0.02$, $p\text{Eta}^2=0.47$) (Table 5.3). There were no between-group differences at POST.

RESTING ARTERIAL HEALTH MEASURES

Resting Arterial stiffness, wave reflections and central haemodynamics

There were no significant differences in AS indices (cfPWV, Alx@75 and cPP) between groups at baseline (Figure 5.3).

Carotid-femoral pulse wave velocity (cfPWV)

In the control group, both right and left cfPWV respectively did not differ significantly across the three time points (Figure 5.3c-d). In the intervention group, the right cfPWV was significantly greater at PRE ($8.65 \pm 0.36\text{m/s}$) than MID ($8.22 \pm 0.44\text{m/s}$) ($F=45.087$, $p<0.001$, $p\text{Eta}^2=0.83$) and POST ($7.72 \pm 0.43\text{m/s}$) ($F=314.270$, $p<0.001$, $p\text{Eta}^2=0.97$) and also significantly greater at MID than POST ($F=53.981$, $p<0.001$, $p\text{Eta}^2=0.86$). Similarly, left cfPWV was significantly greater at PRE ($8.62 \pm 0.33\text{m/s}$) than MID ($8.32 \pm 0.28\text{m/s}$) ($F=105.485$, $p=0$, $p\text{Eta}^2=0.92$) and POST ($7.97 \pm 0.32\text{m/s}$) ($F=85.039$, $p<0.001$, $p\text{Eta}^2=0.90$) and also significantly greater at MID than POST ($F=36.904$, $p<0.001$, $p\text{Eta}^2=0.80$). In the intervention group, total average improvements in right and left cfPWV (ie. ΔcfPWV) from PRE to POST were 10.7% and 7.6% respectively ($p<0.001$) (Table 5.8). Inter-group differences in both right cfPWV ($F=25.029$, $p<0.001$) and left cfPWV ($F=21.058$, $p<0.001$) occurred at POST. Between-participants $p\text{Eta}^2$ values for right and left cfPWV were 0.66 and 0.62 respectively, indicating large effects of the intervention on cfPWV.

Peripheral augmentation index standardised to a heart rate of 75bpm (Alx@75)

For the sake of completion, it should be mentioned that the Alx showed similar trends to the Alx@75. No significant changes in this value were observed in the control group over the 8 weeks. In the intervention group, it improved by 23.3% from PRE to POST ($F=14.131$, $p=0.004$, $p\text{Eta}^2=0.61$) (Table 5.8). There were no between-group differences at any time point in either the Alx or the Alx@75. At each of the three time points, the Alx and Alx@75 showed strong positive correlation ($r=0.987$, $p<0.001$) and therefore, results for Alx@75 will henceforth be presented unless stated otherwise. As illustrated in Figure 5.3a, the Alx@75 did not change across the 8 weeks in the control group but showed a $23.8 \pm 21.1\%$ improvement from PRE ($48 \pm 21\%$) to POST ($38 \pm 22\%$) ($F=13.111$, $p=0.006$, $p\text{Eta}^2=0.59$) in the intervention group.

Table 5.3: Anthropometric, clinical, fitness and quality of life measures at each time point

Parameter	Intervention (n=10)			Control (n=5)		
	PRE	MID	POST	PRE	MID	POST
Body Mass (kg)	88.7 ± 12.1 ^b	88.6 ± 12.1 ^c	87.7 ± 12.1 ^{bc}	90.1 ± 13.3	90.5 ± 13.3	90.9 ± 13.0
BMI (kg/m ²)	27.9 ± 3.5 ^b	27.9 ± 3.6 ^c	27.6 ± 3.6 ^{bc}	27.0 ± 4.2	27.1 ± 4.4	27.2 ± 4.5
Waist Circumference (cm)	98.9 ± 7.2 ^{ab}	92.6 ± 9.2 ^{ac}	90.6 ± 8.4 ^{bc*}	95.5 ± 14.5	94.9 ± 13.0	95.2 ± 13.4*
VO _{2peak} (ml/kg/min)	33.4 ± 5.4 ^b	N/A	38.3 ± 5.8 ^{b*}	31.3 ± 5.0	N/A	31.0 ± 5.4*
Peripheral SBP (mmHg)	123 ± 12	126 ± 12	121 ± 8	121 ± 2	125 ± 7	122 ± 4
Peripheral DBP (mmHg)	79 ± 7	78 ± 5	78 ± 5	77 ± 4	80 ± 6	78 ± 3
Peripheral PP (mmHg)	44 ± 7	48 ± 8	43 ± 4	44 ± 4	46 ± 6	44 ± 5
Peripheral MAP (mmHg)	94 ± 8	94 ± 7	90 ± 8	92 ± 3	95 ± 6	94 ± 2
Heart Rate (bpm)	68 ± 9 ^b	67 ± 9	62 ± 7 ^b	67 ± 7	71 ± 8	71 ± 10
Flexibility (cm)	-8.4 ± 14.6 ^c	-4.5 ± 14.8 ^c	1.3 ± 16.6 ^{bc}	-8.7 ± 14.2	-8.2 ± 14	-8.1 ± 13.4
Fasting glucose (mmol/l)	5.7 ± 0.5	N/A	5.5 ± 0.7	5.6 ± 0.4	N/A	5.7 ± 0.3
Total Cholesterol (mmol/l)	4.4 ± 1.0 ^b	N/A	3.8 ± 0.9 ^b	4.3 ± 0.4	N/A	4.2 ± 0.4
High-density Lipoprotein (mmol/l)	0.94 ± 0.23	N/A	0.95 ± 0.25	1.01 ± 0.12	N/A	0.97 ± 0.09
Triglycerides (mmol/L)	1.5 ± 0.7	N/A	1.5 ± 0.9	1.2 ± 0.5	N/A	1.12 ± 0.5
Low-density lipoprotein (mmol/l)	2.9 ± 0.7 ^b	N/A	2.4 ± 0.7 ^b	2.6 ± 0.3	N/A	2.6 ± 0.2
TC/HDL	4.8 ± 0.9	N/A	4.6 ± 1.2	4.3 ± 0.8	N/A	4.4 ± 0.8

Values are presented as means ± SD; *BMI*, Body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *PP*, pulse pressure; *MAP*, mean arterial pressure; *TC/HDL*, Total Cholesterol:High-density lipoprotein ratio

^a significant difference between PRE and MID: p<0.05

^b significant difference between PRE and POST: p<0.05

^c significant difference between MID and POST: p<0.05

* significant difference between intervention and control groups (at that time point): p<0.05

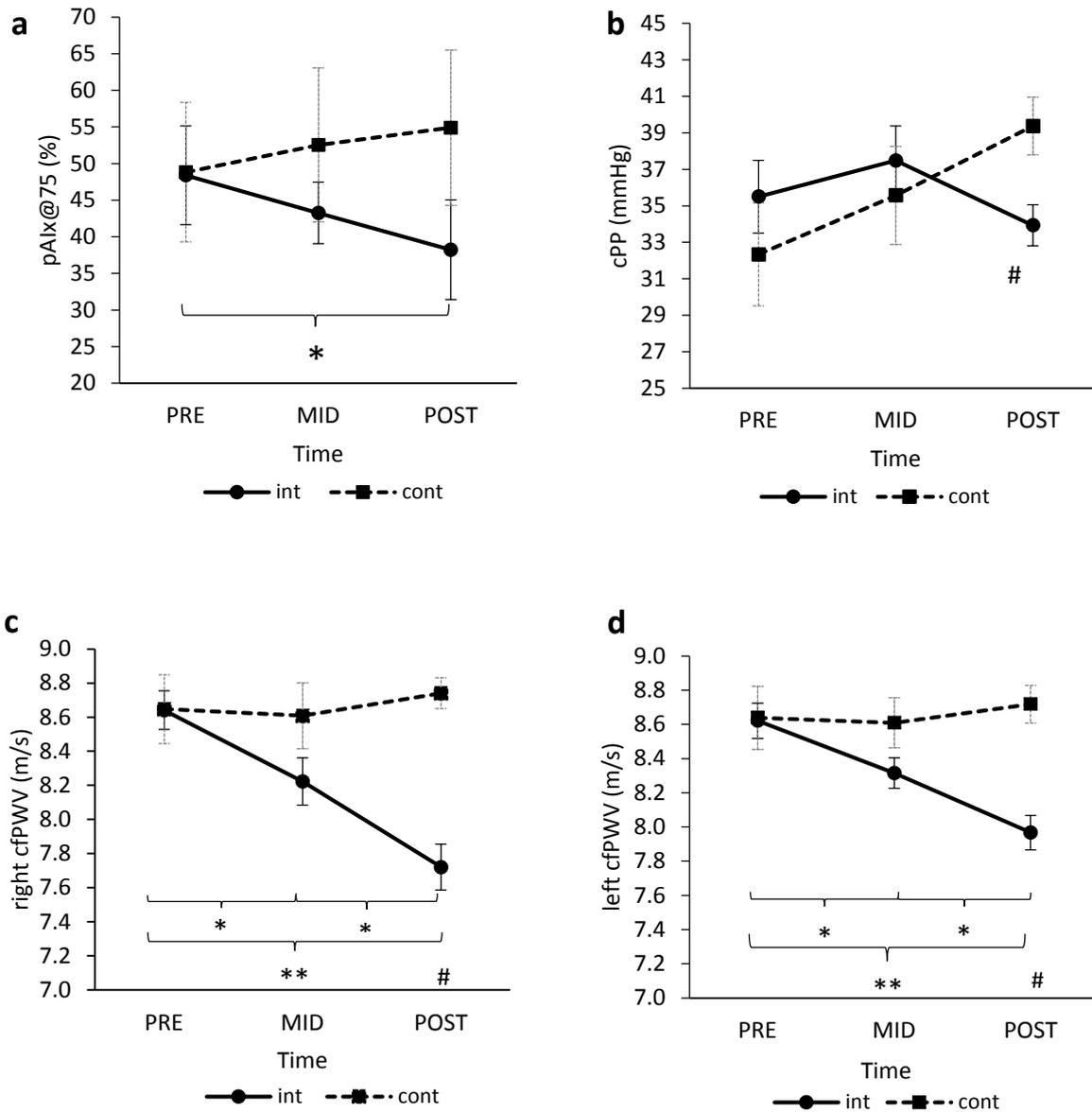


Figure 5.3: Arterial stiffness indices for both groups across time

Group means and standard errors have been illustrated. The figure depicts within- and between-group effects for a) the peripheral Alx@75 b) cPP c) right cfPWV and d) left cfPWV *pAlx@75*, peripheral augmentation index standardised to 75bpm; *cPP*, central pulse pressure; *cfPWV*, carotid-femoral pulse wave velocity

* significant within-group difference in intervention group ($p < 0.05$)

** significant within-group difference in intervention group ($p < 0.001$)

significant between-group difference ($p < 0.05$)

Central Pulse pressure (cPP)

Central PP did not significantly change across the 8 weeks in either of the groups (Figure 5.2b). There was a 1.98% improvement, albeit non-significant in cPP from PRE (35.5 ± 6.7 mmHg) to POST (33.9 ± 3.2 mmHg) in the intervention group (Table 5.8). However, there was a group*time effect for cPP (Figure 5.3b). At POST, cPP was significantly greater in the intervention than in the control group ($F=7.865$, $p=0.015$, $pEta^2=0.38$). In addition, Δ cPP depended on PRE cPP [$F(1,13)=26.292$, $r=0.81$, $p<0.001$]. There were no significant between or within-group differences in either the difference or the ratio between central and peripheral pulse pressures at any time point.

RESTING ARTERIAL ATHEROSCLEROTIC, REMODELLING AND GEOMETRICAL PARAMETERS

There were no significant between-group differences in either right or left cIMT, fIMT, cRI, fRI, cEDD, or cWLR at baseline (Table 5.4).

Intima-media thickness (IMT)

Carotid IMT (cIMT): Mean of means of far wall

The cIMT did not change from PRE to POST in the control group (Table 5.4). Conversely, in the intervention group, both right and left cIMT decreased across the 8 weeks. In this group, significant differences were found between PRE-POST and MID-POST for the right cIMT and significant differences in the left cIMT occurred from PRE-MID and MID-POST. From PRE to POST, right cIMT and left cIMT showed significant decreases of $11.4 \pm 9.5\%$ ($F=13.115$, $p=0.006$, $pEta^2=0.59$) and $13.0 \pm 10.8\%$ ($F=11.534$, $p=0.008$, $pEta^2=0.56$) respectively (Table 5.8). However, there were no significant between-group differences in right or left cIMT at any time point (Table 5.4).

Femoral IMT (fIMT): Mean of means of far wall

There was no change in fIMT changed from PRE to POST in the control group (Table 5.4). In the intervention group, both right and left fIMT decreased across the 8 weeks but the total decrease in right fIMT of 0.077mm ($12.6 \pm 15.3\%$) was not significant (Table 5.8). However, the decreases in left fIMT by 0.0322mm from PRE to MID and by 0.073mm from PRE to POST were significant, resulting in an overall decrease in left fIMT by $13.1 \pm 12.1\%$ ($F=11.29$, $p=0.005$, $pEta^2=0.74$) (Table 5.8). Inter-group differences in left fIMT occurred at POST only ($F=4.862$, $p=0.046$, $pEta^2=0.27$) (Table 5.4).

Resistivity Index (RI)

Carotid RI (cRI)

The cRI remained unchanged in the control group. In the intervention group, the left cRI decreased significantly from PRE to MID and PRE to POST, resulting in an overall decrease

of 0.753 from PRE to POST ($F=14.026$, $p=0.005$, $pEta^2=0.61$), at which point there was a significant inter-group difference in this measure ($F=0.714$, $p=0.022$, $pEta^2=0.34$) (Table 5.4).

Femoral RI (fRI)

Neither the right nor left fRI changed in the control group. In the intervention group, the left fRI decreased significantly from PRE to POST, resulting in an overall decrease of 0.013 ($F=19.286$, $p=0.002$, $pEta^2=0.68$), with significant inter-group differences occurring at both MID ($F=6.434$, $p=0.025$, $pEta^2=0.27$) and POST ($F=11.143$, $p=0.005$, $pEta^2=0.46$) (Table 5.4).

Common carotid end-diastolic diameter (cEDD)

No inter-group differences in either right or left cEDD were observed at baseline (Figure 5.3). Right and left cEDD values at PRE, MID and POST for both groups are illustrated in Figure 5.4a and 5.4b respectively. In the control group, neither right nor left cEDD changed significantly over the three time points. In the intervention group, significant increases occurred from PRE to POST in both right ($5.62 \pm 0.7\text{mm}$ vs $6.01 \pm 0.59\text{mm}$) and left ($5.72 \pm 0.36\text{mm}$ vs $6.15 \pm 0.55\text{mm}$) cEDD and from MID to POST as well, resulting in overall increases of 0.39mm (6.9%) ($F=12.570$, $p=0.006$, $pEta^2=0.58$) and 0.43mm (7.5%) ($F=25.961$, $p<0.001$, $pEta^2=0.74$) respectively. Left cEDD was greater in the intervention than in the control group at POST ($F=6.804$, $p=0.022$, $pEta^2=0.34$) (Figure 5.4b).

Common carotid wall:lumen ratio (cWLR)

Neither right nor left cWLR significantly changed over the course of the 8 weeks in the control group. In the intervention group, right cWLR decreased by 17% from PRE (0.1681 ± 0.0323) to POST (0.1383 ± 0.0277) ($F=23.269$, $p<0.001$, $pEta^2=0.72$). Likewise, left cWLR decreased by 19.2% from PRE (0.19 ± 0.03) to POST (0.15 ± 0.03) ($F=24.729$, $p<0.001$, $pEta^2=0.73$) (Figure 5.4c-d). There was a small but significant between-group difference in right cWLR at POST ($F=9.620$, $p=0.035$, $pEta^2=0.48$) (Table 5.4).

Table 5.4: Arterial remodelling parameters at each of the three time points

Parameter	Intervention (n=10)			Control (n=5)		
	PRE	MID	POST	PRE	MID	POST
Right cIMT far wall average (mm)	0.46 ± 0.06 ^b	0.45 ± 0.08 ^c	0.41 ± 0.07 ^{bc}	0.48 ± 0.16	0.49 ± 0.14	0.49 ± 0.13
Left cIMT far wall average (mm)	0.54 ± 0.08 ^{ab}	0.51 ± 0.08 ^a	0.47 ± 0.09 ^b	0.51 ± 0.13	0.52 ± 0.11	0.52 ± 0.12
Right fIMT (mm)	0.55 ± 0.08	0.50 ± 0.04	0.47 ± 0.05	0.55 ± 0.09	0.55 ± 0.09	0.55 ± 0.08
Left fIMT (mm)	0.54 ± 0.03 ^{ab}	0.51 ± 0.03 ^a	0.47 ± 0.06 ^{b*}	0.55 ± 0.06	0.55 ± 0.09	0.55 ± 0.08*
Right cRI	0.75 ± 0.06	0.74 ± 0.04	0.74 ± 0.05	0.74 ± 0.09	0.74 ± 0.08	0.74 ± 0.07
Left cRI	0.79 ± 0.02 ^{ab}	0.76 ± 0.03 ^a	0.74 ± 0.04 ^{b*}	0.78 ± 0.05	0.78 ± 0.05	0.79 ± 0.04*
Right fRI	0.96 ± 0.01 ^a	0.95 ± 0.01 ^a	0.95 ± 0.02	0.97 ± 0.02	0.96 ± 0.01	0.97 ± 0.02
Left fRI	0.96 ± 0.01 ^b	0.95 ± 0.01*	0.95 ± 0.01 ^{b**}	0.96 ± 0.01	0.97 ± 0.01*	0.97 ± 0.02**
Right cEDD (mm)	5.62 ± 0.70 ^b	5.84 ± 0.62 ^c	6.01 ± 0.59 ^{bc}	5.64 ± 0.75	5.66 ± 0.55	5.56 ± 0.42
Left cEDD (mm)	5.72 ± 0.36 ^{ab}	5.98 ± 0.50 ^{ac}	6.15 ± 0.55 ^{bc*}	5.48 ± 0.33	5.44 ± 0.50	5.40 ± 0.43*
Right cWLR	0.17 ± 0.03 ^b	0.16 ± 0.03 ^c	0.14 ± 0.03 ^{bc*}	0.17 ± 0.04	0.17 ± 0.05	0.18 ± 0.05*
Left cWLR	0.19 ± 0.05 ^{ab}	0.17 ± 0.03 ^{ac}	0.15 ± 0.03 ^{bc}	0.19 ± 0.06	0.19 ± 0.05	0.19 ± 0.05

Values are presented as means ± SD; *cIMT*, common carotid Intima-media thickness; *fIMT*, common femoral intima-media thickness; *cRI*, common carotid resistivity index; *fRI*, common femoral resistivity index; *cEDD*, common carotid end-diastolic diameter; *cWLR*, common carotid wall:lumen ratio

^a significantly lower at MID than PRE: p<0.05

^b significantly lower at POST than PRE: p<0.05

^c significantly lower at POST than MID: p<0.05

* significant difference between intervention and control groups (at that time point): p<0.05

** p<0.005

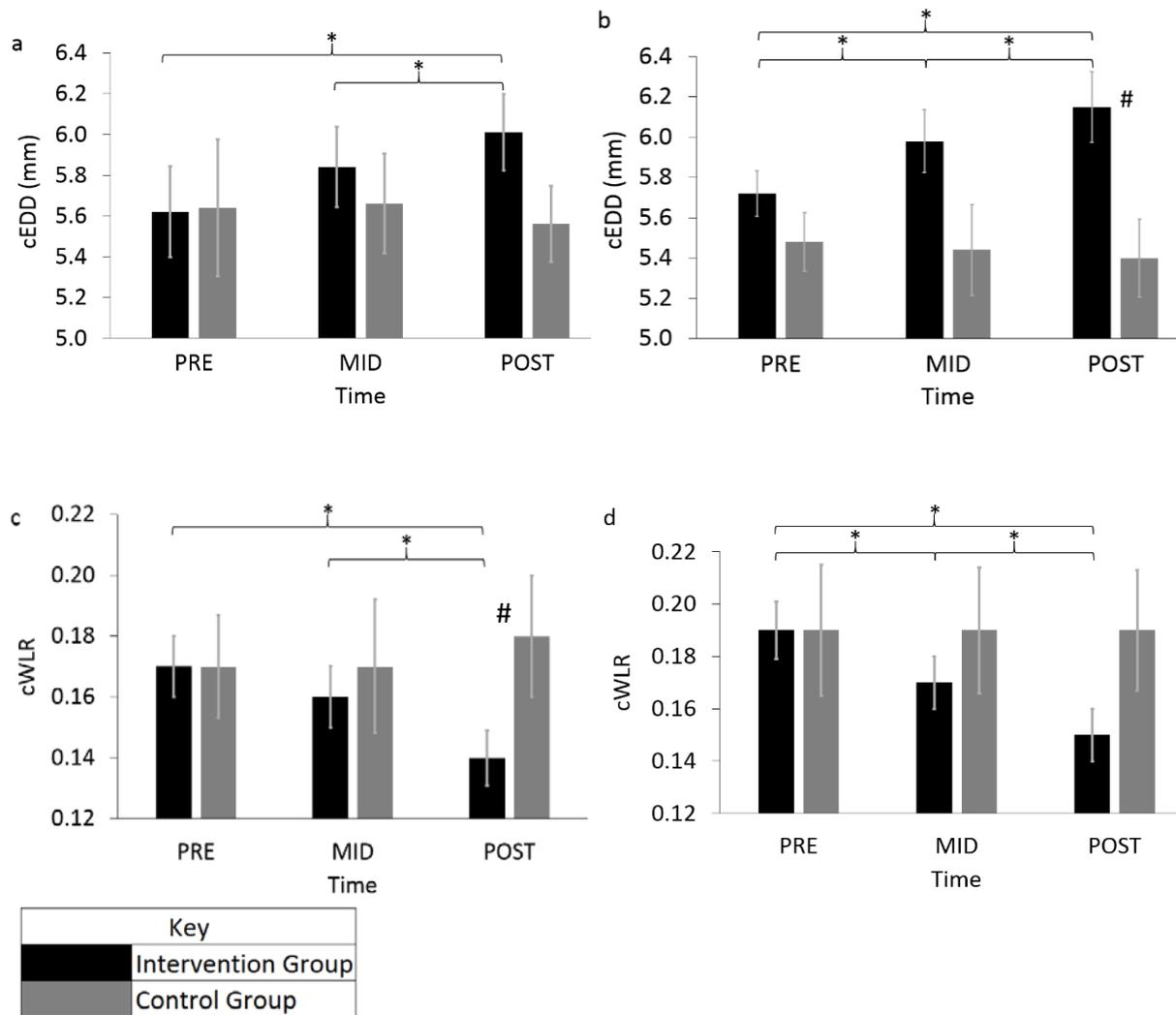


Figure 5.4: Arterial geometrical parameters as a function of time

Group means and standard errors have been illustrated. The figure shows within- and between-group effects for a) right cEDD b) left cEDD c) right cWLR and d) left cWLR for intervention (black bars) and control (grey bars) groups. *cEDD*, common carotid end-diastolic diameter *cWLR*, common carotid wall:lumen ratio

*significant within-group difference in intervention group ($p < 0.05$)

#significant between-group difference ($p < 0.05$)

Time-course of adaptations

Greater improvements in BMI and flexibility were observed within the first four weeks whilst WC, MAP, HR, cPP, cWLR and cfPWV improved to a greater extent within the latter half of the intervention period. Improvements in Alx@75, cIMT, cEDD and fIMT were not obviously different between the first and second four weeks. Figure 5.5 illustrates the general trend in the Alx@75 for both groups across the 8 weeks. However, not enough data was available to carry out statistical analysis of adaptation trends so the reports above are based on absolute average values of the respective index at PRE, MID and POST.

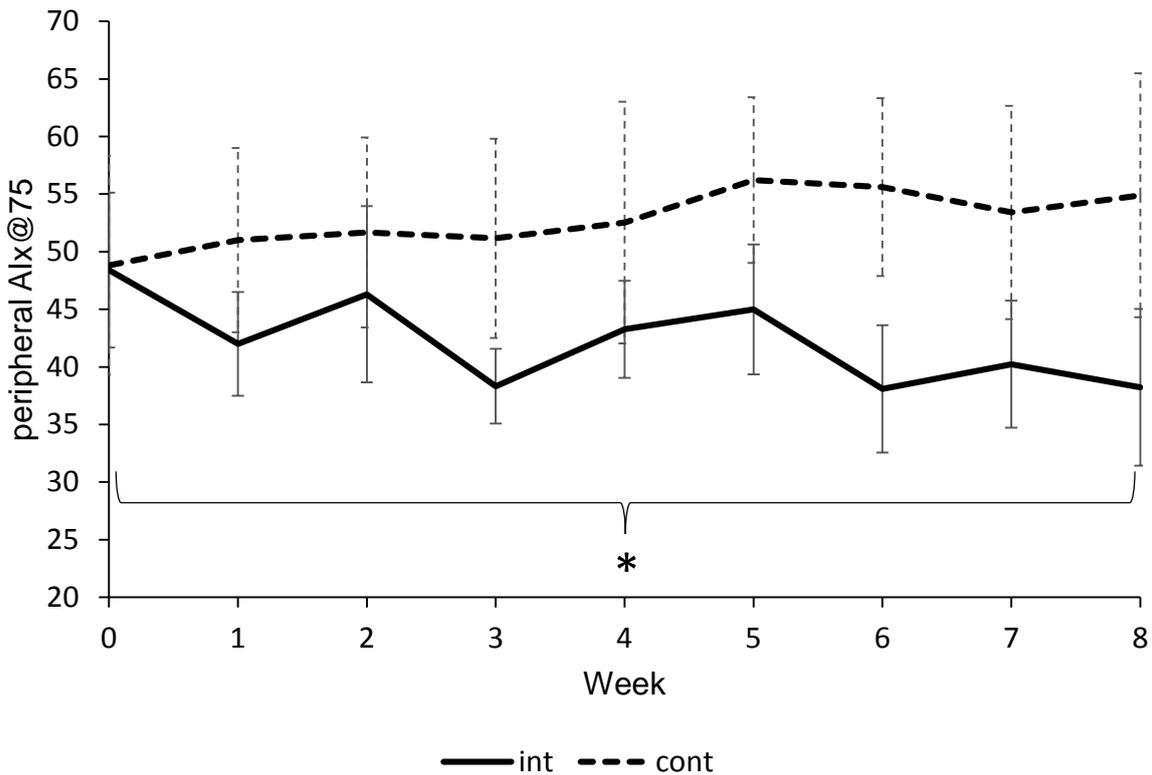


Figure 5.5: Trends in the peripheral Alx@75 across the 8 weeks in both groups

There was no significant change in the Alx@75 from PRE to POST in the control group (dashed line). In the intervention group (solid line), average weekly values can be seen to fluctuate but there was generally a gradual decline in the absolute average value over the 8 weeks such that the value of Alx@75 at POST was significantly lower than it was at PRE despite no significant between-group differences at any time point.

* significant difference between PRE and POST in intervention group: $p < 0.05$

5.3: EXPLORATION OF RELATIONSHIPS BETWEEN SELECTED PHYSIOLOGICAL VARIABLES

Correlations of note amongst all participants: *in most cases, given the highly significant bilateral correlations in absolute values and %changes, relationships will be presented using values from the RIGHT (unless otherwise specified).*

The central and peripheral values for pulse pressure were highly correlated at PRE, MID and POST ($r^2=0.56, 0.93, 0.53$ respectively) ($p<0.05$).

Age was positively correlated to baseline VO_{2peak} ($r=0.55, p=0.035$) but the former did not predict ΔVO_{2peak} . Baseline VO_{2peak} and BMI were negatively correlated ($r=-0.720, p=0.002$) and baseline BMI partly predicted ΔVO_{2peak} ($r=0.66, p=0.037$). Despite no significant relation between VO_{2peak} and either right or left cfPWV at PRE, VO_{2peak} was negatively related to both right cfPWV ($r^2=0.29, \beta=-0.535, p=0.040$) and left cfPWV ($r^2=0.38, \beta=-0.613, p=0.015$) at POST (Figure 5.6). Despite an association between right cfPWV and MAP, inter-group difference at POST as well as trends in right PWV remained significant after correcting for MAP.

The $Alx@75$ and cfPWV were not correlated at any time point. Furthermore, the correlation between the two indices decreased over the course of the 8 weeks in the intervention group ($r=0.16, 0.07, 0.05$ at PRE, MID and POST respectively) ($p>0.05$). Neither cfPWV nor the $Alx@75$ were related to cPP, pPP, ΔPP or pPP/cPP at any time point. However, at MID, peripheral SBP and right and left cfPWV were positively related ($r=0.60$ and $r=0.56$ respectively) ($p<0.05$).

Only 23.6% and 4.7% of the variance in right and left cfPWV respectively was explained by SBP, WC and MAP. The exercise intervention significantly decreased cfPWV bilaterally even after adjusting for these factors.

Heart rate was positively correlated to cWLR at both PRE ($r=0.59, p=0.019$) and POST ($r=0.67, p=0.006$). Heart rate at POST was also positively correlated to WC, cfPWV, cIMT and fIMT and negatively correlated to age and VO_{2peak} (Table 5.5b).

At PRE, MID and POST, cIMT was positively correlated to BMI ($r=0.73, 0.55, 0.55$) and WC ($r=0.55$ at all the time points) ($p<0.05$). At PRE, MID and POST, left fIMT was positively related to WC ($r=0.7, 0.57, 0.5$ respectively, $p<0.05$). No relationships were observed between carotid and femoral IMT or RI or even between IMT and RI.

Table 5.5a depicts significant relationships between outcome measures at POST. This table emphasises five important findings. Firstly, cIMT was positively related to lipid markers (TC and LDL-C), BMI and WC. Secondly, indices of wave reflection (Alx and $Alx@75$) and central haemodynamics (cPP) were positively related whereas the $Alx@75$ was negatively related to height. Thirdly, cfPWV was consistently related to BMI. Fourth, cWLR, an arterial remodelling

parameter, was related to both an atherosclerotic (fIMT) and AS marker (cfPWV) and finally, lower MAP was associated with greater flexibility.

Bilateral correlations in ultrasound-derived measures

Amongst the ultrasound-derived measures (PWV, cEDD, cIMT, fIMT, cRI, fRI), cRI was the only variable that did not show significant bilateral correlation (Table 5.6).

Significant relationships between adaptation magnitudes

Table 5.7 outlines adaptations of correlated magnitudes. The main findings are that first, $\Delta VO_{2\text{peak}}$ was positively related to $\Delta \text{AIx@75}$ ($r=0.66$, $p=0.008$) and ΔcPP ($r=0.54$, $p=0.039$) (Figure 5.7a). Second, although cfPWV and the AIx@75 were not related, ΔcfPWV and $\Delta \text{AIx@75}$ were negatively related in the intervention group ($p<0.05$) (Figure 5.7b). Third, $\Delta VO_{2\text{peak}}$ was positively related to ΔcWLR (right and left) ($p<0.05$). Fourth, ΔcfPWV demonstrated positive relations to ΔBMI , ΔWC and ΔcWLR . Finally, $\Delta \text{AIx@75}$ and ΔcPP were positively correlated to ΔcWLR .

In addition to the relationships outlined in Table 5.7, ΔHR was strongly related to ΔcfPWV , $\Delta \text{AIx@75}$, ΔcPP , ΔcIMT and ΔcWLR ($p<0.05$).

The RPM^{TM} training load was positively related to $\text{POST } VO_{2\text{peak}}$ ($r=0.71$, $p=0.022$), ΔBMI ($r=0.64$, $p=0.048$), ΔWC ($r=0.705$, $p=0.023$) and $\Delta \text{right fIMT}$ ($r=0.68$, $p=0.032$).

Table 5.5a: Significant relationships between outcome measures at POST

variable 1	variable 2	sig (p)	r
BMI	WC	0	0.96
Flexibility	MAP	0.002	-0.69
HDL	MAP	0.036	-0.66
right cfPWV	left cfPWV	0.002	0.84
right cfPWV	BMI	0.037	0.71
left cfPWV	BMI	0.022	0.67
pAIx	cPP	0.033	0.68
pAIx@75	cPP	0.028	0.69
pAIx@75	Height	0.032	0.68
right cIMT	left cIMT	0.001	0.88
right cIMT	TC	0.024	0.70
right cIMT	LDL-C	0.017	0.73
left cIMT	TC	0.011	0.76
left cIMT	LDL-C	0.02	0.72
left cIMT	BMI	0.046	0.72
left cIMT	WC	0.034	0.55
right cEDD	left cEDD	0.04	0.65
right cWLR	right cfPWV	0.038	0.539
right cWLR	right fIMT	0.006	0.669

BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; cIMT, common carotid Intima-media thickness; fIMT, common femoral intima-media thickness; cRI, common carotid resistivity index; fRI, common femoral resistivity index; cEDD, common carotid end-diastolic diameter; cWLR, common carotid wall:lumen ratio; pAIx@75, peripheral augmentation index standardised to a heart rate of 75bpm; cPP, central pulse pressure; cfPWV, carotid-femoral pulse wave velocity; HR, heart rate

Table 5.5b: Significant relationships between resting heart rate and outcome measures at POST

variable	r	p
age	-0.53	0.041
VO _{2peak}	-0.52	0.046
WC	0.53	0.041
cfPWV	0.51	0.05
cIMT	0.57	0.025
cWLR	0.67	0.006
fIMT	0.80	0

WC, waist circumference; cIMT, common carotid intima-media thickness; fIMT, common femoral intima-media thickness; cWLR, common carotid wall:lumen ratio; cfPWV, carotid-femoral pulse wave velocity

Table 5.6: Correlations between bilaterally measured ultrasound-derived indices of arterial stiffness and remodelling

INDEX	r		
	PRE	MID	POST
cfPWV	0.88**	0.90**	0.94**
cIMT	0.80**	0.57**	0.64**
fIMT	0.62	0.76**	0.80**
fRI	0.58	0.64*	0.72**
cEDD	0.54	0.72*	0.83**

Carotid RI was the only ultrasound-derived measure that did not exhibit significant bilateral correlation. *cfPWV, carotid-femoral pulse wave velocity; cIMT, carotid Intima-media thickness; fIMT, femoral intima-media thickness; cRI, carotid resistivity index; fRI, femoral resistivity index; cEDD, carotid end-diastolic diameter*

* $p < 0.005$

** $p < 0.011$

Table 5.7: Significant relationships in improvement magnitudes between outcome measures

variable 1	variable 2	F(1,13)	r	p	95%CI
VO_{2peak}	pAlx@75	9.947	0.66	0.008	0.599, 3.206
	cPP	5.245	0.54	0.039	0.012, 0.402
	right cWLR	11.965	0.69	0.004	0.169, 0.733
Right cfPWV	BMI	17.743	0.76	0.001	1.706, 5.299
	WC	12.33	0.70	0.004	0.312, 1.308
	left cfPWV	56.154	0.90	0	0.859, 1.555
	right cWLR	5.095	0.53	0.042	0.010, 0.477
pAlx@75	right cWLR	11.132	0.68	0.005	0.451, 2.106
cPP	right cWLR	11.089	0.68	0.005	0.402, 1.887
BMI	WC	31.016	0.84	0	0.129, 0.293
	right cWLR	4.281	0.52	0.047	0.001, 0.103
WC	right fIMT	4.902	0.52	0.045	0.005, 0.429

BMI, Body mass index; WC, waist circumference; cPP, central pulse pressure; fIMT, common femoral intima-media thickness; cWLR, common carotid wall:lumen ratio; pAlx@75, peripheral augmentation index standardised to a heart rate of 75bpm; cfPWV, carotid-femoral pulse wave velocity

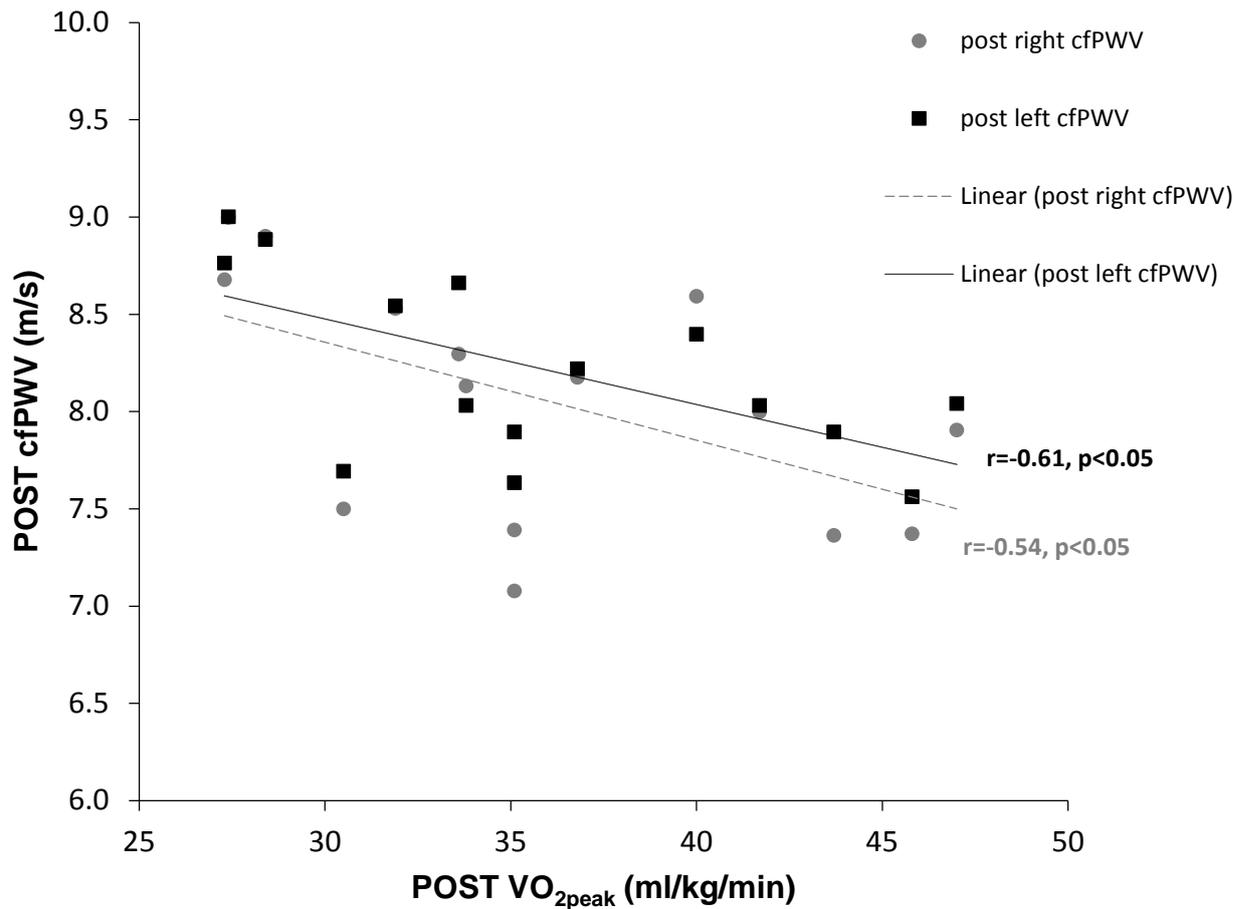


Figure 5.6: Negative correlation between VO_{2peak} and cfPWV at POST

In the intervention group, both VO_{2peak} and cfPWV improved significantly from PRE to POST (ie. average cfPWV reduced whilst VO_{2peak} increased). At the end of the intervention, cardiorespiratory fitness and arterial stiffness were negatively related. *cfPWV, carotid-femoral pulse wave velocity*

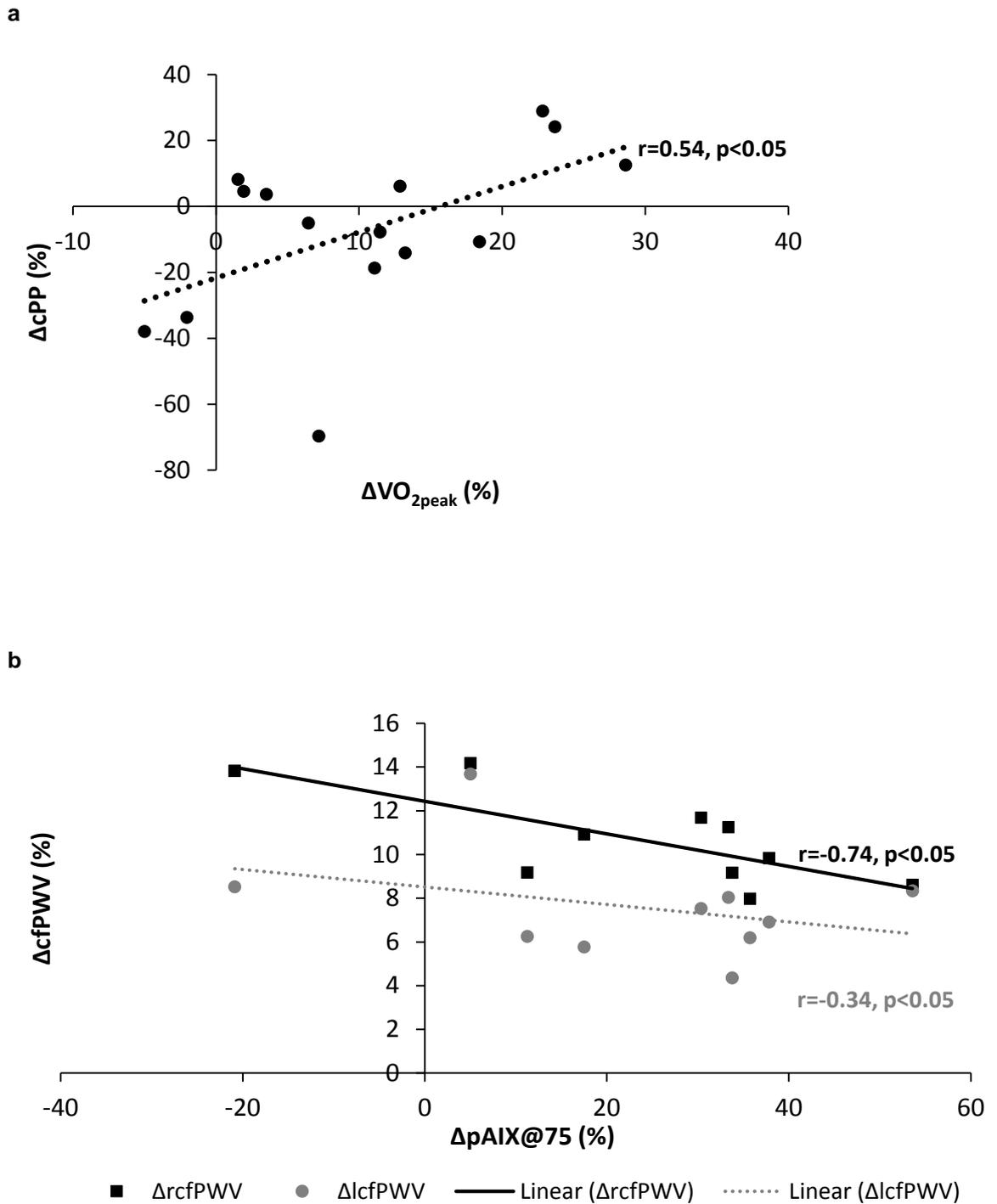


Figure 5.7: Correlations in PRE-POST adaptation magnitudes between variables

All values are percent changes from PRE to POST

a. The positive relationship between improvements in central pulse pressure and cardiorespiratory fitness. b. The negative relationship between two of the main arterial stiffness indices.

ΔcPP , change in central pulse pressure; $\Delta cfpwv$, change in carotid-femoral pulse wave velocity; $\Delta pAIX@75$, change in peripheral augmentation index, $\Delta rcfPWV$ change in right carotid-femoral pulse wave velocity; $\Delta lcfPWV$ change in left carotid-femoral pulse wave velocity

5.4: Specific focus on intervention group results

Improvement magnitudes in the main outcome measures are summarised in table 5.8.

Metabolic Syndrome Risk factors

Table 5.9 outlines the specific MS RF that each individual in the intervention group had at PRE and POST and shows that the number of RFs decreased in 70% of the individuals and remained unchanged in 30%. The risk factor profile did not worsen for any individual. Furthermore, three individuals who would have been classified as having MS at PRE would not have been classified as having it at POST (ie. the number of their risk factors decreased from ≥ 3 to <3). The number of PRE and POST MS RFs were not significantly correlated to absolute values of AS measures at PRE and POST respectively and the % decrease in risk was not related to arterial health indices.

Table 5.8: Improvements in the main anthropometric, fitness, clinical and arterial health measures in the intervention group from PRE to POST

variable	PRE to POST % IMPROVEMENTS (95% CI)
BMI*	1.2 (0.4, 1.9)
WC*	8.4 (4.5, 12.3)
VO _{2peak} *	15.1 (9.1, 20.9)
cPP	1.98 (-9.57, 13.52)
MAP	3.2 (-3.6, 10.0)
HR	8 (3.71, 12.29)
Alx*	23.3 (9.0, 37.7)
Alx@75*	23.8 (8.7, 38.8)
right cfPWV**	10.7 (8.07, 12.07)
left cfPWV**	7.6 (5.53, 9.67)
right cIMT*	11.35 (4.55, 18.15)
left cIMT*	12.99 (4.49, 21.74)
right fIMT	12.64 (1.68, 23.61)
left fIMT*	13.11 (4.49, 21.74)
right cEDD*	6.9 (2.26, 11.54)
left cEDD*	7.5 (4.43, 10.57)
right cWLR*	17.7 (10.7, 24.7)
left cWLR*	19.2 (12.2, 26.2)

BMI, Body mass index; WC, waist circumference; MAP, mean arterial pressure; cIMT, common carotid Intima-media thickness; fIMT, common femoral intima-media thickness; cEDD, common carotid end-diastolic diameter; cWLR, common carotid wall:lumen ratio; pAlx@75, peripheral augmentation index standardised to a heart rate of 75bpm; cPP, central pulse pressure; cfPWV, carotid-femoral pulse wave velocity

* significant improvement from PRE to POST: $p < 0.05$

** significant improvement from PRE to POST: $p < 0.001$

Table 5.9: Framingham Risk Score-based 10-year CV risk factor profiles of participants in the intervention group

ID	AGE	no. MS RFs			CV RISK (%)			VASCULAR AGE (years)		
		PRE	POST	Δ	PRE	POST	%Δ	PRE	POST	Δ
1	40	2 ^{bc}	2 ^{bc}	0	6.7	6.7	0	48	48	0
2	36	1 ^d	0	-1	2.8	2.3	-0.5	36	34	-2
3	32	2 ^{cd}	1 ^c	-1	1.6	1.6	0	30	30	0
4	23	3 ^{ace}	1 ^c	-2	2.8	1.9	-0.9	36	32	-4
5	38	2 ^{cd}	1 ^d	-1	2.8	1.6	-1.2	36	30	-6
6	37	2 ^{bc}	2 ^{bc}	0	1.9	1.9	0	32	32	0
7	30	3 ^{abc}	2 ^c	-2	2.8	2.3	-0.5	36	34	-2
8	24	1 ^c	1 ^c	0	1.4	1.1	-0.3	<30	<30	0
9	34	3 ^{ade}	2 ^{de}	-1	3.3	2.3	-1	38	34	-4
10	42	3 ^{cde}	0	-3	7.9	4.7	-3.2	51	42	-9
Av	33.6	2-3	1-2	1	3.4	2.64	-0.76	38.1	35.1	-2.7

^aWC > 102cm; ^bTriglycerides ≥ 1.7mmol/L; ^cHDL-C < 1.04mmol/L; ^dFasting Blood glucose ≥ 5.6mmol/L; ^eBP ≥ 130/85mmHg. This table indicates that there was an overall decrease in the number of metabolic syndrome risk factors from PRE to POST in the intervention group. 70% of the participants demonstrated an improved risk factor profile whilst 30% showed no change. Cardiovascular risk did not increase in any individuals in the intervention group over the 8 weeks. Six out of the 10 individuals also managed to reduce their vascular/heart age. Cardiovascular risk and vascular age were calculated based on published tables which consider sex, age, HDL-C, TC, pSBP, smoking status, absence/presence of diabetes and treatment for hypertension. *WC, waist circumference; HDL-C, high-density lipoprotein cholesterol, BP, blood pressure; MS, metabolic syndrome; RFs, risk factors; TC, total cholesterol; pSBP, peripheral systolic blood pressure*

CHAPTER 6: DISCUSSION

Arterial disorders account for 80% of all CVD-related deaths, necessitating the need for their early detection and therapies to optimise arterial health. Aerobic exercise (AE) laboratory-based interventions employing various exercise modalities and intensities have shown remarkable success at improving tissue biomarkers of early vascular ageing (EVA) as well as arterial geometry in healthy and clinical populations (14, 46-48, 56, 57, 59, 62, 161, 265, 333, 353, 395, 397, 398, 415, 416). This is the first study to determine the effects of a community-based mixed intensity aerobic interval indoor cycling group-fitness class on cardiorespiratory fitness and indices of arterial health related to EVA. The cohort was limited to community-dwelling, healthy, sedentary males aged 20-45 years in order to eliminate potential confounding factors associated with old age, gender, chronic illnesses and training status. The current findings support the hypothesis that community-based self-paced combined aerobic interval exercise can significantly improve arterial health.

6.1 INTERVENTION CHARACTERISTICS

The concurrent nature of the class was such that overall, it was based primarily on principles of AE, interspersed with regular bouts of both HIIT and SIT. Heart rates during exercise sessions fluctuated from 50-105% individually-determined HR_{peak} . In laboratory-based studies, exercise prescription is usually specified, individually-tailored and monitored so that individuals are required to maintain these pre-determined intensities (absolute/range) for a whole session throughout the intervention, or, progressively increase the volume/intensity in a controlled manner.

Despite the self-paced nature of the exercise, individuals worked at an average of $81 \pm 7\% HR_{peak}$. The current intervention can be classified as 'vigorous' based on previous studies (investigating AE effects on arterial health in healthy, sedentary middle-aged men) which refer to 80-90% HR_{max} (56, 59, 62) and even 75% HR_{max} (49, 342) as 'vigorous'. Amongst these studies, exercise volumes ranged from 20-50 mins per day, 3-7 days per week, with inverse correlations between intensity and volume in trials during which either volume or intensity progressively increased (56, 342). The two studies employing similar exercise prescriptions to the current one were those by Thijssen et al. (59) (80% HR_{max} , 30 mins, 3 times/wk AE) and Heydari et al. (62) (80-90% HR_{max} , 20 mins, 3 times/week HIIT). As discussed later, findings in both these studies were similar to the ones presented here. Interestingly enough, maximal HRs ($94\% \pm 7\% HR_{peak}$, range 88-104% HR_{peak}) often exceeded those of the baseline VO_{2peak} test. In the current study, this could be due to the previously sedentary individuals exerting increasingly more effort as they grew accustomed to exercising. This is further justified by the lack of a plateau effect, whereby average weekly HRs during the course of the intervention gradually increased. When beginning exercise with an activity not carried out on a daily basis (cycling vs walking/jogging/work-related activity), individuals may initially maintain caution, avoiding maximal effort, especially in self-paced activities. Upon increasing familiarity with the exercise and heightened awareness of individual physical limits, the willingness to challenge oneself is likely. In previous controlled-intensity trials,

differences between pre- and post-intervention intensities are not usually relevant and have not been presented.

It is possible that the increasing HR trend observed would not apply to clinical populations or exercising individuals unfamiliar with this type of exercise. On occasion, the training load from just one RPM™ class was greater than the exertion load over the rest of the day indicating that devoting 3% of the day to this sort of exercise, could significantly benefit arterial and overall health and CV risk. It was also interesting to note the positive correlation between RPM™ training load and Δ BMI, Δ WC and Δ fIMT. The more effort an individual exerted, the greater the benefits, especially in the lower limb atherosclerotic marker.

6.2 PRIMARY MEASURES

6.2.1 MAIN FINDINGS

6.2.1.1 CARDIORESPIRATORY FITNESS (CRF)

Future fatal CV events are better predicted by VO_{2peak} than many traditional CV risk factors and an increase in CRF by one MET can decrease non-fatal and fatal cardiac events by 17-29% and 28-51% respectively (217). The improvement in CRF by 15% (1.5METs) in the intervention group is similar to that observed by Ciolac et al. (after a 16 week aerobic interval training intervention) (333) and is greater than most MCAE (60-65% VO_{2max}) interventions in which approximately 8% increases in CRF are observed after 4 (46) or 16 (333) weeks.

In the present study, this 15% improvement in CRF reflects a potentially reduced CV risk in only two months even though neither post-intervention VO_{2peak} nor ΔVO_{2peak} was related to post-intervention FRS-based CV risk or Δ CV risk (ie. CRF increased independently of traditional CV risk factors). Taking individual trainability into consideration, greater improvements in CRF are generally observed after HIIT than MCAE interventions (456) and the absolute increase in VO_{2peak} by 5ml/kg/min in the present study is similar to previous vigorous-intensity endurance training studies (62) but less than the average increase in CRF in purely HIIT programmes (457). This reiterates the description of the current intervention as non-continuous AE with concurrent interval training and sheds light on its potentially superior effectiveness, relative to regular MCAE programmes, at improving CRF.

6.2.1.2 CAROTID-FEMORAL PULSE WAVE VELOCITY (cfPWV)

The observed 10.7% (0.93m/s) and 7.6% (0.65m/s) decreases in right and left cfPWV respectively are similar in magnitude to previous studies using moderate to high-intensity AE interventions (397). The greater improvement in right cfPWV may be due to the higher average baseline cfPWV on the right than the left side and therefore, greater possibility for adaptability and response in the former. Bilateral asymmetry in arterial stiffness and geometry have been attributed to differing anatomy-dependent blood flow and shear stress patterns (458). However, very high positive correlations between bilateral ultrasound-derived indices of arterial health were observed in the present study. A 2015 review reported that AE interventions bring about an average of -0.63m/s

(386) to -0.67m/s (379) improvements in PWV and that greater reductions are observed in peripheral than central AS measures, with interventions lasting longer than 10 weeks (379) and with exercise of at least moderate to high intensity (333). However, reductions in central PWV (0.5m/s) and peripheral PWV (0.8m/s) have even been observed after only 6 days of daily moderate AE for 2 hours each day (398). The effectiveness of the current intervention on the gold standard measure of AS can be considered to be favourable and the intensity robust enough to reduce central AS.

Taken alone, the improvements in cfPWV may independently reduce CV risk given that a decrease in cfPWV by 1m/s reduces CV events and mortality by 14% (22) and that the adjusted relative risk for all-cause mortality is 0.71 (459). Although risk prediction based on traditional CV risk factors is valuable in populations, it may be less so in individuals as the scales used are influenced by non-modifiable risk factors (460).

In agreement with a 2014 meta-analysis (386), the improvements in cfPWV in the intervention group occurred independent of resting HR or MAP as well as anthropometric changes, indicating a primary effect. In young individuals, AE does not significantly alter arterial compliance but amongst older individuals, vigorous AE can modulate age-associated decreases in arterial compliance (49). Unfortunately, this observation could not be investigated further in the present study because 'older' individuals (>50 years) were excluded and no associations between age and cfPWV were observed.

Despite the encouraging results obtained in the present study, there is evidence to suggest that such benefits of exercise on cfPWV may attenuate in training periods of longer durations (greater than 3-6 months) (461, 462). It would be interesting to determine the patterns and magnitudes of operative stiffness adaptations with longer duration (>12 weeks) aerobic interval interventions.

6.2.1.3 AUGMENTATION INDEX (Alx, Alx@75)

It has been suggested that the Alx is a sensitive index for a reduction in AS prior to BP reductions (353), which makes it a useful tool in both public healthcare and clinical settings. In this regard, Donley et al. (161) found a 21% reduction in the Alx@75 in healthy and MS adults after 8 weeks of AE, which is similar to the 24% reduction in the Alx@75 (and 23% reduction in the Alx) in the exercise group observed in the present study. These improvements are greater than most of those reported when similar populations have undergone purely MCAE (6%, 21%, 3% and 9.2% improvements in peripheral Alx) (46, 161, 353, 397) as well as HIIT (3.5% improvement in central Alx) (62). A small sample size is the most likely reason for the absence of post-intervention between group differences in the Alx@75. The findings reported above pertain to healthy individuals and it is possible that exercise-induced improvements in this index may not be observed in overweight or obese individuals, even with vigorous ($80\text{-}90\% \text{VO}_{2\text{max}}$ exercise (67). The average reduction in the Alx with MCAE is -2.63% (386). Furthermore, the means and standard deviations of the Alx@75 reported in the current study were noticeably greater than those of previous studies (62, 161, 394).

Differences in absolute values of the AIx and in Δ AIx between studies whose participants have similar baseline characteristics can only be explained by differences in assessment modes. Previous studies have used principles of applanation tonometry to derive this index, but in the present study, the BP+ device generated the peripheral AIx using an oscillometric cuff-based technique in the absence of a generalised transfer function. Whilst the BP+ device produces reliable and valid results for cBP (203, 463), BP+ AIx validation studies are lacking. Nevertheless, the present absolute values agree with published applanation tonometry-derived normative data of the peripheral AIx amongst healthy populations of different ethnicities (464, 465). One study reported that the correlation between AIx values obtained using different applanation tonometry devices is much weaker than the correlation between inter-device SBP (466). The authors of this study suggested that even slight changes in the pulse waveform could result in large differences in AIx and cBP values so results should be interpreted carefully. Therefore, the present values cannot be deemed inaccurate. The diurnal variation (the gradual decrease over the course of the day) in the AIx (467) must be considered given that weekly inter-subject assessment times could not be standardised in the present study for practical reasons. Depending on RPM™ class times (intervention group) and subject availability (control group), weekly measurements were taken anytime between 5:30am and 6:30pm. However, the issue of diurnal variation would not have affected the PRE, MID and POST values as assessment times for all individuals were the same (8:30-9:00am).

Although a linear positive relation between the AIx and age have been reported, (84), numerous studies have suggested that the AIx changes more with age in younger than older individuals and is therefore a more sensitive index of vascular age in the former population (90). Based on this notion, the large improvement in the AIx@75 observed in the present study can partly be explained by the fact that the current participants were not 'old' (>50 years).

Training prescription is an important consideration when aiming to ensure beneficial and not adverse impacts. The large improvements in the AIx@75 could be due to the high-intensity nature and effective frequency of the exercise. The finding that maximum HRs often exceeded $100\%HR_{peak}$ implies that the high-intensity peaks during the classes were robust enough to substantially improve wave reflection dynamics because greater reductions in the AIx are generally observed with lower volume but high-intensity AE (386). This is in line with greater acute reductions in the AIx@75 within the first 12 hours after a HIIT session compared to an MCAE session (354). The dose of three 45 minute classes per week was potentially optimal as exercise volume is associated with worsening of the AIx (386) so it would be advisable to avoid daily vigorous exercise.

6.2.1.4 CENTRAL HAEMODYNAMICS

Although the 2% reduction in cPP in the intervention group failed to reach statistical significance, the post-intervention between-group difference illustrates that important information regarding left ventricular load can be missed with peripheral BP measurements. The unaltered peripheral PP shows that peripheral BP cannot accurately predict central BP, a better risk predictor. The reduction in cPP in the absence of significant changes in cBP can also be considered relevant in

terms of risk reduction as PP independently predicts risk better than either SBP or DBP alone (79). The positive correlation between post-intervention Alx@75 and cPP illustrates how changes in central haemodynamics can be attributed to modified wave reflections (468). The Alx can act as an intermediate between AS and PP because an increase in AS raises PP via an increase in the Alx. This reflects the independent yet interconnected nature of the three main AS indices as discussed in the following sections.

6.2.2 RELATIONSHIPS BETWEEN ARTERIAL STIFFNESS INDICES

It is important that cfPWV and the Alx are neither used interchangeably nor considered to reflect identical changes in arterial health (469). They are distinct entities which, when considered in conjunction, make up the arterial factors which influence aortic systolic pressure.

The Alx depends on intrinsic AS in addition to the magnitude of reflected waves and the distance to wave reflection sites. An increase in PWV disrupts only the timing of reflected waves, potentially explaining the lack of correlation between the Alx and cfPWV in the present study, even though the two indices have previously been shown to be highly correlated (470). The discrepancy between the current findings and those of Yasmin & Brown (which indicated a positive correlation between the Alx and cfPWV) could be because, in their study, individuals had familial hypertension and applanation tonometry was used to assess AS. In agreement with the current findings, it has been reported that PWV and the Alx are not related in multivariate analysis and that the two indices may be more correlated in stiffer aortas because, in elastic aortas, the Alx is influenced more by wave reflections than AS (471). Indeed, in the present study, the relation between Alx@75 and bilateral cfPWV decreased over the course of the 8 weeks in the intervention group.

The negative relationship between Δ cfPWV and Δ Alx@75 could be due to the small sample size or, alternatively, it can reflect individual responsiveness and what aspects of their vascular health they are more susceptible to alter in response to a training stimulus, which once again relates to the notion of genetically-determined individual 'trainability'. This can lead one to question the plausibility that improvements in one index can occur at the expense of improvements in another. There is evidence to suggest that wave reflection sites move distally with age because as elastic arteries stiffen during ageing, the AS gradient reduces and points of impedance mismatch, which are wave reflection sites, therefore move toward the periphery (171). Based on this, it could be hypothesised that over the course of the intervention, the opposite of this ageing-associated phenomenon occurred. As cfPWV (and presumably elastic artery stiffness) decreased, the AS gradient became steeper such that impedance sites moved closer to the aorta which would counter the direct effects of PWV on wave transit time to and from the aorta, a determinant of the Alx. Further studies using larger samples and varied AS assessment methods are warranted to help determine whether exercise is positively impacting wave reflection timing and amplitude or intrinsic wall stiffness more.

The non-significant reduction and post-intervention between-group differences in cPP could be attributed directly to repetitive carotid and aortic distension during the exercise bouts which would

decrease SNA. This would be attributed to altered baroreflex sensitivity (BRS) because decreased AS increases afferent firing for a given pressure change (42). Therefore, in consonance with the parallel decreases in the other two AS indices, cPP could also have improved due to arterial destiffening-induced reductions in SNA (42).

6.2.3 RELATIONSHIPS BETWEEN CARDIORESPIRATORY FITNESS, ARTERIAL STIFFNESS AND WAVE REFLECTIONS

Over two decades ago, Vaitkevicius et al. reported that AS (indexed by the AIx and cfPWV) was negatively associated with CRF (indexed by VO_{2max}) and that interventions which increase CRF can attenuate the arterial stiffening that occurs during normal ageing (52). The negative post-intervention age-independent correlations between VO_{2peak} and cfPWV and between the magnitudes of changes of ΔVO_{2peak} with $\Delta AIx@75$ and ΔcPP , reflect cross-sectional data demonstrating the ability of AE to reverse age-associated arterial stiffening (51, 52). Interestingly, sport or fitness-related but not leisure-time-associated physical activity is inversely related to AS because the latter does not tend to increase aerobic capacity (472) which indicates that the increase in CRF is paramount in explaining the beneficial impact of AE on AS, a hypothesis supported by the positive relationship between resistance training and AS (47, 65-67, 335).

Aortic stiffness, myocardial performance and functional capacity are believed to be interrelated in a cyclic fashion whereby an increase in aortic stiffness increases cPP which reduces coronary perfusion, myocardial work and performance and thence, exercise capacity (351). As a result, cardiac risk increases whilst fitness decreases which would exaggerate the initial arterial stiffening, thereby initiating a vicious cycle (473). Therefore, it is not surprising that simultaneous decreases in cfPWV, cPP and increased CRF were observed in the present study, with the post-intervention negative correlation between cfPWV and VO_{2peak} . Potential mechanisms (obtained on animal models) explaining the negative correlation between CRF and AS observed in the present study pertain to greater aortic elastin content, no changes in collagen content and a reduced elastin calcium content with greater fitness (this is yet to be confirmed in humans) (392).

6.2.4 PROPOSED MECHANISMS UNDERLYING THE RELATIONSHIP BETWEEN AEROBIC EXERCISE AND ARTERIAL STIFFNESS

Potential mechanisms explaining the decrease in AS with AE and HIIT relate not only to the improved elastin:collagen equilibrium (474) and enhanced NO production (coupled with reduced vasoconstrictor production) (475) but also to the anti-oxidative effects (through superoxide dismutase up-regulation and NADPH down-regulation (476) and anti-inflammatory effects (via increases in anti-inflammatory cytokines IL-4 & 10 and decreases in pro-inflammatory cytokines IL-1 & 6 and TNF- α) (477) of AE. Greater shear-stress-mediated release of NO and a subsequent greater reduction in peripheral vascular resistance with harder exercise has previously been one of the hypotheses used to explain the positive relation between AE intensity and AIx improvements (385). Since MAP remained unchanged, the effects of the present intervention on

the $Alx@75$ could be explained by alterations in structural arterial wall properties instead. However, altered extracellular matrix composition is unlikely because changes in the composition of wall scaffolding proteins occur over many years. The most feasible theories would, therefore, pertain to either decreased IMT or acute increases in PP causing mechanical distension, thereby stretching collagen fibres and altering collagen cross-links (resulting in less uncoiled collagen) (478). Although the acute effects of the exercise on arterial health indices and their determinants (eg. MAP, HR, BRS and post-exercise hypotension) were not assessed in the present study, they may explain longer-term adaptations due to the cumulative effects of repetitive sessions.

The incorporation of various music styles in the RPM™ classes could have potentially directly contributed toward the improved cfPWV and $Alx@75$ (479). Mental stress is associated with CVD (480) and can cause arterial health deterioration (481, 482) whilst positive psychological stimuli can decrease both PWV and Alx (482). Music is being increasingly used as a therapeutic tool in individuals with CVD (483) and a recent study on healthy individuals showed that music can improve arterial function (stiffness and wave reflections) (479). The benefits lasted for as long as the music was being listened to and effects persisted with some types of music. As changes in the Alx and PWV were not correlated in that study, the authors suggest that decreased wave reflections with music are primarily due to reduced wave amplitudes as opposed to reduced transit times. Possible mechanisms underlying this relate to increased beta-endorphin-mediated NO release (484). Since endothelial function influences AS, improvements in the former can favourably alter the latter. The second mechanism relates to the increased aortic and systemic stiffness brought about by noradrenaline and the fact that music therapy in patients with CVD reduces plasma adrenaline and noradrenaline levels (485). Music-induced reductions in SNA and subsequent peripheral artery dilation may also attenuate wave reflection amplitudes (479). Finally, conclusive findings regarding the relationship between AS and BP would determine whether or not the improvements in AS with music can be explained by the music-induced BP reductions (483, 485).

6.3 SECONDARY OUTCOME MEASURES: ARTERIAL STRUCTURAL REMODELLING PARAMETERS

In agreement with studies suggesting extensive outward arterial remodelling with exercise, significant bilateral decreases in cIMT (average 12.2%), cWLR (average 18.5%) and left fIMT (13.1%) as well as increases in bilateral resting cEDD (average 7.2%) were observed in the intervention group only. It has been reported that arterial remodelling is preceded by functional changes (407) but because flow-mediated dilation was not measured, no comment can be made about whether functional or structural changes occurred first. However, exploration of the time course of adaptations revealed no significant difference in the magnitude of structural adaptations within the first and second four weeks. This was in line with a study by Thijssen et al., who assessed carotid and superficial femoral WLR and IMT every two weeks over an 8 week cycling intervention, and showed that improvements in arterial structure occur in a dose-dependent manner (59). More regular (eg. weekly) ultrasonographic assessments might have provided a better understanding of trends in adaptations.

Decreases in fIMT as observed in the current study, coupled with increases in femoral diameter (presently unassessed), are commonly reported following moderate-to-vigorous lower limb exercise (56) and could be justified by reports that firstly, peripheral arteries, such as the femoral and brachial arteries, are relatively more responsive to training stimuli (than central arteries), showing greater adaptability (55), and secondly, such responses appear to be localised, in this case, to the active femoral bed of the lower limb (56). The interesting observation, however, was the systemic remodelling with lower-limb exercise.

6.3.1 POSSIBLE REASONS BEHIND THE SYSTEMIC ARTERIAL STRUCTURAL ADAPTATIONS OBSERVED

If deconditioning results in detrimental arterial adaptations due to muscle atrophy and decreased metabolic demand, it would be rational to believe that beneficial arterial remodelling induced by exercise training must be due to muscular adaptations and increased metabolic demands. Systemic arterial remodelling was observed in the present study despite the range of participant ethnicities, a factor which determines carotid diameter and stiffness (486). This finding was in line with reports that runners and cyclists have greater arterial diameters globally as well as lower systemic arterial wall thickness and WLRs than recreationally active individuals (341). Furthermore, elite squash players have lower brachial, femoral and carotid IMT values than less active individuals and no difference in IMT between the preferred and non-preferred limb despite the larger arterial diameter in the former (340). In agreement with these cross-sectional studies, a 2015 meta-analysis reported that AE interventions involving vigorous exercise do improve cIMT (379). Ranadive et al. observed decreased carotid pulse pressure and cIMT after 8 weeks of moderate to high-intensity AE amongst healthy, sedentary young African-American and Caucasian adults (58). Thijssen et al. reported decreased IMT and WLR in both the carotid and superficial femoral arteries of healthy young males after 8 weeks of high-intensity cycling (59). After 12 and 24 weeks of lower limb AE, decreases in brachial artery (upper limb) wall thickness and WLR were observed amongst young and old sedentary adults (57). Carotid diameter increased by 13% after 8 weeks of RE (417) whilst brachial diameter increased by 5% after 12 weeks of MCAE (265). The improvement of 7.2% in the current study therefore falls between that conferred by MCAE and RE. These reports of systemic arterial remodelling, as in the current study, are supported by the fact that age-associated increases in the carotid and peripheral (brachial, femoral, superficial femoral, popliteal) artery IMT occur to a similar extent and are highly correlated in healthy individuals (487). However, findings regarding the effects of exercise training on carotid remodelling are equivocal (265) and systemic arterial structural modifications have not commonly been observed during AE interventions lasting less than 3 months. There was no change in cIMT after 6 weeks of low-volume SIT and high-volume AE (61) or after 3 months of AE (49).

Although the acute vasodilatory effects of the last exercise bout can last up to one hour post-exercise (488) and thereby explain systemic changes (489), this could not have been the reason

for the present findings given that assessments were carried out at least 24 hours after the last exercise session. There is no definitive conclusion as to whether shear stress is (340, 490) or is not (491) a key stimulus for localised conduit artery remodelling, (340, 490) but since this force was not measured in the present study, no comment can be made regarding these postulations.

The traditional school of thought was that during exercise, blood flow does not increase in inactive arterial beds and may, in fact, decrease due to redistribution to active beds (43). However, there is now substantial evidence showing that arterial function improves systemically with exercise training (43, 326). Possible reasons for the carotid remodelling observed relate to functional changes (systemic haemodynamic stimuli, SNA, vasoconstrictor tone, the inflammatory and anti-oxidative effects of exercise).

Briefly, transmural BP changes during exercise increase circumferential wall strain which is associated with anti-atherogenic remodelling (59). Generalised changes in artery health can be due to the systemic effect that lower limb training has on blood flow and shear stress patterns in inactive arterial beds (318). Exercise-associated increases in pulse pressure and haemodynamic modifications in peripheral arteries also trigger vascular structural (arterial wall) and functional (vasomotor tone) adaptations, even in beds far away from the site of the working muscles (81). However, unlike with resistance arteries, reports of AE-induced changes in diameters of conduit arteries occurring in the trained limbs only (56, 419) suggest that endothelium-dependent arterial remodelling in these arteries is brought about by localised changes in shear stress as opposed to global changes in MAP (318, 492).

Although chronic BP elevations (associated with hypertension) can cause conduit and peripheral arterial wall thickening (487), episodic increases in BP induced by repetitive, acute exercise bouts, likely exert different effects which would explain reports that RE, which generally induces a greater pressor response than AE, results in relatively greater reductions in wall thickness than the former (265). Participants in the present study, who continued to show arterial adaptations throughout the 8 week intervention, likely have normal endothelial function, based on the contention that arteriogenesis is self-limiting and depends on normal NO production (493).

An interesting factor which could explain altered vascular adaptation in studies such as the present one is that the more perceived control over training (intensity, duration, time, type) that one has, the lower the exercise-induced increase in SNA (RAAS activation, catecholamine release, HR and BP) (494). The self-paced nature of the current exercise (excluding instructor-driven motivation), may affect arterial adaptations differently to laboratory settings where exercise prescription is controlled externally.

The proposed reason for the inability of short-term interventions to reduce cIMT is that exercise cannot affect the age-related increase in carotid distending pressure which maintains wall stress (342). However, carotid pressure has been used as a surrogate for aortic pressure so although the former was not directly assessed in this study, it is likely that it too decreased during the intervention as cBP decreased. Carotid diameter increases during and after an acute bout of strenuous exercise whilst the carotid pressure:diameter ratio decreases (430). Carotid pressure

is an independent determinant of cIMT (495) so a decrease in the former would improve the latter. Literature states that carotid sinus baroreceptors are more sensitive to dynamic than static pressure, that BRS is negatively related to cIMT and that high-intensity AE is more effective at improving vagal tone than low-intensity AE via resetting of the carotid baroreflex (62, 496). Given this information, it is very likely that over the course of the 8 weeks, the fluctuating, high-intensity nature of the class reset the carotid baroreflex and decreased carotid pressure (and cIMT), phenomena which could be attributed to persistent and accumulated effects of repetitive exercise sessions. However, a previously unexplored concept is that the uniqueness of spin classes lies in the intermittent standing and sitting with oscillating intensity. Thus, despite cycling being primarily a lower limb exercise, the continuous postural changes means that there is engagement of upper limb, abdominal, back and neck musculature. Contraction of the neck muscles may have directly increased cEDD (due to local metabolite production) and reduced cIMT and cWLR via the same mechanisms by which contractions of peripheral skeletal muscles cause remodelling in muscular arteries (discussed below). Furthermore, changes in cIMT may reflect adaptations to changes in pressure as opposed to atherosclerotic ones. It is possible that the increased cEDD lowered distending pressure, resulting in the positive IMT adaptations. Repetitive post-exercise hypotension may have a role to play in longer-term adaptations so the measurement of BP right after the exercise session may have helped determine this. The increased cEDD would explain the reduced cRI, which is affected by both resistance and compliance.

Alterations in local autonomic function, (extensively reviewed by Michelini et al. (497)) directly induced by contraction of the neck muscles surrounding the carotid bifurcation (thyrohyoid, superior belly of omohyoid, sternohyoid, sternothyroid, sternocleidomastoid) in such indoor cycling programmes definitely warrant exploration and may explain the extensive carotid remodelling. Improved carotid compliance and cardiovagal BRS with rowing (327) could also potentially be explained by contraction of secondary muscles which are supposedly 'inactive' during rowing.

All the presented responses most likely reflect reversible adaptations as opposed to atherosclerotic changes which highlights the importance of regular and consistent exercise to avoid inward arterial modelling which occurs quickly with physical inactivity (37). Only 4 weeks of detraining decreases superficial femoral and carotid artery resting diameters in healthy young adults (417). Due to time constraints, there was no follow-up once the intervention period was over so it is unknown whether the intervention-induced reductions in cIMT persisted or if they were reversible.

6.3.2 POTENTIAL MECHANISMS UNDERLYING THE OBSERVED ARTERIAL REMODELLING

It should be mentioned that despite the widespread use of resting cEDD as an index of arterial remodelling, it is regulated by SNA as well as paracrine and circulating hormones. Since exercise influences both these opposing vasodilator and vasoconstrictor pathways as well as arterial

diameter independently, cEDD may not be an ideal index of arterial structure and remodelling (419). As for arterial wall thickness, it is not surprising that cIMT and fIMT were not related. The two arteries are exposed to different hydrostatic pressures and respond uniquely to atherogenic and anti-atherogenic influences (498) deeming it best to measure IMT at both these sites to best determine the subclinical atherosclerotic status, especially in low-risk populations (498). Unfortunately, current high-resolution ultrasound technology does not allow differentiation between intimal and medial layers so further investigation into mechanisms which potentially explain adaptations (eg. intimal atherosclerosis, medial VSMC hypertrophy/hyperplasia, medial collagen content, endothelial alterations) cannot be carried out. Nevertheless, the proposed processes underlying the arterial remodelling would relate to acute increases in muscle blood flow and velocity during the exercise sessions (499), brought about by muscle contractions. Over time, this repetitive acute stretching of the conduit arteries and changes in shear patterns (reduced pro-atherogenic retrograde flow and increased anterograde as well as oscillatory flow) upregulate eNOS gene expression and enhance endothelium-dependent release of NO (56). An increase in arterial diameter serves to improve blood flow efficiency and to attenuate increases in wall stress and transmural pressure which occur due to the repetitive, intermittent exercise bouts (500). By virtue of the present results, the intensity of the current intervention would appear to be sufficient to reach the threshold required for increased oscillations in shear stress and consequent NO-dependent arterial remodelling (318). However, at high-intensity exercise, endothelial function may not be enhanced due to the contrasting anti-inflammatory and anti-oxidative effects of exercise (326). Nevertheless, arterial remodelling ultimately balances vasoconstrictor and vasodilator pathways and homeostatically-regulated shear patterns in a way that atherosclerotic risk is reduced (318). In the present study, shear patterns and endothelial function were not investigated and therefore, the mechanisms explained above are hypotheses based on existing literature. In light of this autonomic nervous system (ANS) involvement, it would be interesting for future long-term studies employing aerobic interval interventions to investigate whether training-induced alterations in the ANS affect cognitive function (which has previously been linked to measures of AS) (501), neural plasticity or neuronal circuitry (497).

6.3.2.1 RELATIONSHIPS BETWEEN AND AMONGST PRIMARY AND SECONDARY OUTCOME MEASURES

In contrast to cross-sectional studies, (343-345) there was no inverse relationship between CRF and IMT in the present study. This may be because of the smaller sample size in the current study, the fact that two of these three studies were carried out on an older population or because all the above studies compared sedentary individuals to those who had engaged in chronic regular physical activity such that VO_{2max} values differed considerably (compared to the relatively smaller range in the present study).

In previous research, peripheral artery diameter has explained up to 70% of the training-induced improvement in ΔVO_{2max} which illustrates the relationship between exercise-induced arterial remodelling and adaptations in central haemodynamics (502). However, no direct relationship

between arterial remodelling parameters and CRF was observed in the present study, in agreement with previous reports (502).

Disparity amongst inter-study findings makes it difficult to conclusively ascertain whether changes in arterial stiffness and remodelling are related (379). In the present study, Δ cfPWV, Δ Alx@75 and Δ cPP were positively correlated to Δ cWLR but not to Δ IMT, indicating an association between AS and arteriogenesis and not atherosclerosis. This is consistent with reports that PWV is related to carotid plaques but not to cIMT and that PWV and IMT reflect different aspects of arterial damage, especially in these younger adults (503).

In addition to being the result of reduced cfPWV, the significant improvements in the Alx@75 in the intervention group between PRE and POST could also be brought about by more efficient ventricular-vascular coupling and decreased left ventricular load due to the adaptive arterial remodelling. Munir et al. (181) reported a significant decrease in the peripheral Alx immediately after cycling exercise and this was associated with increased femoral diameter and blood flow. Despite not being measured in the current study, this mechanism could be an alternative explanation for the decreased Alx@75 and could explain why the large effect size for the significant reduction in the Alx@75 from PRE to POST in the intervention group, despite the negative correlation between Δ cfPWV and Δ Alx@75 (for reasons described in section 6.2.2).

It is difficult to establish causal relationships but the correlated findings between indices of the different EVA characteristics in the current study indicate global improvements in arterial health with this exercise. The change in cfPWV is said to be related to the number of CV risk factors in individuals with normal cIMT suggesting that Δ cfPWV is more sensitive than Δ cIMT (379) and changes in cfPWV are more easily observed, as in the current study.

6.3.2.2 COULD RPM™ HAVE A RESISTANCE EXERCISE COMPONENT?

Individuals are often required to pedal against high loads during the HIIT efforts in RPM™. It can therefore be argued that RPM™, despite being classified as AE, also incorporates a degree of RE. Unfortunately, no lower limb measures of strength were carried out in the present study. However, a previous study showed that 8 weeks of RE increases the resting cEDD of healthy young adults by 13% (417) and in the current study, cEDD increased by 6.9% (right) and 7.5% (left). These values were greater than those observed in previous longitudinal AE studies (some of which have reported no significant increase in cEDD) (57, 59), justifying the possibility of an RE component in RPM™.

In a study by Cook et al., better carotid (central) artery compliance in habitual rowers than sedentary controls was associated with greater cardiovascular BRS (327). The authors concluded that the combined strength and endurance training nature of rowing meant that the AE component negates the detrimental effects of the RE component on arterial compliance. In addition, post-exercise AS is greater after upper-body than lower-body RE (372). Based on these two findings,

it would be interesting to determine whether correlations exist between levels of C-reactive protein (indicating the degree of RE), (504) and Δ cfPWV after both acute and long-term RPM™.

6.4 TERTIARY OUTCOME MEASURES

In the intervention group, BMI and WC reduced over the two months as expected and this could be attributed to the exercise as participants were required to maintain normal dietary habits during the entire study period. In agreement with previous findings, Δ BMI, Δ WC and Δ cfPWV were all positively related (146). Waist circumference plays a role in determining aortic PWV, as confirmed by the current findings of a positive correlation between WC and cfPWV. However, the disadvantage of assessing cfPWV using Doppler ultrasonography is that the crude surface distance from the carotid to the femoral point is measured (139) and therefore depends on body habitus. Although the role of obesity on PWV is overestimated in such cases, cfPWV is still directly related to visceral adiposity (505). Improvements in cfPWV were significant even after adjusting for WC which, together with pSBP and MAP explained less than 24% of the variance in cfPWV. Central obesity modifies arterial wall properties in a way that AS increases as a direct result. The 8cm reduction in WC has significant clinical implications since the relative risk of CV events rises by 2% with a 1cm increase in WC (506) and the risk of mortality rises by 16% with every 10cm increase in WC, even if the BMI is within the normal range (507). Interval training has previously been shown to be more effective than low-intensity MCAE at reducing total as well as abdominal fat (62). The minimal (1kg) decrease in body mass in the intervention group could be explained by a change in body composition and an increase in lean muscle mass over the 8 weeks (in light of the drop in WC). Reports of an inverse association, independent of CRF, between AS (central and peripheral PWV) and muscle strength in young men (338) would support the idea of an increase in lower limb muscle strength in the present study. Likewise, although weight loss and lower cfPWV are associated independently of changes in traditional risk factors, (508), exercise training improves arterial health without causing as much weight loss as dietary manipulation in obese men (509).

Chronic exercise training results in 3-4 and 1-2mmHg reductions in pSBP and pDBP respectively (478). This is due to the vasodilation in working skeletal muscles during acute exercise bouts being propagated to larger vessels (510). In the intervention group only, peripheral SBP and DBP decreased by 2 and 1 mmHg respectively in only two months and despite not reaching statistical significance, implies that the maintenance of this type of training likely has long-term practical benefits. Additionally, the decrease in HR by 8% (6bpm) in only eight weeks demonstrates the efficacy of this exercise prescription at improving aspects of autonomic function (increasing vagal tone whilst reducing the chronic influence of SNA) and left ventricular function (511). However, this contention is not supported by any direct evidence in the present study. Nevertheless, it was interesting to note that post-intervention HR was positively correlated to AS and negatively correlated to CRF. The decreased HR may have resulted in arterial de-stiffening via decreased

sympathetic tone and/or less elastin breakdown in arterial walls (30). This justifies the notion of systemic effects of the intervention programme, where integrated benefits occurred.

Hypercholesterolaemia has been linked to increased cPP and systemic AS (Aix) (130). In particular, LDL-C but not HDL-C is related to AS (wave reflections and central haemodynamics). As hypercholesterolaemia is related to endothelial dysfunction, it can be reversed, as demonstrated in the present study. Although LDL-C was not related to AS in this study, reductions in cIMT and both LDL-C and TC were positively correlated, independent of BP. This has been previously observed (347) and it makes sense given that LDL-C accumulation in arterial walls underlies atherosclerosis using cIMT as the gold standard index.

Increased trunk flexibility has recently been shown to be independently correlated to AS and the contribution of the former to the latter compares to the prediction of AS by CRF (206). Despite not being correlated to AS indices, trunk flexibility did improve significantly in the intervention group only. Although the mechanisms underlying this association have not been fully elucidated, both structural and functional factors may underlie the prediction of AS by trunk flexibility after adjusting for BP (207). It is possible that the increased flexibility and cfPWV would have been due to better equilibrium between collagen and elastin in both muscles and arteries because vessels can deform in a similar way that muscles do during stretching (42). Conversely, neural factors regulating SNA may determine both AS and flexibility (512), as evidenced by the negative relationship between MAP and flexibility. Cortez-Cooper et al. attributed the increased carotid compliance after stretching to a decrease in carotid pulse pressure (399). The acute effects of stretching on compliance (via altered pulse pressure) can explain the relationship between flexibility, cPP, and cfPWV in the long-term.

Based on the FRS, the calculated CV risk reduction of just under 1% in the intervention group only, coupled with a reduced vascular age of an average of nearly 3 years, provides evidence of the effectiveness of this community-based exercise intervention at improving overall health whilst maintaining regular dietary habits and physical activity levels outside the planned exercise sessions. Including cfPWV to this risk prediction in multivariate analysis would have improved stratification accuracy (12) but this was outside the scope of the present study. In the scientific community, there is no accepted consensus about associations between cfPWV and CV RFs. However, individual MS risk factors other than BP do not directly and independently affect cfPWV. Instead, a clustering of these risk factors acts in synergy to increase cfPWV (513). Metabolic syndrome has previously predicted PWV and IMT in adults and has been proposed as a surrogate marker for high CV risk (514) so the decrease in the number of MS RFs in most of the participants in the intervention group is encouraging. However, contrary to previous findings of a positive correlation between cfPWV and the number of atherosclerotic risk factors (513), the fact that the number of or change in risk factors was not related to cfPWV or cIMT in the present study may be due to the emerging hypothesis described in section 3.10, that exercise may confer protection against these existing factors as opposed to directly eliminating them. It is also likely that MS risk factor clustering affected localised PWV in other, especially muscular, arteries which are more sensitive to their effects (515).

CHAPTER 7: CONCLUSIONS, IMPLICATIONS AND FUTURE DIRECTIONS

7.1 PERSPECTIVES AND CONTRIBUTION TO KNOWLEDGE: WHAT THIS STUDY ADDS

- **Self-regulated and self-paced, group-fitness moderate to high-intensity aerobic interval (HIIT and SIT) indoor cycling classes carried out in existing community settings are similarly (but not as equally) efficient as more controlled laboratory-based interventions at improving arterial health, cardiorespiratory fitness, and basic CVD risk profiles**
- Previous laboratory-based findings have practical application to everyday life, that is, to pre-existing community settings where exercise is undertaken in typically unmonitored, less-controlled environments
- The mode and intensity of exercise are unique to this training as it is one of the only structured aerobic interval exercise programmes which incorporates different types of interval training
- The self-paced nature (excluding instructor-driven motivation) elicited significant improvements in arterial and overall health indicating that individuals, including previously sedentary ones, are not prone to 'slacking' if the exercise environment is not rigorously controlled and monitored
- As sedentary individuals begin a new self-paced exercise programme, their intensity gradually increases over a 2 month period despite session-to-session and weekly fluctuations in intensity (gauged by average %HR_{peak})
- Group-fitness classes confer similar adaptations to actual sports training (as opposed to leisure-time activity)
- This population of healthy young to middle-aged adults is highly responsive to the current exercise prescription in terms of arterial health and subsequent CV risk
- There is a strong age-independent negative relationship between cardiorespiratory fitness and carotid-femoral pulse wave velocity
- Changes in arterial stiffness and structural modification/remodelling indices need not be related in terms of magnitude or time course of adaptation despite similar underlying mechanisms
- Lower limb training is capable of conferring systemic arterial health benefits, possibly due to contractions of secondary muscles
- It could be hypothesised that improvements in cfPWV and the Alx@75 are negatively correlated because as central arteries become more compliant, the AS gradient becomes steeper and sites of impedance mismatch occur closer to the aorta, thereby countering the effect of increased pulse wave transit time brought about by reduced cfPWV

7.2 PRACTICAL IMPLICATIONS

Arterial stiffness is a modifiable risk factor and this population is capable of demonstrating substantial CV adaptations with exercise. Perhaps the most salient finding is that arterial health can be improved systemically with self-paced mixed-intensity aerobic training with HIIT and SIT components. The study supports the idea of the 'trainability' of this particular population of community-dwelling, healthy, sedentary young and middle-aged male adults. As described in Chapter 3 and shown in Chapter 5, lifestyle interventions related to diet and exercise can improve both arterial health and CRF which, at 36 years of age, are associated both with each other and with CVD. Fortunately, despite the foundations of CV risk being present from adolescence (516), the gradual onset of CVD means that interventions beginning in early to mid-adulthood can potentially prevent its occurrence by reducing risk and can even reverse existing damage.

There are encouraging overall compliance rates with community-based group-fitness classes which effectively improve CRF, arterial health, and general health. One of the main objectives of this research was to determine the effects of an intervention which was not laboratory-based. In these controlled environments, exercise timing and intensities are specifically-tailored and regulated to maintain intensities within a certain range. However, in the current intervention programme, participants regulated their own exercise times, attendance and intensity so it was possible that compliance and/or effort would not have been sufficient enough to accurately gauge the haemodynamic effects of the exercise. This in itself would have deemed group-based fitness classes in the community setting impractical for this sedentary cohort. Given that HRs often exceeded HR_{peak} values, it is evident that sedentary individuals were willing to take responsibility for their own participation and push themselves during the classes as they would in a stricter environment. The current findings of improved arterial health can be used to further potentiate the growth of the fitness and group-fitness industries because in previous research carried out employing group-fitness classes, outcome measures have related to traditional risk factors and no study has considered the benefits on arterial health, an independent CV risk predictor, accorded by these programmes.

Self-paced AE which includes different interval training modalities is particularly suitable for this cohort and should be encouraged within communities. The unique characteristics and structure of this exercise programme heighten its effectiveness, given that the magnitudes of benefits observed were often greater than previously reported with purely MCAE interventions. The lower intensity AE between the HIIT peaks allowed recovery, an important aspect when starting an exercise programme after being physically inactive for over half a year. These individuals would possibly worry about participating in purely HIIT programmes, which might also be unsafe given their CRF. The current intervention eases them into the transition whilst challenging them at the same time. In practical settings, progressive volume and intensity HIIT is carried out and in these types of indoor cycling classes, individuals can continue to push themselves as they get fitter and more used to the exercise. The exercise volume is not demanding for those with time constraints as it requires less than 2.5 hours per week. Cycling

has the added benefit in that it can be carried out even with a wide range of injuries (ie. it is 'body-friendly') due to its non-weight-bearing, low-impact nature.

Education about both arterial health, as well as this type of concurrent aerobic interval exercise, is warranted. It should be made known to individuals (including clinicians) that improving arterial function is just as important as improving more obvious and commonly assessed aspects of cardiovascular health. Furthermore, there needs to be increased awareness of training which combines as many exercise modalities as possible, instead of focusing solely on AE, RE, HIIT or SIT.

Continuous improvements, albeit attenuated ones, in arterial and overall health, are observed with training. As meta-analyses have reported that larger improvements are observed with interventions of longer durations (379, 386), it is important to maintain this activity. Merely two months of this physical activity was enough to show significant improvements in arterial health and CRF. This is particularly encouraging for individuals who may be at high risk for developing CVD.

Even a marginal improvement is a step forward. According to literature stating the extent of improvements in arterial health needed to significantly reduce risk, the changes seen within 8 weeks were sufficient enough to conclude that CV risk was reduced. **Arterial de-stiffening and improvements in the metabolic syndrome risk profile can occur simultaneously.** Most pharmacological therapies target specific, single aspects of CV health whilst AE can confer the widespread, integrated effects.

The constant changes in posture required in this type of cycling may explain the systemic effects via altered carotid baroreflex sensitivity and pressure. Despite cycling being primarily a lower limb activity, the observed carotid artery remodelling could be due to constant pressure fluctuations in the common carotid artery brought about by engagement of the surrounding neck musculature when straining during postural changes (continuous sitting and standing). **The Alx is a sensitive marker of arterial health in this population and changes can be detected before changes in BP but diurnal variation should be considered.** Likewise, the benefits of an intervention can be detected earlier if the Alx is assessed. Changes in cfPWV are more obvious than IMT. The large reductions in cfPWV and slight changes in IMT coupled with the absence of a correlation between the two indicates that if changes in IMT are not observed, it does not mean that there is a lack of effect on arterial health.

7.3 RECOMMENDATIONS FOR FURTHER WORK

Despite there currently being extensive knowledge in this field of research of exercise and arterial health, significant further work is required. Future studies investigating the effects of group-fitness exercise on arterial health could include the following:

Randomised controlled trials of longer durations, larger sample sizes, and more standardised assessment techniques: these would enable better determination of the time-course of adaptations and potential plateauing effects after more than two months. It would also reduce the likelihood of inter-study discrepancies. Cross-over or follow-up studies could help determine whether the exercise-induced benefits persist after reverting to a sedentary lifestyle for a few weeks. However, since it may be unethical to encourage a sedentary lifestyle amongst individuals who recently started becoming active, it would be more advisable to carry out a follow-up study instead. Follow-up longitudinal studies employing a similar exercise intervention to the current one could enable the determination of CV risk and mortality reductions based on improvements in CV risk factor profiles.

Acute effects and autonomic function: Investigating the effects of aerobic with concurrent interval exercise on haemodynamics both during and in the few minutes after this sort of training would shed light on possible explanations for the long-term improvements, especially in terms of endothelial function (by measuring nitric oxide levels), carotid diameter and stiffness as well as baroreflex sensitivity. It would also be interesting to investigate whether acute changes to this specific type of training can be altered by long-term engagement in this and/or other types of exercise. Comparison of aerobic interval exercise to other AE modes can also be made in individuals with differing physical activity statuses.

To date, the physiological mechanisms underlying changes in arterial health in response to exercise training are unclear. The processes presented here are merely educated speculations based on previous research and therefore warrant further exploration. In the future, it will hopefully be possible to be able to distinguish between intimal and medial layers of the arterial wall. Investigations into local stiffness parameters will indicate whether central or peripheral arteries benefit more from concurrent aerobic interval training.

Different populations: Despite the positive results, it is possible that they are specific to this population so future studies could determine the effects of this same intervention on women, older individuals, and clinical populations. It would also be interesting to know whether ethnicity would play a role in trainability with this type of exercise.

How this exercise affects hyperaemia-induced flow-mediated dilation: Including this assessment into future studies would give a complete picture of the effects of this type of exercise on EVA characteristics

Genetic influences and individual trainability: Sometimes, expected correlations between the characteristics of a subject and an outcome do not occur depending on whether the individual is adaptable and how she/he adapts. For example, there is much debate as to whether the AIx and cfPWV are related. Despite observing improvements in both, the magnitudes of change were negatively correlated. This might be because exercise affects either wave reflections or intrinsic wall stiffness to different extents in different individuals.

The RE component of this exercise and body composition changes: It could be argued that HIIT and SIT involve some aspects of resistance training. Blood markers such as endothelin-1

(which increases with RE) as well as inflammatory markers could be measured both acutely and weekly. Simultaneous incorporation of DXA scanning would be useful to determine whether the lack of significant change in body mass despite a decrease in waist circumference could be due to an increase in lean muscle mass, especially in the lower limb.

Benefits of this type of training on other health indices: Now that the general cardiovascular impact of this type of exercise has been demonstrated, the effects on psychological and different physiological (eg. respiratory, neurological, muscular, cognitive) aspects can be considered. As described in section 6.3.2, it would be interesting to investigate how AS and cognition are related and how they are influenced by such aerobic interval exercise.

Comparison of exercise alone to diet and exercise: The benefits in the current study accrued without changes to diets. Further studies could determine the extent to which dietary manipulation, especially in relation to salt and alcohol intake, can alter the effects of exercise alone.

Comparison of different group-fitness programmes: The exercise environment in a community-based, group-fitness setting is very different that to a controlled laboratory one. Therefore, although the benefits of various modes of exercise on arterial health are known, future studies could determine the effects, on arterial health, of various modes of group-based exercise, which differ in the mix of AE, RE, anaerobic activity, stretching/flexibility and dance on arterial health. This would reveal a wide scope for research due to the diverse range of fitness classes offered globally.

Different non-invasive assessment methods: Measurement methods and devices used in this study were very specific. Future studies could determine whether similar results are obtained when PWV and the AIX are assessed by applanation tonometry. Conversely, it may be useful to use the BP+ device to measure BP, central haemodynamics, and the AIX, as it is portable and simple to use.

Flexibility and AS: Is there a cause-and-effect relationship between the two with similar underlying mechanisms? Would incorporating stretching components into aerobic interval exercise programmes help reduce AS even more? Would incorporating stretching into resistance training programmes attenuate the detrimental effects of the RE on arterial health indices?

7.4 LIMITATIONS

The main disadvantage of the present study was the sample size. Although *a priori* analysis suggested that a sample size of $n=10$ in the intervention group was adequate enough to achieve statistical power, the same number of individuals in the control group would have been preferred. However, previous studies have excluded a control group altogether so it was useful to observe that no significant differences in outcome measures occurred during the intervention period within this group.

Since subject recruitment posed a challenge and was staggered, it was impossible to conduct a matched-pairs experiment as initially planned. However, group allocation was randomised and there were no significant baseline differences in any characteristics or outcome measures.

Due to the extensive time demands already placed upon the participants as well as the investigator, who had to visit the gym at subject-dependent times each week, the study did not investigate arterial function so it was difficult to ascertain whether changes in arterial health occurred due to altered structure, tone or both. However, the acute effects of exercise were minimised by ensuring that at least 24 hours elapsed between an exercise session and any data collection. Secondly, vascular tone changes do not usually affect arterial wall thickness but since the latter significantly improved in the study, arterial health changes could be attributed to changes in arterial structure.

Given the freedom that participants in the intervention group had in choosing class times and the fact that the augmentation index, central haemodynamics, and heart rate were measured on a weekly basis, the inter-individual measurement times could not be standardised (measurement times ranged from 6:00am to 6:00pm). However, weekly intra-individual measurement times were kept constant as were inter-and intra-subject measurement times at PRE, MID and POST. Furthermore, given the motive of the practical application of previous findings of the current study, measurements taken at different times of the day was not deemed a major limitation as it would represent a more realistic situation.

In the present study, participants were simply requested to maintain their current dietary practice. It is acknowledged that this may have potentially posed two problems. Firstly, participating in exercise would have motivated individuals to initiate a healthier lifestyle and it is possible that they would have altered the quality and quantity of meals/snacks to support weight loss or a reduced WC. These minor adjustments could potentially have accounted for part of the improvements in outcome measures. However, all participants consistently reported that their diets were 'the same'. Secondly, it has been reported that exercise can influence appetite and energy intake (amount and composition) with reduced energy intake observed after 12 weeks of HIIT in overweight men, independent of changes in the perception of appetite or appetite-related peptides (517).

In acute settings, energy intake is influenced by factors such as the time delay between an exercise session and a meal/snack (518) as well as post-exercise drink macronutrient content (519). Therefore, both acute and longer-term effects of the RPM™ classes could have altered appetite (control) and energy intake. 'Normal' dietary practice would have meant that perhaps, the new energy expenditure levels of the individuals was not being matched by energy intake or else, unconscious changes in appetite and diet alone could have potentially influenced arterial health measures, WC, BP, lipid profiles (as fat intake decreases after HIIT) (520).

Finally, it is possible that the current findings are specific to healthy men and that the same adaptations cannot be observed amongst women or individuals with *a priori* chronic illnesses.

7.5 CONCLUSIONS

The current results obtained using a novel approach to investigating exercise and arterial health demonstrate the practical application of laboratory-based findings to public healthcare and pre-existing societal settings where exercise intensities are self-selected and typically unmonitored. The simultaneous incorporation of two different interval training modalities into a moderate-intensity AE session lies in contrast to previous studies employing single-modality (or concurrent AE and RE) exercise and aerobic interval training with only one mode of interval exercise. Improved cardiorespiratory fitness and target organ damage-related tissue biomarkers of EVA were observed, as was systemic adaptive outward arterial remodelling and improved CV risk factor profiles. The current findings from this community-based, self-paced vigorous concurrent exercise intervention confirmed the repeatability of laboratory-based findings in everyday community settings and provide evidence that exercise induces beneficial arterial adaptations thereby conferring substantial vasculoprotection. 'Real-world' exercise carried out in pre-existing environments is a feasible and effective approach to enhance arterial health in healthy, sedentary men.

'Longevity is a vascular question, which has been well expressed in the axiom that man is only as old as his arteries'

Sir William Osler, 1891.

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APPENDIX A: ETHICS APPROVAL LETTER



A U T E C
S E C R E T A R I A T

26 August 2014

Andrew Kilding
Faculty of Health and Environmental Sciences

Dear Andrew

Re Ethics Application: **14/242 Effects of exercise on indices of arterial health.**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 25 August 2017.

As part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through <http://www.aut.ac.nz/researchethics>. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 25 August 2017;
- A brief report on the status of the project using form EA3, which is available online through <http://www.aut.ac.nz/researchethics>. This report is to be submitted either when the approval expires on 25 August 2017 or on completion of the project.

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this.

To enable us to provide you with efficient service, please use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

All the very best with your research,

Kate O'Connor
Executive Secretary
Auckland University of Technology Ethics Committee

Cc: Shivani Sethi sset023@aucklanduni.ac.nz

APPENDIX B: INDICES USED TO ASSESS ARTERIAL STIFFNESS AND WALL THICKNESS

The primary methods of assessing each index, along with the disadvantages and advantages of the index and its assessment methods are outlined

parameter	definition	main assessment method(s)	advantages of index & assessment methods	disadvantages of index & assessment methods
Arterial compliance	absolute change in diameter or area for a given pressure change; measure of local AS	invasive or non-invasive, usually via ultrasound, MRI or PWA	straightforward index with good prognostic value; differentiates between intrinsic wall properties and effects on elasticity of distending pressure; provides indices at any given BP; stress-strain and PV relationships obtainable; non-invasive, direct assessments are valid & affordable; assessed at several different arteries; accounts for many parameters eg diameter, thickness; local CCA AS is moderately associated with aortic stiffness, independent of traditional CV RFs	incremental arterial pressure is required and is usually extrapolated from brachial BP but due to amplification, might not accurately represent local BP so AT is normally used to obtain carotid/aortic BP using a GTF; assessments are usually technically challenging and time-consuming and operator bias is likely
Arterial distensibility	relative change in diameter or area for a given pressure change; a measure of local AS	MRI, ultrasound or most commonly, from a mathematical equation	easily calculated from an equation so no practical assessment required	evaluation is usually indirect: impractical to accurately measure arterial volume and changes in volume, therefore, the index is calculated from an equation

parameter	definition	main assessment method(s)	advantages of index & assessment methods	disadvantages of index & assessment methods
Incremental elastic modulus (EM) <i>Young's EM</i>	pressure change required for 100% stretch from resting diameter; inverse of distensibility; a measure of strain on the arterial wall; a measure of local AS <i>Incremental EM per unit area; measure of the intrinsic stiffness of arterial wall material; measure of local AS</i>	usually via ultrasound or MRI	local CCA AS is moderately associated with aortic stiffness, independent of traditional CV RFs	pertain to disadvantages of ultrasound and MRI summarised in Appendix B
β stiffness index	Ratio of the natural logarithm of SBP/DBP to relative change in diameter; represents compliance, independent of the effect of BP; measure of local AS	ultrasound: a) echo tracking with radiofrequency tracking or Doppler processing; b) B-mode ultrasound with automatic image processing and simultaneous electrocardiography; c) M-mode ultrasound	B and M-mode ultrasound are inexpensive and can be used in clinical settings; local CCA AS is moderately associated with aortic stiffness, independent of traditional CV RFs	B and M-mode ultrasound do not measure changes in arterial diameter accurately; the use of brachial BP as a surrogate for central BP leads to inaccurate results due to PP amplification so AT is normally used to obtain carotid/aortic BP using a GTF

parameter	definition	main assessment method(s)	advantages of index & assessment methods	disadvantages of index & assessment methods
Systemic arterial compliance	absolute change in diameter or area for a given pressure change; measure of systemic AS	ultrasound (Doppler velocimetry) and common carotid AT		prognostic value of the index has not been determined yet; inaccuracies arise due to the use of brachial BP used as a surrogate of central BP
Capacitative compliance	<i>the relationship between pressure change and volume change in the arteries during the exponential component of diastolic pressure decay; a measure of systemic AS</i>	<i>PWA (Diastolic pulse contour analysis and modified Windkessel model)</i>	the methods used for its assessment are based on a simple theoretical model	
Oscillatory compliance	<i>the relationship between oscillating pressure change and oscillating volume change around the exponential pressure decay during diastole; a measure of systemic AS</i>			<i>the doubtful validity of modified Windkessel model to calculate the index</i>

parameter	definition	main assessment method(s)	advantages of index & assessment methods	disadvantages of index & assessment methods
PWV	the velocity of travel of the pulse along a length of the artery; a measure of regional/local AS; gold standard measure of AS; influenced by arterial wall elasticity, artery geometry and blood viscosity	invasive angiography (local); mechanical sensors, AT (regional); radiological: ultrasound, MRI (local)	several assessment methods and sites exist: can be measured from pressure, distension or flow waveforms obtained transcutaneously at carotid/femoral arteries; aortic PWV (cfPWV) is most prone to age-related stiffening; can be measured invasively or non-invasively; assessment methods are all simple, valid, reproducible and fairly affordable; widely applied in clinical/research settings; PWV independently predicts CV risk (highest prognostic value from all predictors); largest amount of clinical evidence providing predictive value of AS for CV events	comparisons between studies and meta-analyses should be made with caution due to the huge array of assessment methods; central arteries are inaccessible; difficult to determine foot of waveform; inaccuracies in surface measurements of distance between arteries; limited to larger arteries because accuracy decreases if recording points are close together; underestimation of aortic PWV with age as it becomes more tortuous; assessments are usually time-consuming and require operator training; peripheral PWV has unknown predictive values, questionable validity and high sensitivity to heart rate; non-invasive data suffer more drift and noise than data obtained invasively; values are influenced by HR and BP
Local	<i>average PWV over a long segment composed of arteries with varying mechanical properties</i>	<i>ultrasound, MRI, invasive aortic catheterization</i>	<i>takes into account heterogeneity in AS and changes in AS along arterial tree; specific assessment of arteries which are prone to early atherosclerotic alterations can be carried out; direct measurement of path length</i>	<i>equipment is expensive</i>
Regional	<i>PWV over one particular segment of the arterial tree</i>	<i>specialised devices, ultrasound</i>	<i>assessed easily using specialised commercial devices which are well-validated and more affordable; regional PWV is correlated to atherosclerotic changes</i>	<i>external, coarse distance measurements affected by body habitus; exact position of arterial abnormalities cannot be determined; biochemical alterations and initial variations in elastic properties cannot be detected; direct contact (of sensors/probes) with skin is required</i>

parameter	definition	main assessment method(s)	advantages of index & assessment methods	disadvantages of index & assessment methods
Pulse pressure (PP)	SBP-DBP	calculated from either sphygmomanometer-or automated device-derived cBP	surrogate for AS; an important independent predictor of risk; can be obtained simply and practically using automated devices; useful in clinical settings	cannot assess AS adequately enough when used alone because of PP amplification (especially in younger individuals)
AIx	difference between second and first systolic peak expressed as % of PP	EITHER 1. radial artery AT to obtain a peripheral pressure waveform to which a GTF is applied to obtain a central pressure waveform OR 2. carotid AT (no GTF needed); OR 3. specialised automated oscillometric devices	a measure of wave reflections and the impact they have on central aortic pressure and the load imposed on the left ventricle; can be obtained easily and affordably via non-invasive methods and if via carotid artery, directly (no GTF required); the AIx independently predicts all-cause and CV mortality in ESRD as well as death and MI	readings can be influenced by posture and height; carotid AT requires technical expertise and is difficult to carry out in obese individuals; with radial AT, inaccuracies arise due to the use of a GTF
Central haemodynamics	cSBP, cDBP, cPP, cAP, cAIx at aortic root	radial artery AT to obtain peripheral pressure waveform to which a GTF is applied to obtain a central pressure waveform OR specialised automated oscillometric devices	better predictors of CV events than pBP; unlike pBP, cBP influences cIMT; can be obtained simply and practically using automated devices; waveforms provide absolute values of wave reflection characteristics	readings can be influenced by posture and height; carotid AT requires technical expertise; with radial AT, inaccuracies arise due to the use of a GTF
Ambulatory Arterial Stiffness Index (AASI)	AASI = 1-(the regression slope of DBP on SBP)	calculated from the recording of 24-hour ambulatory BP	it is a stronger predictor than PP for fatal stroke	AASI is not independently related to cfPWV and is influenced by nocturnal BP reductions; relative to cfPWV, the AASI has a lower predictive value for CV mortality and stroke; further validation studies needed

**APPENDIX C: TECHNIQUES AND EQUIPMENT USED TO ASSESS THE ARTERIAL HEALTH INDICES
ASSESSED IN THE PRESENT STUDY**

method and indices derived from it	sub-method	comments about methodology	advantages	disadvantages
<p>Ultrasound <i>all indices relating changes in vessel D or area to local distending P (compliance, distensibility, β stiffness index, incremental EM, Young's EM);</i> <i>local/regional PWV</i> IMT RI EDD</p>	<p>waveforms over two different arteries are either obtained simultaneously or separately using ECG synchronization (local PWV can also be assessed by calculating the ratio of change in flow and change in CSA during a cardiac cycle)</p>	<p>IMT: marker of structural compromise; B-mode ultrasonography used to measure combined thickness of tunica intima and media; usually carotid near and far walls and common femoral far wall;</p>	<p>For PWV: direct measurement of path length</p> <p>For IMT: standardised protocol so can compare inter-study results; simple; widely available; good resolution; findings from a localised segment can be generalised; yields few missing values; easy to image</p>	<p>For PWV: reliable identification of the foot of the waveform and a high enough sampling frequency are required for accurate results; expensive equipment; distance measurement depends on body habitus; requires operator expertise</p>
<p>MRI <i>All indices relating changes in vessel D or area to local distending P</i> Local PWV IMT RI EDD</p>			<p>simple; technique learned easily; enough temporal and spatial resolution to study aortic systolic pulse wave; accurate path length determination; measurements from inaccessible arteries can be made; no groin exposure; can provide diagnostic information about biochemical properties of the local arterial wall</p>	<p>expensive and therefore not practical for routine clinical use; lack of availability; time-consuming; can only currently be applied to relatively large arteries</p>

method and indices derived from it	sub-method	comments about methodology	advantages	disadvantages
<p>Pulse wave analysis (PWA) <i>capacitative & oscillatory compliance</i></p> <p><i>central pressures (cSBP, cDBP, cPP)</i></p> <p><i>central/peripheral AIx</i></p>	<p>1. <u>AT</u>: use of a micromanometer-tipped probe to compress the artery between a sensor and underlying structures; intra-arterial pulse pressure is transmitted through the arterial wall to the sensor and the pressure waveform is digitalized</p> <p>2. <u>specialised electronic devices</u> using either oscillometric principle or microphone to record Korotkoff sounds; pressure recorded from brachial artery</p> <p>a). O'rouke PWA system</p>	<p>assess systemic AS by evaluating mechanical properties of the arterial tree; based on arterial viscoelastic heterogeneity; P or V waveform analysis using sphygmograph, vasculograph; various invasive devices or non-invasive AT; central P waveform can be reconstructed from peripheral waveform using a GTF</p> <p>radial/carotid AT to obtain peripheral pressure waveform. GTF based on Fourier analysis is applied to derive corresponding central waveform</p>	<p>aortic, carotid and radial AIx values are strongly correlated; no GTF needed; simple, easy to use; self-measurement; ambulatory; good reproducibility and intra/inter-test reliability</p> <p>non-invasive; relatively inexpensive; well-validated; better reproducibility than automated sphygmomanometers; radial AT is well-tolerated and simple; carotid AT is non-invasive and is a direct measurement</p>	<p>AS oversimplification; controversy about GTF and accuracy of calibration of radial AT through brachial BP; technical expertise required for carotid AT; no prospective study testing validity of superficial AT; underestimates pressure determined invasively; overestimates compliance measures; less sensitive than invasive measures at detecting differences in compliance between individuals; all components not well defined so questionable validity; values of compliance can be influenced by regional circulatory properties (eg arterial length, wave reflection sites); hard to interpret tonometry-derived values so questionable reliability</p>

method and indices derived from it	sub-method	comments about methodology	advantages	disadvantages
PWA CONTINUED...	bi) systolic pressure pulse contour analysis	Radial AT	simple; affordable; rapid; not much operator bias; good quality of validation; good inter- and intra-observer reproducibility; used in clinical/research settings; good reproducibility; can be reassuring that high pBP is due to exaggerated PP amplification (if cBP is normal);	gender-specific GTFs may be more reliable; low prognostic value
	bii) diastolic pressure pulse contour analysis	Windkessel model used to assess diastolic pressure decay to get large vessel and peripheral compliance	simple; reproducible; well-validated; well-tolerated; non-invasive; wide clinical application; no significant operator bias; evaluation of compliance of both proximal and distal arteries (capacitative and oscillatory) possible; a sensitive marker of early vascular disease; affordable	relatively less accurate than other methods because the diastolic component of the waveform on which it depends on is less reliably recorded than systolic component
	c) pressure wave analysis on digital artery	Use servocontrolled pressure cuff		questionable accuracy because recorded waveforms can be dampened derivations of cBP
	d) digital volume PWA	photoplethysmography to measure the transmission of IR light through the finger to obtain volume waveform	simple; equipment is easily portable; relatively inexpensive; useful for epidemiological studies; reduction in movement artefacts	dampening of the peripheral pulse; temperature-dependent changes in peripheral circulation; not well validated (in terms of risk prediction and its relationship with central AS); can only be applied to peripheral sites; technical operator bias likely when assessing obese individuals

method and indices derived from it	sub-method	comments about methodology	advantages	disadvantages
<p>Specialised commercial devices</p> <p><i>PWV</i></p>	<p>eg. Sphygmocor, PulsePen, Complior</p>	<p>commonly used to measure regional PWV; some enable PWA in addition to PWV assessment; mostly based on the use of pressure mechanotransducers and AT</p>	<p>pressure sensors are considered the gold standard for PWV measurement; well-validated; affordable</p>	<p>only measures regional PWV; errors due to distance estimation (external, coarse measurements depend on body habitus); distortion of acquisition signal; difficult to make valid measurements with AT over the carotid artery; quality of recordings depends on operator expertise; hard to assess in obese people; direct contact with skin required (may cause discomfort as the artery is compressed throughout the cardiac cycle); only used in research/clinical settings by well-trained operators</p>
<p><i>indices assessed via PWA</i></p>	<p>eg. BP+</p>	<p>automated oscillometric device</p>	<p>no use of GTF; self- and home-based measurements possible (operator-independent); good inter- and intra-test reliability; valid; good reproducibility; acceptable accuracy; affordable</p>	<p>height of individual not accounted for; readings possibly affected by posture</p>
<p>Aortic angiography</p>		<p>threaded catheterization from a peripheral artery; <i>a direct measure of aortic pulse wave</i></p>	<p>direct therefore most accurate values obtained</p>	<p>invasive and expensive and therefore, its use cannot be justified in routine clinical settings</p>
<p>Optical methods</p>	<p>Currently emerging</p>	<p>various methods have been proposed (described elsewhere)</p>	<p>local PWV can be assessed; no direct skin contact required; due to the good penetration ability of IR light, pulse waveforms are acquired from deep within the skin and therefore, good signals can be acquired even in obese individuals; simple architecture; lower production costs</p>	<p>still in early stages of validation</p>

APPENDIX D: INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE – LONG FORM



SPORTS PERFORMANCE
RESEARCH INSTITUTE, NEW ZEALAND
AN INSTITUTE OF AUT UNIVERSITY

For Administrative Use
Participant ID:

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

The purpose of this questionnaire is to give an indication of the kinds of physical activities that you do as part of your everyday life. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport. Think about all the **vigorous** and **moderate** intensity activities that you did in the **last 7 days**.

Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.

Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

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

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

Yes

No

Skip to PART 2: TRANSPORTATION

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

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities such as heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**?

Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

No vigorous job-related physical activity

Skip to question 4

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

_____ **hours per day**

_____ **minutes per day**

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

_____ **days per week**

No moderate job-related physical activity

Skip to question 6

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

_____ **hours per day**

_____ **minutes per day**

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

_____ **days per week**

No job-related walking

Skip to PART 2:

TRANSPORTATION

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

_____ **hours per day**

_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

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

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

_____ days per week

No traveling in a motor vehicle

Skip to question 10

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

_____ hours per day

_____ minutes per day

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

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

_____ days per week

No bicycling from place to place

Skip to question 12

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

_____ hours per day

_____ minutes per day

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

_____ **days per week**

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

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

_____ **hours per day**

_____ **minutes per day**

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

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

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

_____ **days per week**

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

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

_____ **hours per day**

_____ **minutes per day**

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

_____ **days per week**

No moderate activity in garden or yard

Skip to question 18

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

_____ **hours per day**

_____ **minutes per day**

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

_____ **days per week**

No moderate activity inside home

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

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

_____ **hours per day**

_____ **minutes per day**

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

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

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

_____ **days per week**

No walking in leisure time

Skip to question 22

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

_____ **hours per day**

_____ **minutes per day**

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

_____ **days per week**

No vigorous activity in leisure time

Skip to question 24

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

_____ **hours per day**

_____ **minutes per day**

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

_____ **days per week**

No moderate activity in leisure time

Skip to PART 5: TIME SPENT

SITTING

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

_____ **hours per day**

_____ **minutes per day**

PART 5: TIME SPENT SITTING

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

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

_____ **hours per day**

_____ **minutes per day**

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

_____ **hours per day**

_____ **minutes per day**

This is the end of the questionnaire. Thank you for completing it!

APPENDIX E: JUSTIFICATION OF 'SEDENTARY' DEFINITION AS USED IN THE PRESENT STUDY

The first instinct was to classify those performing less than 30 minutes of moderate physical activity on most days of the week (at the time of recruitment) as 'sedentary' as per ACSM and international guidelines for adults (18-64 years old). This would be equivalent to <150 minutes of moderate-intensity aerobic exercise or <75 minutes of vigorous exercise per week or an equivalent combination of the two, in addition to muscle strengthening exercise ≥ 3 days per week. However, although the IPAQ is a straightforward questionnaire, inaccurate recall, misinterpretation of questions or dishonesty could result in misleading values of total physical activity per week on average. This could have potentially resulted in individuals being misclassified as sedentary (according to ACSM guidelines) by a narrow margin and vice versa.

In the majority of previous studies which have stated their definition of sedentary, <60min/wk (approximately equivalent to <2days/week) has been used because these individuals would be inactive enough to be 'high risk' and would be definitely classified accurately, even with an error of one hour per week. However, it would have been harder to recruit participants if this definition was used because firstly, not many individuals would do less than one hour of physical activity per week if all domains of activity (as in the long form IPAQ) are included and secondly, these individuals would likely be unhealthy if they were, in fact, engaging in less than 60 minutes of physical activity per week and would thus not fulfil the 'apparently healthy' inclusion criterion. Furthermore, as potentially 'high risk' individuals, it would not have been safe or ideal to make them undergo either fitness tests or high-intensity exercise (RPM).

Initially, the preferred option was to classify individuals as sedentary if they engaged in less than 90 minutes of physical activity per week. However, for reasons stated above pertaining to recruitment difficulty, sedentary in this study was classified as engaging in less than 2 hours of moderate aerobic activity per week, as determined by the long form IPAQ. This was thought to be a compromise between ACSM guideline and the most commonly used definition to date. It would be unlikely that individuals would be misclassified and it would also increase the likelihood that individuals were apparently healthy (and not 'high risk') and that it was safe for them to exercise.

APPENDIX F: PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of any other reason why you should not do physical activity?

If
you
answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to **all** PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____

DATE _____

SIGNATURE OF PARENT
or GUARDIAN (for participants under the age of majority) _____

WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.



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APPENDIX G: MODIFIED SIT-AND-REACH TRUNK FLEXIBILITY TEST



APPENDIX H: STUDY ADVERTISEMENT FLYER



SPORTS PERFORMANCE
RESEARCH INSTITUTE, NEW ZEALAND
AN INSTITUTE OF AUT UNIVERSITY

ARTERIAL HEALTH RESEARCH

WANTED: 20-45 YEAR OLD INACTIVE MALES



- **HEALTHY non-smokers**
- **SEDENTARY - currently doing <2hours of moderately hard exercise per week**

1.

- Simply maintain current lifestyle for 8 weeks

2.

- 3 assessments at AUT Millennium at weeks 0, 4, 8

3.

- One weekly 5min session.. (at a location convenient for you)

REWARDS FOR PARTICIPATION:

- ✓ 8 weeks free access to Les Mills RPM classes 3x/wk
- ✓ Free 2 week gym trial at Les Mills after study completion
- ✓ No joining fee if you opt to join Les Mills afterwards
 - ✓ \$50 monetary compensation
 - ✓ **FREE** fitness & basic blood tests

If interested, please contact either:

Primary Researcher

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Dr. Andrew Kilding

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APPENDIX I: PARTICIPANT INFORMATION SHEET



25th March 2015

Project title

The effects of a group fitness aerobic interval exercise training programme on arterial health in healthy sedentary male adults

An invitation

Hi! My name is Shivani Sethi and I am currently doing my research-based Master's degree in Sport and Exercise at AUT. My research will be supervised by Associate Prof. Andrew Kilding (Sports Performance Research Institute New Zealand) and Dr. Andrew Lowe (IBTec). We invite you to participate in this research which investigates the effects of a specific exercise programme on arterial health.

Purpose of this research

It is well known that exercise may benefit the cardiovascular system (heart and blood vessels) in many ways and can reduce the risk of cardiovascular disease (CVD). CVD has been identified as the leading cause of death in NZ, accounting for approximately 40% of deaths annually. Many of the well-known predictors of CVD (eg. high blood pressure, high fasting blood glucose levels, elevated blood cholesterol levels, obesity) have been studied in an attempt to eliminate them as risk factors. One lesser-known independent predictor of CVD is arterial stiffness (AS), which is just as important a risk factor but has not been investigated to a great extent.

Arteries are blood vessels which carry blood away from the heart to the rest of the body so it is vital that they remain healthy in terms of structure and function. AS simply describes how rigid the walls of your arteries are.

Description of this research: The structure and function of arteries can be modified by exercise training programmes of various lengths and characteristics. This research will focus on community-dwelling sedentary individuals (those who do not engage in regular physical activity) to determine the effects that a specific exercise training programme has on arterial health.

RPM™ (Raw Power In Motion) is a 45 minute indoor cycling group-fitness ('spin') class trademarked by Les Mills, a New Zealand-based gym well-reputed internationally for its various group fitness classes. The type of exercise is known as high-intensity interval training (HIIT) or simply interval aerobic exercise: 'Interval' indicates that it is based on short, hard work (exercise) periods interspersed with short recovery periods. 'Aerobic' indicates that it is rhythmic activity which uses large muscle groups. Before, during and after this 8 week intervention, various indices of AS will be assessed by employing reliable, modern technology.

How was I identified and why am I being invited to participate in this research?

As a community-dwelling, sedentary (non-exercising) male between 20-40 years old, you have been invited to be part of this research. Please ensure that at the time you are being recruited for this study, you either:

- Engage in NO regular physical activity (that is, you lead a physically inactive lifestyle and sit or stand still for most of the day) OR
- Engage in LESS THAN a total of either:
 - 2 hours (120 minutes) of moderate (medium) intensity aerobic activity per week OR
 - less than 1 hour of vigorous (hard) aerobic activity per week OR
 - an equivalent combination of moderate and vigorous aerobic activity per week **AND**
 - You have not engaged in a formal exercise programme for the past 6 months

Aerobic exercise ('cardio') includes activities such as walking, cycling, swimming or low-impact aerobics which involves the use of large muscle groups and gets your heart rate up and makes you breathless (the extent of breathlessness depending on the intensity of the physical activity).

Even if you meet the criteria for leading a 'sedentary' lifestyle, you will not be eligible to take part in this research if:

- You smoke or use tobacco
- You have a chronic illness: any history of or current cardiovascular-related disease (eg. coronary artery disease, atherosclerosis), high blood pressure (BP \geq 160/100mmHg), any illness involving the brain (eg. stroke/psychological disorders), kidney disease (or on dialysis), morbid obesity, diabetes type I or II, thyroid disease, abnormal blood fat profile or high cholesterol levels
- You are on long-term prescribed medication used to treat cardiovascular-related, metabolic, hormonal or neurological chronic illnesses
- You have a current injury or illness which may hinder your participation and ability to exercise
- You cannot communicate fluently in written and spoken English
- You indicate that you may not be available throughout the intervention period or may not be able to attend weekly exercise/assessment sessions
- You take antioxidant supplements regularly (these include vitamins A, C, and E, beta-carotene and/or selenium)
- You are not willing to do the particular indoor cycling classes in a group-fitness setting
- You have personal, cultural or religious sensitivities regarding any of the assessment procedures

What will happen in this research?

Preparation for visit 1:

Confirmation of 'sedentary' status using IPAQ

You should have received a consent form and International Physical Activity Questionnaire (IPAQ), with this information sheet. Please ensure that before your first visit (or at the beginning of your familiarization session), you have completed, signed and dated the consent form. The consent form confirms that you have understood the facts, procedures, and implications of the research, are willing to participate in this research and have given us permission to carry out the tests on you.

The IPAQ is a simple questionnaire which will enable us to gauge your current physical activity levels in order to determine whether you meet the criteria for leading a 'sedentary' lifestyle as required for

participation in this study. Please complete the IPAQ and email it back to us if you think you would like to participate.

Familiarization session arrangement

If you meet all the inclusion criteria, you will be invited to come to AUT Millennium for a familiarization session. A time will be arranged via e-mail/phone regarding the time of your first visit. Please ensure that you bring your exercise gear with you for the first visit. Also, please try to avoid strenuous physical activity for 24 hours before the first visit and please ensure you attend the first session after an overnight fast (9-11 hours).

Visit 1 - Familiarization and baseline testing:

After the study background and protocol have been outlined to you, you will have a chance to ask any questions. If you think you would like to participate, you will have to submit the consent form formally. You will then be required to fill out a Physical Activity Readiness Questionnaire (PAR-Q) which will enable us to determine whether it is safe for you to exercise based on your answers to specific health-related questions. Some general details and basic measures (height, weight, waist circumference, resting heart rate, resting blood pressure & arterial stiffness) will then be taken.

Next, your bloods will be taken using a simple finger-prick method (see page 6). The results will enable us to classify your CVD risk using a Cardiovascular Risk Assessment (CRA) form.

The distance between your neck and upper thigh (groin area) will be taken using a tape measure. You will then be required to lie down on your back or sit up slightly (if needed) with an ECG attached to you whilst ultrasound recordings are carried out on various parts of your body (neck, upper thigh, and arm). This will require approximately 1 hour (see page 6 for further details).

You will then be required to perform the 'sit-and-reach test' which is a measure of trunk flexibility. It just involves sitting down on the ground with your legs straight out and reaching as far forward as possible.

Finally, you will be required to perform a ' $\text{VO}_{2\text{peak}}$ ' test which will provide us with information about your fitness levels. The test will involve cycling at progressively increasing intensities until you can no longer continue. Note: If your blood pressure is elevated ($>160/100\text{mmHg}$), you will be referred to your GP and you will not be allowed to participate further. The test will start very easy and will continue to a high intensity. The test lasts about 12 minutes. During the test, you will wear a heart rate monitor around your chest and a face mask with a mouthpiece (which resembles a snorkel) for breathing analysis.

Finally, you will be given a Food Frequency questionnaire (FFQ) which will give us an idea about your dietary habits.

Group allocation

Following visit 1, you will be randomly allocated to either the 'control' group or 'intervention' group. It is important you understand that you may be asked to serve as a control subject in this study (see below).

What happens if I get allocated to the control group?

If you are randomly allocated to the control group, you will complete all the same procedures and assessments as the intervention group. Refer to page 6 and page 8 (table 1) for a summary of the assessment sessions and to page 7 (figure 1) for a visual representation of when the measurements will be taken. All your assessments will take place at the AUT Millennium Institute of Sport and Health.

The only inter-group difference is that the intervention group will undergo 8 weeks of exercise training, 3 times per week, whereas you will continue with your normal lifestyle. You will be requested to maintain your current, normal physical activity, and dietary habits for the entire intervention period and will be required to keep a physical activity diary (which will be provided during the familiarization session).

Once you have completed all pre-, mid- and post-intervention assessments, you will be invited to participate as the 'intervention group' and do what those initially allocated to the intervention group did for the first 8 weeks (i.e. 3 weekly Les Mills RPM classes for 8 weeks). No formal physiological assessments will be carried out at this time but you will be given free membership at Les Mills Victoria Street/Takapuna for 8 weeks.

What happens if I get allocated to the intervention group?

You will be required to participate in three RPM sessions per week (for 8 consecutive weeks) at the Les Mills Takapuna or Victoria Street gym. These can be done at any time of day according to your convenience but each session must be separated by at least 36 hours. A timetable of classes will be provided.

Once a week, your basic arterial stiffness measurement (no. 1, page 5), body mass and blood pressure measurements will be carried out at Les Mills Vic St/Takapuna approximately before your last exercise session of the week.

The other arterial health measures (no. 2, page 5) will be carried out at the AUT Millennium Institute of Sport and Health (Weeks 0, 4 and 9). These ultrasound-based arterial health assessments will be carried out at your convenience, but in week 4, it will not be on a day that you have done RPM.

At the end of 8 weeks (excluding week 0), a final assessment session will take place where bloods, ultrasound assessments, and the fitness test will be repeated. *Refer to page 6 and page 9 (table 2) for a summary of the assessment and RPM sessions and to page 7 (figure 1) for a visual representation of when the measurements will be taken.*

You will be requested, aside from the RPM classes, to maintain your current, normal physical activity, and dietary habits for the entire intervention period and will be required to keep a physical activity diary (which will be provided during the familiarization session).

You will also be required to wear a heart rate monitor during each RPM session. You will be responsible for ensuring that you have done so as the information obtained from it will firstly enable us to determine, the intensity at which you worked at during each session and secondly, will be used to monitor your attendance to RPM. Your heart rate monitor will be collected after the end of your last RPM session each week and a new one will be available to you before your first class the following week.

Physiological assessment procedures: Refer to figure 1 on page 7

Basic arterial stiffness, blood pressure and heart rate measurement: This will be similar to a normal blood pressure measurement which you are likely to be familiar with. It simply involves placing a cuff around your arm so that a reading can be taken. There will be very mild discomfort as the cuff compresses your arm, but this will not last more than a few seconds! These measurements will be taken 10 times in total (before and after the 8 week intervention phase as well as weekly, throughout the 8 weeks).

Arterial health measures: Before, halfway through, and at the end of the intervention, you will be required to come in to get an ultrasound assessment of your arteries done. There will be 2 separate tests being carried out. One will involve ultrasound assessment of the arteries in your arm whilst the other will involve the neck area (where you can feel your pulse) and the top of the lower limb, near the groin. The names of the measures to be determined are 'intima-media thickness' (IMT, the thickness of your artery walls), 'pulse wave velocity' (PWV, the speed at which your blood travels) and carotid artery diameter. You will be fully clothed for all procedures and there will be nothing invasive. Ultrasound will simply involve putting gel on a probe which will be rolled on the surface of the skin to give an image on a computer-like screen. You will not feel any pain and may feel only very slight discomfort, if any. At the same time, an ECG will be set up to non-invasively measure the electrical activity of your heart. This will involve placing 3 small patches called electrodes on your chest and abdomen. Please note that it may be necessary to shave or clip some hair so the patches stick to the skin. These ultrasound-based arterial health measures will be taken 3 times in total (before, halfway through and after the intervention period).

Bloods: Don't worry! Your blood sample will be taken using a very basic prick of your finger, so there will be no needles and hardly any visible blood. Samples will be taken 3 times in total (pre, mid and post-intervention). They will enable us to fill out a CRA form and compare the results before and after 8 weeks. If you wish that we return your blood samples to you upon completion of the study, please let us know before the study starts.

VO_{2peak} test: This is simply just a fitness test. It will be carried out once during session 1 and at the end of the 8 week study period. It involves cycling in the laboratory while wearing a snorkel-like mask to measure your breath. The test starts easy and gets progressively harder until you cannot continue. The test is maximal which means that it usually stops when you are completely exhausted and cannot continue pedalling.

If you are in the intervention group, your heart rate (HR) during the RPM sessions will be monitored. You will be assigned a HR strap and will be requested to wear it at every class you book into (see page 5). You will be given instructions as to how to use and store it.

Your body mass will be recorded 10 times in total (before, weekly and after the 8 week intervention phase as well as weekly, throughout the 8 weeks).

Note on assessment timings and preparation: All your ultrasound-based assessments will be carried out at the same time of day, at your convenience. This should ideally be in the morning (between 8:30-11:30am) as a fasted state (of 9-11 hours) is preferred for these tests. Due to this, refreshments (drinks and a light snack) will be provided upon completion of testing. You will also be required to avoid vitamin supplementation throughout the study and will be asked to avoid caffeine, alcohol, and high-fat meals for at least 12 hours before testing.

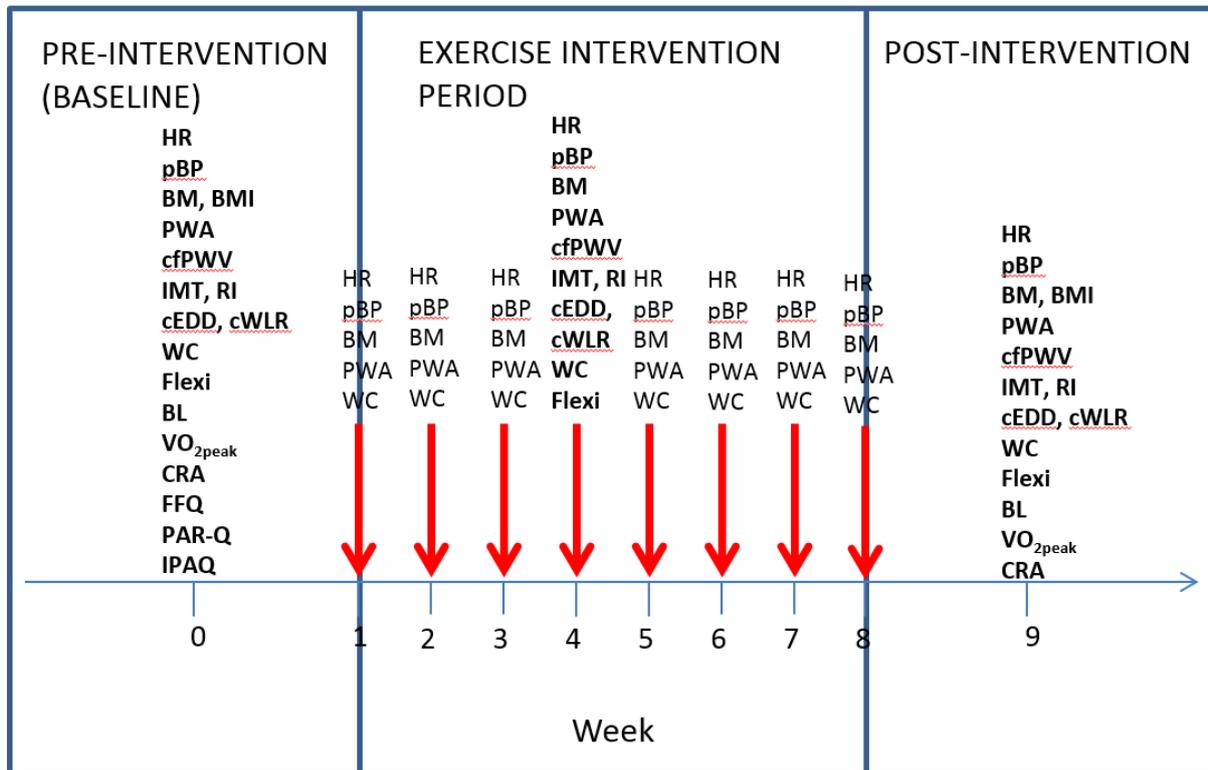


Figure 1: General plan of the study indicating timeline of assessments

The assessment protocol was identical between the control and intervention groups. PRE, baseline (pre-intervention, week 0); MID, mid-intervention (end of week 4); POST, post-intervention (end of week 8). HR, heart rate; pBP, peripheral blood pressure; WC, waist circumference; BMI, body mass index; BM, body mass; Flexi, flexibility; BL, bloods; CRA, cardiovascular risk assessment; IPAQ, International Physical Activity Questionnaire; PAR-Q, Physical Activity Readiness Questionnaire; FFQ, Food Frequency Questionnaire; PWA, BP+-derived measures of arterial health: central blood pressure & augmentation index' cfPWV, carotid-femoral pulse wave velocity; IMT, common carotid and common femoral intima-media thickness; RI, common carotid and common femoral resistivity index; cEDD, common carotid end-diastolic diameter; cWLR, common carotid wall:lumen ratio

Table 2: Summary of physiological assessment session timings and locations - CONTROL GROUP

Week no.	Measures	Location
0	Familiarization and baseline testing: Consent, IPAQ, PAR-Q, HR-QOL, FFQ, BM, AS, HR, BP, BL, CRA, IMT, PWV, VO _{2peak}	AUT Millennium
1	HR, BP, BM, AS	AUT Millennium
2	HR, BP, BM, AS	AUT Millennium
3	HR, BP, BM, AS	AUT Millennium
4	HR, BP, BM, AS, IMT, PWV	AUT Millennium
5	HR, BP, BM, AS	AUT Millennium
6	HR, BP, BM, AS	AUT Millennium
7	HR, BP, BM, AS	AUT Millennium
8	HR, BP, BM, AS	AUT Millennium
9	HR, BP, BM, AS, IMT, PWV, VO _{2peak} , BL, CRA, HR-QOL	AUT Millennium

HR, resting heart rate; BP, resting blood pressure; BM, body mass; AS, arterial stiffness; BL, blood chemistry; VO_{2peak}, fitness test; CRA, Cardiovascular Risk Assessment; HR-QOL, health-related quality of life; FFQ, Food Frequency Questionnaire; PAR-Q, Physical Activity Readiness Questionnaire; IPAQ, International Physical Activity Questionnaire

If you have any personal or cultural issues regarding the above procedures please let the primary researcher know prior to the study so that these can be accommodated for.

Table 1: Summary of sessions – INTERVENTION GROUP

NB: In weeks 4 and 8, you will be doing your normal 3 RPM™ sessions in addition to having to come to AUT Millennium for physiological assessments.

Week no.	Measures	Location	RPM sessions at Les Mills Vic. St. (intervention group only)
0	Familiarization and baseline testing: Consent, IPAQ, PAR-Q, HR-QOL, FFQ, AS, HR, BP, BM, BL, CRA, ultrasound VO _{2peak}	AUT Millennium	No RPM
1	HR, BP, BM, AS	Les Mills Vic. St/Taka	3xRPM technique classes
2	HR, BP, BM, AS	Les Mills Vic. St/Taka	3xRPM classes
3	HR, BP, BM, AS	Les Mills Vic. St/Taka	3xRPM classes
4 – session 1	HR, BP, BM, AS	Les Mills Vic. St/Taka	3xRPM classes
4 – session 2	ultrasound	AUT Millennium	
5	HR, BP, BM, AS	Les Mills Vic. St/Taka	3xRPM classes
6	HR, BP, BM, AS	Les Mills Vic. St/Taka	3xRPM classes
7	HR, BP, BM, AS	Les Mills Vic. St/Taka	3xRPM classes
8	HR, BP, BM, AS	Les Mills Vic. St/Taka	3xRPM classes
9	HR, BP, BM, AS, ultrasound, VO _{2peak} , BL, CRA, HR-QOL	AUT Millennium	No RPM

HR, resting heart rate; BP, resting blood pressure; BM, body mass; AS, arterial stiffness; BL, blood chemistry; VO_{2peak}, fitness test; CRA, Cardiovascular Risk Assessment; HR-QOL, health-related quality of life; FFQ, Food Frequency Questionnaire; PAR-Q, Physical Activity Readiness Questionnaire; IPAQ, International Physical Activity Questionnaire

If you have any personal or cultural issues regarding the above procedures please let the primary researcher know prior to the study so that these can be accommodated for.

What are the discomforts and risks?

You may feel a small pinch on your finger when blood is being drawn but this should last less than 2 seconds and depending on you as an individual, you may bleed extremely little or slightly more from the site of the prick on your finger. Again, there should not be a lot of blood and it should not bleed for more than a few minutes, if that!

During testing: the only discomfort you may feel is when a cuff placed around your arm inflates and compresses your artery during the arterial stiffness measurements. This will last for no longer than 15 seconds. The cuff will not be too tight and it is totally safe! During the ultrasound assessments, you may feel slight pressure from the probe on your neck and groin but this would be extremely light pressure and should not be uncomfortable. As you will need to be tested when in a fasted state, you may be hungry before and during the assessment procedures (weeks 0, 4 and 9) but you will be provided with free drinks and snacks at the end of the assessment session!

During exercise: during the fitness test, exercise intensity may be quite high and you will feel tired and breathless and your muscles may even feel fatigued. However, the test will occur in a controlled, supervised environment.

If you are in the intervention group, during the indoor cycling (rpm) sessions, you may feel tired, thirsty, fatigued and have slightly sore muscles from previous sessions but this is normal when exercising, particularly if you are new to exercise. We understand that you may not be familiar with exercise but you can rest assured that these sessions will also be carried out in safe, controlled environments. In addition, you will be given technique classes to ensure that you are exercising safely and have proper form when cycling. As with any physical activity, there is always a small chance of injury but we will try to avoid this by ensuring proper technique, a warm-up and cool-down.

What are the benefits?

You will benefit from being part of this study as you will receive a lot of information about different aspects of your health status (eg. blood profile, fitness levels and cardiovascular, in particular, arterial health). The tests are normally very costly and are therefore not carried out often on healthy people in the general public.

You shall be introduced to exercise in a safe way and although you will not be able to participate in other physical activity during the study, it will hopefully make you interested in exercise and enable you to keep it up after the study has been completed.

For those initially allocated to the control group for the first 10 weeks, a 2 week free Les Mills gym trial at the end of the study will be provided and there will be no joining fee if you decide to obtain Les Mills membership.

For those initially allocated to the control group for the first 10 weeks, free 4 week access to RPM™ rides will be provided as will a 2 week free Les Mills gym trial at the end of the study, plus no joining fee if you decide to become a member of Les Mills.

What compensation is available for injury or negligence?

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

How will my privacy be protected?

All the data obtained during this study will only be available to the researchers involved. If the data is published in the public domain your name as a subject will not be revealed and you will remain anonymous. The only information you will need to provide to a third party would be your personal details (ie. who you are and your contact details). This is required to allow you free access into the Les Mills gym.

What are the costs of participating in this research?

The cost of participating in this study will be the time you will have to put aside for it. If you are initially allocated to the intervention group, you will need to set aside a total of 24 hours for the exercise sessions and a total of about 6 hours for testing sessions (this time excludes travel time to and from the venue). If you are initially allocated to the control group, you will need to come in for a total of about 9 hours during the first 10 weeks and then set aside 24 hours during the next 8 weeks (includes exercise and assessment sessions) when you have the opportunity to go through the intervention.

Will I receive feedback on the results of this research?

At the end of the study, verbal feedback will be given to you and written feedback can be provided upon request. Your results from the study will only be shared with your doctor if you are at any health risk and you grant us permission to do so.

What do I do if I have concerns about this research?

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor:

Name: Associate Professor Andrew Kilding

E-mail: andrew.kilding@aut.ac.nz

Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEK, Kate O'Connor, ethics@aut.ac.nz, 921 9999 ext 6038.

Whom do I contact for further information about this research?

Researcher contact details

Name: Shivani Sethi; E-mail: shivani.sprinz@gmail.com

Project Supervisor contact details

Name: Dr. Andrew Kilding; E-mail: andrew.kilding@aut.ac.nz

Approved by the Auckland University of Technology Ethics Committee on the 26th August 2014, AUTEK Reference number 14/242.

APPENDIX J: CONSENT FORM



Consent Form

Project Title: *Investigation into the effects of a group fitness interval aerobic exercise training programme on arterial health in healthy sedentary male adults*

Project Supervisors: *Assoc. Prof. Andrew Kilding and Dr. Andrew Lowe*

Researcher: *Shivani Sethi*

- I have read and understood the information provided about this research project (Information Sheet dated 25th March 2015).
Yes/No
- I have had an opportunity to ask questions and to have them answered.
Yes/No
- I am in good health and am not currently suffering from any injury or illness which may impair my physical performance
Yes/No
- I agree to provide blood and samples and will inform the researchers before participation if I require my samples to be returned after analysis.
Yes/No
- I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
Yes/No
- I am happy for my details to be passed to Les Mills for the purpose of admission to classes as part of the 8 week intervention.
Yes/No
- I agree to take part in this research.
Yes/No
- I agree to allow the use of my collected data to be used for research, including journal publications and post-graduate thesis
Yes/No
- I understand my data collected will be de-identified prior to analysis and will be held for the purpose of research only (by the names researcher and supervisor) for a period of three years.
Yes/No

Participant signature: _____

Participant name: _____

Date: _____

Project Supervisor Contact Details:

Associate Professor Andrew Kilding
Sports Performance Research Institute, New Zealand (SPRINZ)
AUT Millennium
17 Antares Place, Mairangi Bay, 0632
Contact: 09 921 9999 x 7056
Email: andrew.kilding@aut.ac.nz

For Administrative Use
Participant ID:

Approved by the Auckland University of Technology Ethics Committee on the 26th of August 2014

APPENDIX K: EXAMPLE OF ACTIVITY LOG FOR INTERVENTION GROUP



SPORTS PERFORMANCE
RESEARCH INSTITUTE, NEW ZEALAND
AN INSTITUTE OF AUT UNIVERSITY

ACTIVITY LOG

This is an activity log which you are required to **keep throughout the duration of the study**. It will provide an indication of your activity levels during this period so we can ensure that you technically remain 'sedentary' throughout the intervention other than doing the 3 RPM classes each week. It will be **checked on a weekly basis** so please bring it with you to the last RPM session of each week, when you will be tested at Les Mills Takapuna/Victoria Street.

Please try to be as accurate and as honest as possible when filling in the log. You can either keep it handy and fill it in as you do any activity or you can fill it in at night and try to recall what you did that day.

You will be required to complete this log on **3 days every week**. Please try and include:

1) A weekday when you do not do an RPM session

2) A weekday in which you go for an RPM session

3) A weekend day (or any day that you consider your normal 'weekend day') **with/without an RPM session**

For any one day, note down each physical activity, the length of time you did it for and how hard you thought it was. To gauge the intensity (column 4), an **RPE scale** has been provided with this activity log and it will assist you in filling out this section. When recording RPE, **think about the intensity of the overall activity session** and how it felt, not just a certain time point in it. **Include any low, medium and high-intensity physical activities** (ie. Easy, medium and difficult physical activities). **Examples of these have been provided** with this log. Please indicate approximately **how many hours you slept** that day (12am at the start of that day until the time you woke up that day plus the time you slept that day until 12am the next day). Some everyday activities have been listed to help you out.

Underneath the main table for the activity log for each week is a small table indicating the **RPE for JUST the 3 RPM sessions** for the week. Please indicate the date you attended the RPM session, your subjective, perceived intensity of the session (see RPE scale) and add any further comments!

Please note: when recording the intensity (RPE) for the RPM session, **think about the session as a whole and how the overall class felt** (ie. try not to simply think of the single hardest point and allocate a number to that specific feeling).

RPE scale

This scale will enable you to gauge the intensity of each physical activity that you do. It is very subjective so try and pick the number which best represents how you felt during each activity

RPE	DESCRIPTION
0	Complete Rest; Nothing at all; Sleeping; sitting quietly
1	Very, very easy; Very light; Just noticeable; Standing
2	Easy; Weak; Light
3	Moderate
4	Somewhat hard
5	
6	Hard; Strong; Heavy
7	
8	Very hard; very strong
9	Near maximal
10	Maximal; Extremely hard; about to give up
11	All-out effort; unable to continue; Exhaustion; Absolute maximum; Highest possible

DAY OF THE WEEK	Activity	Duration (hours or mins)	Intensity (RPE)
Day 1	Sleeping		
Weekday	Sitting		
RPM	Standing		
	Walking		
	RPM		
Day 2	Sleeping		
Weekday	Sitting		
No RPM	Standing		
	Walking		
Day 3	Sleeping		
Weekend day	Sitting		
(RPM/no RPM)	Standing		
	Walking		

Date (on the Monday):

WEEK:

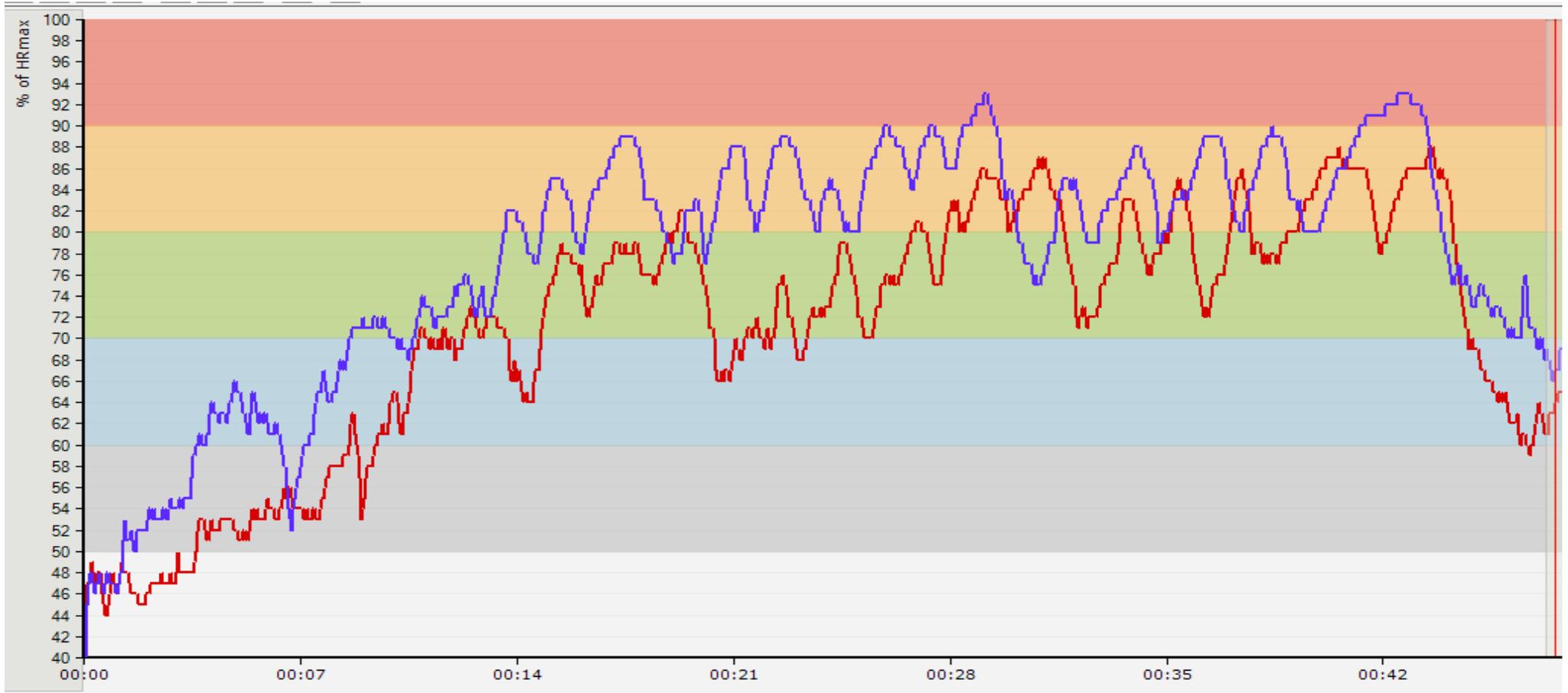
	Date	Intensity (RPE)	Comments
RPM session 1			
RPM session 2			
RPM session 3			

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Participant ID:

APPENDIX L: OFFICIAL DESCRIPTION OF AN RPM™ CLASS

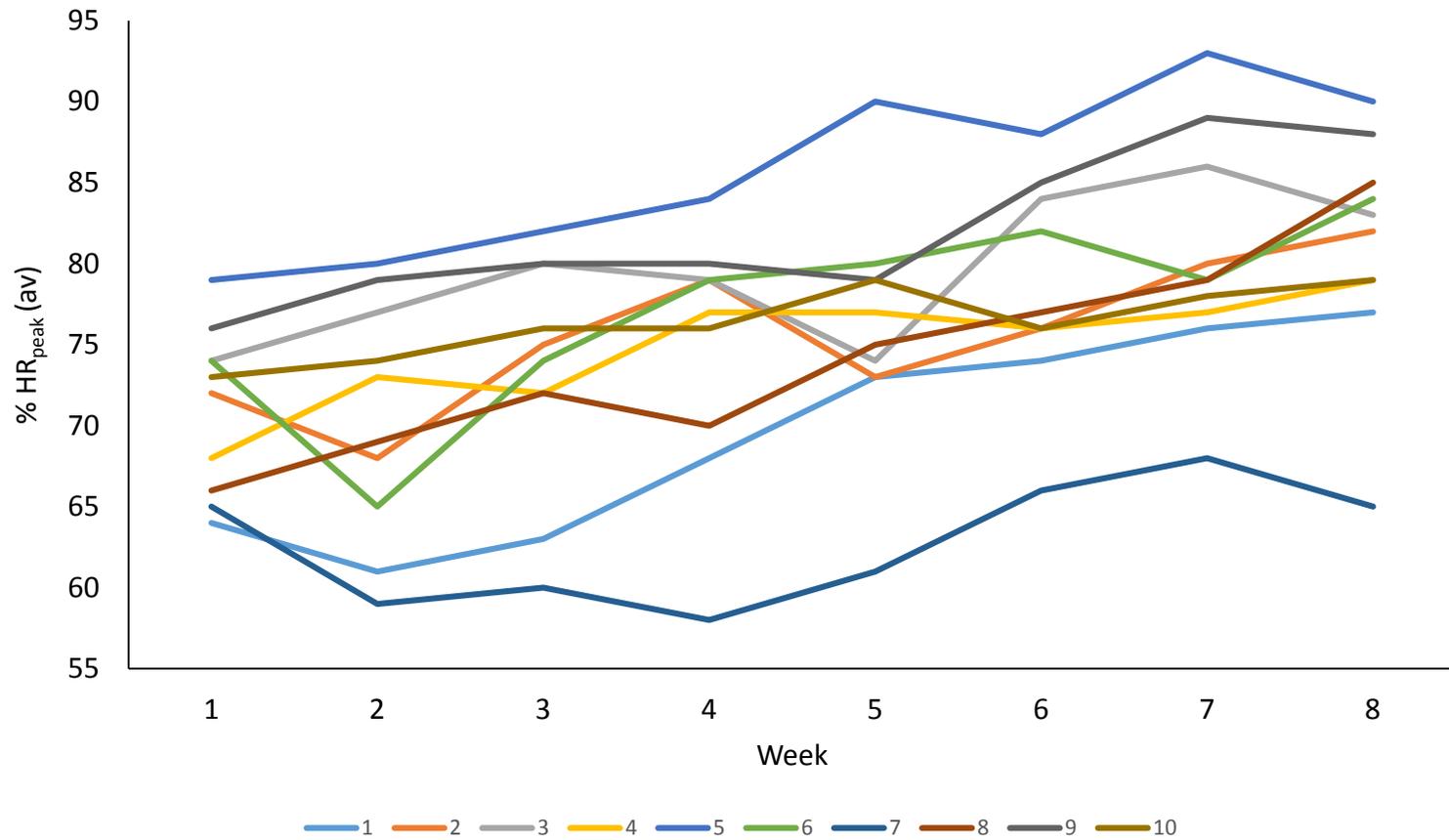
TRACK #	TRACK NAME	TRAINING OBJECTIVE
1	Pack Ride	To warm the riding muscles by establishing base and working resistance. We prime the neuro muscular system by riding at a moderate pace.
2	Pace	To use speed and resistance to enter an aerobic training zone and get familiar with the different cadences (leg speed), resistance (load) and riding positions which will be experienced in the class.
3	Hills	To develop muscular strength and power through increased load and slower cadences in Seated and Standing Climbs.
4	Mixed Terrain	To actively recover from the hill track then focus on challenging our aerobic fitness through the integration of speed intervals and climbs.
5	Intervals	To drive into an anaerobic training zone through short blocks of high-intensity (big resistance/load) interspersed with recovery periods. This is the most physically demanding track of the class.
6	Speed Work	To first experience active recovery following the interval track, then train speed endurance – gradually building effort with repeated sprints
7	Mountain Climb	To increase strength endurance through big resistance and slower cadence. This track will occasionally include power intervals – combining high loads with increased pace for short periods.
8	Ride Home	To flush the legs and stretch our working muscles to enhance recovery.

APPENDIX M: EXAMPLE OF RAW HEART RATE DATA FROM POLAR TEAM²



Sample overlay of heart rate data for a single subject, collected using the Polar Team² unit during a 45 minute RPM™ class in week 1 (red) and week 8 (purple). HR_{peak} week 8 > week 1 ($p < 0.05$). Note: similar HR patterns are seen between classes, but they are not exact overlays due to the unique music and choreography of each class.

APPENDIX N: AVERAGE WEEKLY INTENSITY DATA FOR EACH SUBJECT OVER ALL 8 WEEKS



Average weekly RPM™ heart rate (as %HR_{peak}) for all ten participants in the intervention group (separate lines) over the 8 weeks. The mean %HR_{peak} (average) of the three weekly classes was calculated for each individual. There was a general trend for average heart rates during a class to increase each week over the 2 months and average intensities in week 8 were greater than week 1 ($p < 0.05$).