Arterial Stiffness and Abdominal Aortic Aneurysm: The Effect of Acute Exercise

A thesis presented by

Maria Christina Perissiou

BSc. Sports and Exercise Science,
National and Kapodestrian University of Athens, Greece

MSc. Biology of Exercise,
National and Kapodestrian University of Athens, Greece

Primary supervisor: A/Prof Chris Askew
Co-supervisor: Dr Tom Bailey
Associate Supervisors: Prof Jonathan Golledge, A/Prof Anthony Leicht

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

School of Health and Sport Sciences
Faculty of Science, Health, Education and Engineering
University of the Sunshine Coast

December 2017
Student ID: 1082564
Abstract

**Abdominal aortic aneurysm (AAA)** is a localised dilatation of the abdominal aorta leading to an increase in normal diameter by >50%. AAA accounts for approximately 2% of all deaths worldwide (Upchurch and Schaub 2006). Surgical repair is the only current treatment option but only available for patients with large AAA (≥55mm) (Nordon, Hinchliffe et al. 2011) leaving patients with small AAA (≤55 mm) with no treatment options. Increased arterial stiffness is a key factor characterising the development and growth of an AAA (Raaz, Zollner et al. 2015) and likely contributes to elevated cardiovascular mortality in patients with small AAA (Golledge, Muller et al. 2006, Bath, Gokani et al. 2015). Therefore, arterial stiffness could be used as an important treatment target in order to delay aneurysm progression and reduce cardiovascular risk. Arterial stiffness is reduced with increased cardiorespiratory fitness (Gando, Murakami et al. 2016) and aerobic-exercise training in older adults (Fujie, Sato et al. 2014). These adaptations are attributed to the frequent, repetitive haemodynamic perturbations associated with each bout of exercise (Green, Hopman et al. 2017). A single bout of exercise transiently lowers arterial stiffness in healthy adults (Kingwell, Berry et al. 1997, Mutter, Cooke et al. 2017), is commonly accompanied by short-term improvements in arterial function and central blood pressure (Millen, Woodiwiss et al. 2016) and may be dependent on exercise intensity (Tordi, Mourot et al. 2010). However, in adults with cardiovascular comorbidities commonly observed in patients with AAA, excessive increases in arterial stiffness during and after exercise (Shim, Yang et al. 2011, Gkaliagkousi, Gavriilaki et al. 2014, Bunsawat, Ranadive et al. 2017) may transiently exacerbate cardiovascular risk (Schultz, La Gerche et al. 2017). To date, little is known about the haemodynamic effects of acute exercise in patients with AAA. Determining the arterial stiffness response to exercise would provide better understanding of the short-term risks and benefits of exercise in patents with AAA, and an insight into the potential efficacy of exercise for reducing arterial stiffness and cardiovascular risk. Hence, this thesis aimed to determine the effect of a single bout of exercise on the post-exercise arterial stiffness response between patients with AAA and healthy older adults. As patients with AAA are older and with low levels of cardiorespiratory fitness, this thesis also explored the effects of cardiorespiratory fitness and exercise intensity on post-exercise arterial stiffness, in healthy older adults. Additionally, this thesis aimed to establish the reliability of its measurements by assessing the reliability of post-exercise changes in arterial stiffness in older healthy adults.
**Study one** investigated the reliability of arterial stiffness indices in ten healthy older adults at rest and during 60 min recovery after a single bout of moderate intensity continuous aerobic exercise. At rest, immediately after and during exercise recovery, intraclass correlation coefficient (ICC) values indicated excellent reliability for carotid-femoral pulse wave velocity (PWV) (0.87 to 0.91), good reliability for augmentation index corrected for heart rate (AIx75) (0.64 to 0.87) and adequate reliability for reflection magnitude (RM) (0.41 to 0.60). This study established the reliability of arterial stiffness indices at rest, immediately after exercise and during supine recovery from a bout of moderate-intensity cycling exercise in healthy older adults. Establishing the precision of post-exercise changes in arterial stiffness indices in older adults, justified and strengthened the use of these measures in Studies 2 and 3.

**Study two** assessed the effects of cardiorespiratory fitness and exercise intensity on post-exercise arterial stiffness in fifty-one older adults (age: 72 ±5 y) who were stratified into fitness tertiles ($\overline{V}O_2$peak: low-, 22.3 ±3.1; mid-, 27.5 ±2.4; high-fit 36.3 ±6.5 ml.kg⁻¹.min⁻¹). In a randomised, counter-balanced order, participants underwent moderate-intensity continuous (40% of peak power output; PPO) or higher-intensity interval cycling exercise (12×1 min at 70%, separated by 1 min at 10% PPO) or a control (no-exercise) protocol. PWV, AIx75 and RM were assessed at rest and for 90 min following each protocol. AIx75 at rest was higher in the low-fit group compared to the higher-fit group (P<0.010). There were no differences in PWV and RM between fitness groups. PWV and RM were lower after higher-intensity interval exercise compared to control in all fitness groups (P<0.001). PWV after moderate-intensity continuous exercise was not different compared with control in the low-fit group (P=0.057), but was lower in the mid and higher-fit groups. Post-exercise AIx75 was higher than control (P<0.001) in all the fitness groups. This study demonstrates that submaximal exercise may reveal important differences in arterial stiffness between adults with higher and lower levels of cardiorespiratory fitness. Higher-intensity interval exercise may be an effective strategy to reduce post-exercise PWV and RM in older adults of all fitness levels. Establishing the effect of exercise intensity and cardiorespiratory fitness on the arterial stiffness response to exercise in older healthy adults, enabled this thesis to further explore if the presence of AAA affects this response to exercise.

**Study three** assessed the post-exercise arterial stiffness response between patients with AAA (n=22; AAA diameter: 36 ±5 mm; age: 74 ±6 y) and healthy older age-matched adults (n=22; age: 72 ±5y). The exercise protocols used in study three were the same as those adopted in study two. At rest, PWV was higher in AAA compared with healthy older adults (P<0.001),
whilst AIx75 and RM were not different between groups. There were no differences in post-exercise changes in arterial stiffness between patients with AAA and healthy adults. Post-exercise changes for AIx75 were similar compared with control. Conversely, post-exercise, PWV and RM were lower compared with control at all time-points (P<0.05). After higher-intensity interval exercise, PWV was lower at 0 (P=0.015) and 40 (P=0.044) min post compared with after moderate-intensity continuous exercise. In patients with AAA and healthy older adults, a single bout of exercise similarly lowers PWV and RM compared to control. Higher intensity interval exercise tends to further reduce post-exercise PWV in both groups.

The results of these series of studies provide new knowledge on the post-exercise arterial stiffness response in healthy older adults and patients with AAA. The findings of this thesis suggest that post-exercise arterial stiffness is similar between patients with small AAA and age-matched, healthy adults. Importantly, higher-intensity interval exercise may be an effective strategy to lower post-exercise PWV in older adults of low cardiorespiratory fitness and patients with AAA. Based on the suggestion that acute post-exercise reductions in arterial stiffness contribute to chronic vascular adaptations with training, this thesis suggests that exercise intensity and cardiorespiratory fitness should be considered when prescribing an exercise intervention, in order to improve vascular health in older healthy adults and patients with AAA.
Declaration of Originality

I, Maria Christina Perissiou, certify that the work in this thesis entitled "Arterial Stiffness and Abdominal Aortic Aneurysm: The Effect of Acute Exercise" has not previously been submitted for a degree, or as part of requirements for a degree, to any university or institution other than the University of the Sunshine Coast.

I also certify that this thesis is an original piece of research, and the work included within this thesis is my own. Where work has been done conjointly with other persons, my contribution is clearly stated and the contribution of other persons is clearly acknowledged and recognised.
Acknowledgements

I would like to firstly thank my Primary Supervisor, A/Prof. Chris Askew. I will always remember googling Sunshine Coast in order to explain to my parents where I am going. Chris, you offered me an opportunity that changed my life and made a dream of mine come true. These 3 and a half years were a great experience and I will always be grateful for that. Your support, experience and guidance throughout my PhD journey were a significant help for my candidature. Importantly, you helped me to grow up (better late than ever!), as a researcher and as a person. Thank you for everything.

To my co-supervisor Dr Tom Bailey. I feel so lucky that I had you by my side during this journey. And what a journey that was! After endless hours in the lab, about a million e-mails, countless meetings and numerous mood swings of mine, this thesis came together. Your help, support, patience and humour (yes, it is not that bad) through this roller coaster journey made my PhD manageable and possible. I will always be grateful for all these and your friendship through this. It’s been a great pleasure working with you. Thank you Tom.

I would like to thank my co-supervisors Prof. John Golledge and A/Prof. Anthony Leicht for their guidance and support during my candidature. Your experience and (always) fast feedback have been a great and valuable help through my PhD.

I would like to thank the staff at Nambour General Hospital, the Royal Brisbane and Women’s Hospital and Sunshine Vascular Clinic for their support in this project. I would also like to thank the University of the 3rd Age in helping to recruit participants for this research project. A big thank you to the participants of this thesis. None of these would be possible without them!

To Mark, my research brother. Who knew that after three and a half years in Australia I will become a Bristol Rovers fan! You are a great teammate and a true friend! It was great to share with you all the long hours in the lab and the office, the long football debates, travels and the joys and frustrations of this PhD. A big thank you also to the rest of our VasoActive research team! Supporting each other through our candidatures made certainly things easier. I am looking forward to the future when we all catch-up over a beer and have a good laugh about our joint experience!

To my family back home. You have always supported and believed in me and this means everything to me. Through these three and a half years you have been always there for me and helped me to go through all the difficulties I had to encounter. I love you guys. In one word, ευχαριστώ.

To the Okinja Rd. gang, my local family. Thank you for being there sharing all these great adventures with me across the Coast, Australia and even overseas! Thank you for keeping me sane and taking me out “just for a beer” whenever I was lost in my PhD bubble, even though we all knew that was a lie. You are great friends and I will always value our friendship.
Contents

Abstract .......................................................................................................................... 2
Declaration of Originality ........................................................................................... Error! Bookmark not defined.
Acknowledgements ...................................................................................................... 6
Contents .......................................................................................................................... 7
List of figures ................................................................................................................ 11
List of tables .................................................................................................................. 12
List of abbreviations .................................................................................................... 13
1. Introduction ............................................................................................................. 15
   1.1. Aims of this thesis .............................................................................................. 17
2. Literature review ...................................................................................................... 18
   2.1 Abdominal aortic aneurysm ................................................................................ 18
       2.1.1 Definition of abdominal aortic aneurysm ..................................................... 18
   2.2 Structure of the healthy aorta ............................................................................ 18
   2.3 AAA pathophysiology ....................................................................................... 19
   2.4 Biomechanical determinants of AAA ................................................................. 20
   2.5 AAA epidemiology and risk factors .................................................................... 22
   2.6 Mortality in AAA ............................................................................................... 24
       2.6.1 Aneurysm rupture mortality risk ................................................................. 24
       2.6.2 Cardiovascular disease mortality risk .......................................................... 25
       2.6.3 AAA treatment ............................................................................................ 25
       2.6.4 Exercise training in patients with AAA ....................................................... 28
   2.7 Arterial stiffness .................................................................................................. 30
       2.7.1 Measures of arterial stiffness ...................................................................... 31
       2.7.2 The effect of age on arterial stiffness ............................................................ 36
       2.7.3 The effect of sex on arterial stiffness ............................................................. 40
       2.7.4 Cardiovascular disease and arterial stiffness ............................................... 40
       2.7.5 The importance of arterial stiffness in AAA ............................................... 42
   2.8 Arterial stiffness and exercise ............................................................................. 43
       2.8.1 The relationship between cardiorespiratory fitness and arterial stiffness ..... 43
       2.8.2 Aerobic exercise training and arterial stiffness ............................................. 44
       2.8.3 Aerobic exercise training and arterial stiffness in older adults ................. 44
2.8.4. Aerobic exercise training and arterial stiffness in patients with cardiovascular disease ................................................................. 45
2.8.5. Aerobic exercise training and arterial stiffness in patients with hypertension ................................................................. 45
2.9. Acute arterial stiffness response to a single bout of exercise ................................................................. 46
  2.9.1. Arterial stiffness response to a single bout of exercise in healthy young adults 47
  2.9.2. Acute arterial stiffness response to exercise and cardiovascular risk ............. 48
  2.9.3. Reliability of arterial stiffness indices in response to exercise ...................... 48
  2.9.4. Characteristics and factors that affect the arterial stiffness response to a single bout of exercise ................................................................. 49
2.10. Summary .................................................................................................................................................. 52

3. Reliability of arterial stiffness indices at rest and following a single-bout of moderate-intensity exercise in older adults ................................................................. 54
  3.1. Introduction .................................................................................................................................................. 55
  3.2. Methods ...................................................................................................................................................... 56
    3.2.1. Participants .............................................................................................................................................. 56
    3.2.2. Experimental Overview ..................................................................................................................... 57
    3.2.3. Maximal incremental cycling test ...................................................................................................... 57
    3.2.4. Moderate-intensity continuous exercise (visits 1-3) ......................................................................... 58
    3.2.5. Arterial stiffness measurements: ......................................................................................................... 58
    3.2.6. Statistical Analyses: ............................................................................................................................ 60
  3.3. Results ......................................................................................................................................................... 61
  3.4. Discussion .................................................................................................................................................. 66
    3.4.1. Reliability of resting arterial stiffness indices ...................................................................................... 66
    3.4.2. Time-course of the changes in arterial stiffness after exercise ......................................................... 67
    3.4.3. Reliability of arterial stiffness immediately after exercise ................................................................. 67
    3.4.4. Reliability of arterial stiffness indices during exercise recovery ...................................................... 68
    3.4.5. Clinical significance ............................................................................................................................ 68
    3.4.6. Limitations ............................................................................................................................................. 69
    3.4.7. Conclusion .......................................................................................................................................... 69

4. Effects of Exercise Intensity and Cardiorespiratory Fitness on the Acute Response of Arterial Stiffness to Exercise in Older Adults ........................................................................... 70
  4.1. Introduction ............................................................................................................................................... 71
  4.2. Methods ................................................................................................................................................... 72
4.2.1. Experimental Overview..................................................................................72
4.2.2. Participants..................................................................................................72
4.2.3. Maximal incremental cycling test.................................................................73
4.2.4. Experimental exercise and control protocols................................................73
4.2.5. Arterial stiffness measurements.................................................................73
4.2.6. Statistical analyses......................................................................................73

4.3. Results ...........................................................................................................74
4.3.1. Participant characteristics...........................................................................74
4.3.2. Heart rate, mean arterial pressure and perceived exertion during the experimental protocols ..........................................................75
4.3.3. Arterial stiffness and hemodynamic indices at baseline and in response to exercise...........................................................................76
4.3.4. Baseline arterial stiffness indices..................................................................76
4.3.5. Post-exercise arterial stiffness and hemodynamic indices.........................76

4.4. Discussion ......................................................................................................83
4.4.1. Arterial stiffness at rest ..............................................................................83
4.4.2. Post-exercise arterial stiffness......................................................................84
4.4.3. Conclusions.................................................................................................87

5. Arterial Stiffness Response Following a Bout of Exercise in People with and without Abdominal Aortic Aneurysm..............................................88

5.1 Introduction ..................................................................................................89

5.2 Methods ..........................................................................................................90
5.2.1 Participant recruitment ..............................................................................90
5.2.2 Maximal incremental cycling test ...............................................................91
5.2.3 Experimental exercise and control protocols...............................................91
5.2.4 Arterial stiffness measurements.................................................................91
5.2.5 Statistical analysis......................................................................................91

5.3. Results ...........................................................................................................92
5.3.1. Participant characteristics...........................................................................92
5.3.2. Heart rate, mean arterial pressure and perceived exertion during the exercise protocols..........................................................94
5.3.3. Central blood pressure and arterial stiffness indices at baseline and in response to exercise...........................................................................94
5.3.4. Baseline central blood pressure and arterial stiffness indices...............94
5.3.5. Post-exercise central blood pressure and arterial stiffness indices ..........94

5.4. Discussion ..................................................................................................................101
  5.4.1. Arterial stiffness at rest ..........................................................101
  5.4.2. Post-exercise arterial stiffness..............................................102
  5.4.2. Conclusion......................................................................................104

6. General discussion.......................................................................................................105
  6.1 Arterial stiffness measures are reliable at rest and in response to a bout of exercise in older adults ..........................................................105
  6.1 Acute response of arterial stiffness to exercise ..................................................107
  6.2 Interpretation of post-exercise change in arterial stiffness indices and the importance of the control protocol ..........................................................109
  6.3 Acute response of arterial stiffness to exercise is intensity dependent ............111
  6.4 Acute response of arterial stiffness to exercise depends on cardiorespiratory fitness ..................................................................................................112
  6.5 Response of arterial stiffness to exercise is similar in patients with AAA and healthy older adults .................................................................114
  6.6 Methodological considerations and research limitations .............................115
  6.7 Summary of findings .........................................................................................116

7. References ..................................................................................................................117

8. Appendix ....................................................................................................................161
  8.1 Published version of Chapter 3 ........................................................................161
  8.2 Published version of Chapter 4 ........................................................................169
List of figures

**Figure 1.** An abdominal aortic aneurysm (Source: www.vascularweb.org)..........................18

**Figure 2.** Carotid-femoral pulse wave velocity (PWV) .........................................................32

**Figure 3.** The effect of intra-arterial infusion of endothelin-1 (ET-1) on aortic wave velocity (PWV) .................................................................................................................................34

**Figure 4.** Representation of the central arterial waveform ..................................................36

**Figure 5.** Regression curves representing the effect of age on carotid-femoral pulse wave velocity for males and females ..........................................................38

**Figure 6.** Regression curves representing the effect of age on augmentation pressure ..........39

**Figure 7.** Regression curves representing the effect of age on augmentation pressure ..........39

**Figure 10.** Absolute PWV, Alx75 and RM at rest and post-exercise ..............................64

**Figure 11.** Delta RM, response of low-fit, mid-fit and high-fit to control, moderate continuous and high-intensity interval exercise ..............................................................82

**Figure 12.** Delta PWV response of AAA and healthy groups to no-exercise control, moderate-intensity continuous and higher-intensity interval exercise .................................98

**Figure 13.** Delta Alx75 response of AAA and healthy groups to no-exercise control, moderate-intensity continuous and high-intensity interval exercise .................................99

**Figure 14.** Delta RM response of AAA and healthy groups to no-exercise control, moderate-intensity continuous and high-intensity interval exercise ..............................................100
List of tables

**Table 1.** Participant characteristics and maximal incremental test results..........................62

**Table 2.** Delta of PWV, Alx75 and RM from baseline at 0, 20, 40 and 60 min post exercise. ..............................................................................................................................................................63

**Table 3.** Reliability of absolute arterial stiffness indices at rest, immediately post exercise and during exercise recovery ..................................................................................................................................................................................64

**Table 4.** Participant characteristics............................................................................................................................75

**Table 5.** Arterial stiffness at baseline and after control, moderate continuous- and high-intensity interval exercise in low-, mid- and high-fit groups...............................................................78

**Table 6.** Heart rate and blood pressure indices at baseline and after control, moderate continuous- and high-intensity interval exercise in low, mid and high fit groups............79

**Table 7.** Characteristics of AAA patients and healthy adults. .............................................................93

**Table 8.** Arterial stiffness at baseline and after no-exercise control, moderate-intensity continuous and higher-intensity interval exercise in patients with AAA and healthy older adults .................................................................................................................................96

**Table 9.** Heart rate and blood pressure indices at baseline and after no-exercise control, moderate-intensity continuous and higher-intensity interval exercise in patients with AAA and healthy older adults..........................................................97
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA</td>
<td>Abdominal aortic aneurysm</td>
</tr>
<tr>
<td>AIx</td>
<td>Augmentation index</td>
</tr>
<tr>
<td>AIx75</td>
<td>Augmentation index corrected for heart rate</td>
</tr>
<tr>
<td>ANP</td>
<td>Atrial natriuretic peptide</td>
</tr>
<tr>
<td>AP</td>
<td>Augmented pressure</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>(e)</td>
<td>Elastic modulus</td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
</tr>
<tr>
<td>EVAR</td>
<td>Endovascular aneurysm repair</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow mediated dilatation</td>
</tr>
<tr>
<td>(h)</td>
<td>Wall thickness</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>High sensitivity C-reactive protein</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>(L)</td>
<td>Length</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>LMM</td>
<td>Linear mixed model</td>
</tr>
<tr>
<td>LRP-1</td>
<td>Low density lipoprotein receptor-related protein 1</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Pb</td>
<td>Backward pressure wave</td>
</tr>
<tr>
<td>Pf</td>
<td>Forward pressure wave</td>
</tr>
<tr>
<td>PP</td>
<td>Pulse pressure</td>
</tr>
<tr>
<td>PPO</td>
<td>Peak power output</td>
</tr>
<tr>
<td>PWV</td>
<td>Pulse wave velocity</td>
</tr>
<tr>
<td>(r)</td>
<td>Radius</td>
</tr>
<tr>
<td>(ρ)</td>
<td>Blood density</td>
</tr>
<tr>
<td>RC</td>
<td>Reliability coefficient</td>
</tr>
<tr>
<td>RM</td>
<td>Reflection magnitude</td>
</tr>
<tr>
<td>RPE</td>
<td>Rating of perceived exertion</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>(t)</td>
<td>Time</td>
</tr>
<tr>
<td>(v)</td>
<td>Volume</td>
</tr>
<tr>
<td>$\dot{V}O_2$</td>
<td>Rate of oxygen uptake</td>
</tr>
<tr>
<td>VSMC</td>
<td>Vascular smooth muscle cell</td>
</tr>
<tr>
<td>WSS</td>
<td>Wall shear stress</td>
</tr>
</tbody>
</table>
1. Introduction

Abdominal aortic aneurysm (AAA) is the localised dilatation of the abdominal aorta surpassing normal diameter by >50% (Upchurch and Schaub 2006). In Western countries, the prevalence of AAA reaches around 10% among those over the age of 65 years (Sakalihasan, Limet et al. 2005) and is responsible for ~2% of all deaths worldwide (Nordon, Hinchliffe et al. 2011). A progressive expansion of the untreated AAA leads to aneurysm rupture that often leads to death. Surgical or endovascular repair is currently the only effective treatment to prevent AAA rupture and aneurysm-related death but is generally only considered for patients with large AAA (≥55mm in diameter) (Lederle, Wilson et al. 2002). Consequently, there are no current therapeutic options available for patients with small AAA which still pose a small risk of rupture (Baxter, Terrin et al. 2008). Besides the risk of rupture as aneurysm diameter progresses, patients with small AAA also have a higher risk of cardiovascular complications and events compared with healthy older adults (Bath, Saratzis et al. 2017). Thus, there is a strong need to develop therapies that target AAA progression and cardiovascular risk.

Increased arterial stiffness has been shown to contribute to the development and progression of AAA (Hoegh and Lindholt 2009, Durmus, Kazaz et al. 2014). Increased arterial stiffness describes the reduced capability of an artery to expand and recoil in response to changes in pressure (Cecelja and Chowienczyk 2012), and is suggested to contribute to cardiovascular disease development (McEniery, Yasmin et al. 2005, Cecelja and Chowienczyk 2012). Carotid to femoral pulse wave velocity (PWV) quantifies the velocity of generated pulse waves between the two arterial segments and is the gold-standard index for the non-invasive measurement of aortic stiffness (Mitchell, Parise et al. 2004, Wilkinson, McEniery et al. 2010, Lee Stoner 2012). Additionally, systemic arterial stiffness is commonly measured by quantifying wave reflection characteristics, represented by augmentation index (AIX) and the novel reflection magnitude (RM) (Nichols and Singh 2002, Westerhof, Guelen et al. 2006). Increases in basal PWV, AIX and RM are independent predictors of the future risk of cardiovascular events (Mitchell, Hwang et al. 2010, Chirinos, Kips et al. 2012). Hence, reducing arterial stiffness might be an important strategy for reducing overall cardiovascular risk and delaying AAA progression in patients with AAA.

Higher cardiorespiratory fitness is associated with lower basal arterial stiffness in older healthy adults (Gando, Murakami et al. 2016). Additionally, regular exercise is reported to be effective
for reducing arterial stiffness at rest, in healthy individuals (Montero, Vinet et al. 2014) and in those with established cardiovascular conditions such as hypertensive individuals (Montero, Roche et al. 2014) and patients with chronic heart failure (Chrysohoou, Angelis et al. 2015). The benefits of regular exercise on vascular structure and function are commonly attributed to the repeated, transient hemodynamic perturbations observed in response to a single bout of exercise (Green and Smith 2017, Romero, Minson et al. 2017). Transient reductions in arterial stiffness are evident after a single bout of sub-maximal exercise in younger and middle aged adults (Kingwell, Berry et al. 1997, Millen, Woodiwiss et al. 2016, Mutter, Cooke et al. 2017) and are suggested to provide a window of benefit (Luttrell and Halliwill 2015) including a transient reduction in central blood pressure (Millen, Woodiwiss et al. 2016). However, the effect of acute aerobic exercise on arterial stiffness has yet to be established in patients with small AAA and been poorly explored in healthy older adults.

Patients with AAA have been reported to have low levels of cardiorespiratory fitness (Kothmann, Batterham et al. 2009, Myers, Powell et al. 2011), when compared to age-specific normative data (Stensvold, Sandbakk et al. 2017). While higher levels of cardiorespiratory fitness are associated with lower basal arterial stiffness in older adults, the influence of cardiorespiratory fitness on the post-exercise arterial stiffness response, is not well defined. Younger individuals with low cardiorespiratory fitness, demonstrate a prolonged post-exercise increase in PWV (Moore, Berrones et al. 2016). Such increases in arterial stiffness are associated with increased cardiovascular risk during the recovery from intense exercise (Rognmo, Moholdt et al. 2012, Schultz, Davies et al. 2013) and may be a concern in a population of high cardiovascular risk such as older adults and patients with AAA. To date, there have been no comparisons of the post-exercise arterial stiffness response between older adults with lower and higher levels of cardiorespiratory fitness.

In younger adults, acute high-intensity exercise is reported to induce greater post-exercise reductions in PWV and AIX compared with moderate intensity exercise (Tordi, Mourot et al. 2010, Hanssen, Nussbaumer et al. 2015) and may infer greater post-exercise benefits for cardiovascular function. Conversely, studies in adults with increased basal arterial stiffness, demonstrated that continuous high-intensity exercise leads to a prolonged increase in PWV post-exercise (Gkaliagkousi, Gavriilaki et al. 2014, Bunsawat, Ranadive et al. 2017) which have been associated with increased cardiovascular risk during the recovery from intense exercise (Rognmo, Moholdt et al. 2012, Schultz, Otahal et al. 2013). High-intensity interval exercise has emerged as a useful intervention for clinical populations that normally cannot
tolerate long bouts of high intensity exercise (Francois and Little 2015). However, the effect of higher-intensity interval exercise on the acute post-exercise arterial stiffness response is to date unknown in older adults and patients with AAA.

Non-invasive, oscillometric technology (SphygmoCor Xcel; AtCor Medical, Sydney Australia) allows the estimation of PWV and wave reflection characteristics and is increasingly being used in research (Williams, Cockcroft et al. 2014). To support the use of post-exercise measures of arterial stiffness in clinical research and practice, the reliability (precision) of these measures needs to be established across repeated assessments in older adults. To date, the reliability of PWV and AIx is well established in young and middle aged adults at rest (Hwang, Yoo et al. 2014) and immediately after exercise (Keith, Rattigan et al. 2013, Lim, Faulkner et al. 2016). However, the reliability of post-exercise changes in arterial stiffness in older adults has yet to be established.

By characterising the transient changes in arterial stiffness following a bout of exercise, this thesis will provide further knowledge regarding the short-term risk and benefits of exercise in patients with AAA and healthy older adults. This will aid to identify a safe and effective exercise prescription that could limit cardiovascular related mortality in both populations and reduce aneurysm progression in patients with AAA.

1.1. Aims of this thesis
The main aim of this thesis was to better understand the arterial stiffness responses to exercise in healthy older adults and patients with abdominal aortic aneurysm (AAA). Three primary research aims were explored within this thesis:

1. To establish the reliability of the acute arterial stiffness response to exercise in healthy older adults (Chapter 3).

2. To determine the effect of exercise intensity and cardiorespiratory fitness on the acute arterial stiffness response to exercise in healthy older adults (Chapter 4).

3. To compare the acute arterial stiffness response to exercise in patients with small AAA and healthy adults of a similar age (Chapter 5).
2. Literature review

2.1 Abdominal aortic aneurysm

2.1.1 Definition of abdominal aortic aneurysm

‘Aneurysm’ is derived from the Greek word ανευρυσμα (aneurusma), meaning widening, and is defined as a permanent and irreversible localised dilatation of a blood vessel (Upchurch and Schaub 2006). The development of an aneurysm often arises from congenital areas of weakness in the arterial wall and the aneurysm enlarges over time (J. Thubrikar 2001). Common aneurysm locations include the Circle of Willis in the brain (Kayembe, Sasahara et al. 1984), the popliteal artery behind the knee (Wright, Matchett et al. 2004), the renal arteries (Martin III, Meacham et al. 1989) and the aorta. Aortic aneurysms affect both the thoracic and the abdominal aorta (Upchurch and Schaub 2006).

An abdominal aortic aneurysm (AAA) is a localised dilatation of the abdominal aorta surpassing the normal diameter by >50% (Upchurch and Schaub 2006). AAAs are primarily located in the infrarenal abdominal aorta, proximal to the aortic bifurcation (Figure 1) (Nordon, Hinchliffe et al. 2011). An AAA is considered ‘small’ when the outer aortic diameter exceeds 30 mm but is not greater than 55 mm (Cosford and Leng 2007). Aneurysms with a diameter greater than 55 mm are classified as ‘large’ and have an increased risk of rupture, a disastrous event with high mortality rate (80%) (Nordon, Hinchliffe et al. 2011).

![Abdominal aortic aneurysm](Source: www.vascularweb.org)

Figure 1. An abdominal aortic aneurysm (Source: www.vascularweb.org).

2.2 Structure of the healthy aorta

The tunica intima is the innermost layer of the aortic wall and consists of endothelial cells that are in direct contact with the blood as it flows (Gray 1918). The endothelium produces
numerous paracrine hormones, including nitric oxide (NO), which regulates vasodilation and endothelin-1 (ET-1) which regulates vasoconstriction of the aortic wall (Green, Maiorana et al. 2004). The function of the endothelium is vital in maintaining aortic health and is also important for regulating blood flow and blood pressure during periods of high metabolic demand. Endothelial dysfunction is implicated in the pathogenesis of several cardiovascular diseases (Vallance and Chan 2001), including AAA (Siasos, Mourouzis et al. 2015).

The tunica media is the middle layer of the aortic wall and is composed by layers of smooth muscle cells (Gray 1918). The elastic properties in the tunica media increase the distensibility of the vessel, and contribute to a reduced blood pressure by dilating and recoiling during systole and diastole respectively (Didangelos, Yin et al. 2010). The vascular extracellular matrix (ECM) is a group of extracellular molecules located in the tunica media that regulates the structure and compliance of the aortic wall. The stability of the ECM is vital in maintaining arterial homeostasis and increasing vessel distensibility (Didangelos, Yin et al. 2010). The ECM predominantly consists of elastin and collagen proteins which maintain a structure that is both compliant and rigid (Frantz, Stewart et al. 2010). Instability of the ECM is considered critical for AAA pathogenesis (Didangelos, Yin et al. 2010).

The tunica adventitia is the outer layer of the vascular wall; it consists of a network of collagen and mast cells called the vasa vasorum (Gray 1918). Only recently important functions of the adventitia were identified. More specifically, the adventitia seems to participate in the cell trafficking through the aortic wall and the signalling between vascular endothelial cells, smooth muscle cells and the local tissue environment (Majesky, Dong et al. 2011). In addition, this layer is involved in both the regulation of the dynamic lumen size (via medial smooth muscle tone) and the inward or outward wall-remodelling response (Zaromitidou, Siasos et al. 2016). Growing evidence indicates that vascular inflammation is initiated in the adventitia and progresses inward towards the intima, a process that supports the ‘outside-in’ hypothesis (Maiellaro and Taylor 2007).

2.3 AAA pathophysiology

The pathophysiology of an AAA is complex and still not well understood. However, inflammation is considered critical factor for AAA formation. An increase in pro-inflammatory cytokines and matrix metalloproteinases (MMPs) in the aortic wall, is suggested to contribute in the remodelling of the aortic ECM and linked to AAA pathogenesis (Pearce and Shively 2006, Azevedo, Prado et al. 2014). AAAs are characterised by dilation of all layers of the
arterial wall as a result of loss of elastin, apoptosis of smooth muscle cells, and compensatory collagen deposition (Brady, Thompson et al. 2004, Cosford and Leng 2007). Alterations in the ECM composition observed in the aneurysmal aortic wall have also been detected in non-aneurysmal aortic segments (Baxter, Davis et al. 1994); hence AAA is characterised as a local manifestation of a systemic disease.

2.4 Biomechanical determinants of AAA
Understanding the biomechanical behaviour of the aortic tissue reveals an important insight into AAA formation and progression. Formation of an aneurysm in the abdominal segment of the aorta can be explained through differences in the histological structure of the infrarenal aorta and the thoracic aorta. The diameter of the aorta decreases from the root (the section of the aorta attached to the heart) to the bifurcation (the point at which the abdominal aorta splits into the left and right common iliac arteries), where the wall of the abdominal aorta contains a lesser proportion of elastin (Chiesa, Melissano et al. 2011). Decreased elasticity of the abdominal aortic wall decreases its ability to deform elastically, which eventually leads to aneurysm formation (Long, Rouet et al. 2005). Technically induced elastin degradation in the abdominal aorta in rat models leads to formation of an AAA (Nackman, Karkowski et al. 1997). Hence degradation of the ECM evident in histological samples of the abdominal aorta is one of the major factors in aneurysm development.

Differential haemodynamic forces along the length of the aorta may also relate to AAA formation. Most relevant to AAA pathophysiology is the marked difference between resting aortic wall shear stress (WSS) in the thoracic aorta and the abdominal aorta. WSS is the tangential force exerted by moving blood along the endothelial surface, which regulates arterial wall remodelling (Schiffrin, Tedgui et al. 2014). In suprarenal aortic segments, flow is antegrade throughout the cardiac cycle, providing continuous high WSS. In the infrarenal aorta, WSS values are slightly lower because a reverse flow is present during diastole (Greve, Les et al. 2006). In a non-compliant vascular system, the pressure wave travels faster through the stiff aortic walls; thus the reverse flow that is present in the abdominal aorta during late systole causes even lower WSS values (Yang, Cho et al. 2014). It has been suggested that low WSS disturbs homeostasis in the ECM, leading to aortic wall remodelling (Langille 1996). For example, in rodent AAA models, smooth muscle cell apoptosis and elastin and collagen degeneration are higher when aortic WSS is low compared with high WSS (Hoshina, Sho et al. 2003). The accelerated loss of elastin due to wall remodelling in the medial layer of the abdominal aorta results in stiffer aortic walls and an imbalance between wall strength and wall
stress that leads to AAA formation (Vorp and Geest 2005). Moreover, a recent study using three-dimensional AAA geometry generated from images from computed tomography angiography reported that low WSS predominates at sites of AAA rupture (Boyd, Kuhn et al. 2016). Hence, compliance of the aortic wall and WSS play a significant role in AAA development and rupture.

An additional important haemodynamic risk factor for AAA progression is peak wall stress. Peak wall stress is the area in the aneurysmal wall where highly localised stress exists at a discontinuity with the rest of the aneurysmal wall. Peak wall stresses calculated in pre-surgical (large) AAA models are significantly higher compared with peak wall stresses in small AAA models (47.5 ± 4 N/cm² vs 36.9 ± 2 N/cm²) (Fillinger, Raghavan et al. 2002). Studies have also explored the stress-to-strength balance in the aortic wall using three-dimensional reconstruction and the finite element method (a computerised method for predicting an element’s reaction to an applied force). Results demonstrated a higher peak wall stress and a lower minimum wall strength in ruptured AAAs than in non-ruptured AAAs (Vorp and Geest 2005). Hence, AAA is regarded as a case of material failure in which the applied load is excessive and the tensile strength of the material is inadequate (Blanchard 1999, J. Thubrikar 2001).

Measuring peak wall stress and WSS in an aneurysmal aorta appears to provide important information regarding AAA formation and progression; however, it is a complicated procedure (Fillinger, Raghavan et al. 2002, Fillinger, Marra et al. 2003). Accurate analysis of AAA wall stress requires information regarding parameters such as aortic geometry and variability of wall thickness. In addition, governing equations representing the relevant physical laws (momentum balance and conservation of mass) (J. Thubrikar 2001) and material parameters (Sonesson, Sandgren et al. 1999) are needed in order to solve accurately this multifactorial equation of wall stress in AAA. Although finite element analysis and computer-generated modelling has been successfully used to map the biophysical distribution of wall stresses for aneurysms of various sizes and different degrees of tortuosity (Doyle, Morris et al. 2008, Scotti, Jimenez et al. 2008), this technique has not been widely accepted or used in clinical practice. To date no study has been able to measure peak wall stress or WSS in vivo in an AAA owing to the high risk and multifactorial complexity of measuring the flow inside an aneurysm (Scotti, Jimenez et al. 2008).
Aortic stiffening is a measure of the ability of the aorta to expand and recoil in response to pressure changes. Evidence using finite element analysis identified segmental aortic stiffening as an early pathomechanism that generates increased aortic wall stress and triggers aneurysmal formation and growth (Raaz, Zollner et al. 2015). Furthermore, a recent study using magnetic resonance imaging to recreate a four-dimensional flow in the abdominal aorta demonstrated a significant negative correlation between WSS and aortic wall stiffness (Kolipaka, Illapani et al. 2017). Increased aortic stiffness plays an important role in the formation and progression of the AAA (Wilson, Lindholt et al. 2001, Wilson, Lee et al. 2003, Kadoglou, Papadakis et al. 2012) and can be easily measured non-invasively (Hwang, Yoo et al. 2014). Arterial stiffness (as a broader topic) is introduced in Section 2.7 and the importance of arterial stiffness in AAA is discussed in detail in Section 2.7.5.

2.5 AAA epidemiology and risk factors
Population-based studies have demonstrated that AAAs account for nearly 2% of all deaths and are estimated to occur in 2% to 6% of the Western adult population (Bergqvist, Björck et al. 2008, Avci, Vos et al. 2012). Indeed, after controlling for other risk factors, earlier studies reported a negative correlation between AAA prevalence and African Americans, Hispanics and Asians (Gillum 1995, Kent, Zwolak et al. 2010, Desai, Dua et al. 2014).

Male sex is a well-established risk factor for AAA with a 4 to 1 male to female ratio (Scott, Bridgewater et al. 2002, Kent, Zwolak et al. 2010). However, prevalence of overall aneurysm rupture reaches nearly 30% in females, resulting to a similar mortality rate as in males (Vouyouka and Kent 2007). Absence of AAA screening in women, since women have a higher rate of undiagnosed cardiovascular disease, may justify the greater rupture and mortality rate (Mikhail 2005). Furthermore, because women are generally smaller in body size, some studies have suggested that an aneurysm of a certain diameter in a woman represents more advanced disease than an aneurysm of the same size in a man (Forbes, Lawlor et al. 2006, Lo, Bensley et al. 2013).

Age is also a prominent AAA risk factor. Peak incidence of AAA occurs among men aged about 70 years (Brewster, Cronenwett et al. 2003). Clinically relevant aneurysms (>30 mm in diameter) are present in about 1% of men aged between 55 and 64 years, and the incidence increases by 2–4% per decade thereafter (Singh, Bonaa et al. 2001). AAAs are anticipated to become even more common as both the proportion and the number of older individuals in the general population continue to rise (Lindholt, Juul et al. 2005).
Hypertension is one of the most common risk factors for AAA (Cornuz, Sidoti Pinto et al. 2004, Nordon, Hinchliffe et al. 2011) indicating an increase in the haemodynamic forces that contribute to aneurysm formation (Fillinger, Raghavan et al. 2002). Earlier studies showed that higher central (aortic) blood pressure was associated with both faster AAA progression (Ruegg, Mason et al. 2010, Bhak, Wininger et al. 2015) and aneurysm rupture (Fillinger, Marra et al. 2003). Conversely, authors of a recent meta-analyses demonstrated that hypertension is not associated with augmented AAA development (Takagi, Ando et al. 2017). The authors suggested that the extensive use of antihypertensive medications may be accountable of the lack of relation between hypertension and AAA development; however, no direct comparison was made.

Studies have strongly associated AAA prevalence with hypercholesterolemia. Both elevated total cholesterol and low high-density lipoprotein (HDL) cholesterol have been significantly related to AAA prevalence (Naydeck, Sutton-Tyrrell et al. 1999, Forsdahl, Singh et al. 2009). The risk of developing an AAA is 70% lower in individuals with a serum HDL cholesterol level of >1.79 mmol/L compared to the risk in those with a serum HDL level of <1.20 mmol/L (Singh, Bonaa et al. 2001). Therefore, a low level of serum HDL cholesterol is probably a high-risk factor for AAA formation.

To date, smoking is deemed as the most distinguished lifestyle-related risk factor for AAA (Bhak, Wininger et al. 2015). The incidence of AAA disease is much greater in smokers than in non-smokers: in a large (n = 114,567) screening study, a history of smoking was associated with an odds ratio of 2.97 (95% CI 2.65–3.32) for AAAs of 30–39 mm and 5.07 (95% CI 4.13–6.21) for AAAs ≥40 mm (Lederle, Johnson et al. 2000). Additionally, smoking has been related with a ~20% increase in aneurysm development (Brady, Thompson et al. 2004), whilst smoking cessation has been shown to reduce AAA prevalence (Kent, Zwolak et al. 2010). Thus, it is apparent that smoking is an important factor influencing AAA development and progression.

Family history is also a risk factor for AAA suggesting that genetic factors play an important role in AAA development. Population-based studies have found that a positive family history of AAA is associated with a risk approximately double than of those without a family history (Larsson, Granath et al. 2009). In a case-control study of 98 cases of AAA and 102 controls, a positive family history was associated with an increased risk of AAA (odds ratio of 4.77, 95% CI 1.26–18.1) (Blanchard 1999). In addition, genome-wide association studies have associated
genetic mutations with AAA formation (Golledge and Kuivaniemi 2013). Further studies exploring the association between genetic mutation and aneurysm formation may provide an insight into the potential mechanisms of AAA pathogenesis.

Patients with AAA are typically diagnosed with cardiovascular co-morbidities. Recent findings found a high prevalence of ischaemic heart disease (42%), coronary artery disease (34%), myocardial infarction (31.3%), stroke (22.8%) and heart failure (6.2%) in patients with AAA (Van Kuijk, Flu et al. 2009, Bath, Gokani et al. 2015). This relationship is bidirectional; patients with coronary artery disease have an 8.4% higher risk of developing an AAA compared with the risk in healthy individuals (Elkalioubie, Haulon et al. 2015). Shared risk factors may be an important component of this relationship. If the underlying pathophysiology of AAA formation is due to degeneration of the aortic media (Kuivaniemi, Ryer et al. 2015), then cardiovascular risk factors (e.g. smoking, hypertension, atherosclerosis) that contribute to this process may add to an underlying AAA predisposition. Hence, AAA represents a local manifestation of poor cardiovascular health and alteration of the modifiable risk factors for AAA may reduce AAA prevalence.

2.6. Mortality in AAA

2.6.1. Aneurysm rupture mortality risk

Ruptured AAAs cause approximately 2% of all deaths per year globally (Sidloff, Stather et al. 2014), with rupture occurring when bleeding is present exterior to the wall of the aneurysm, (Brown, Zelt et al. 2003) which can be often a catastrophic event. The majority of patients with AAA rupture die before reaching the hospital, and only 50% survive following surgical repair that follows rupture (Bradbury, Makhdoomi et al. 1997). A separate and serious consequence of AAA that can also lead to a rupture is ‘dissection’. Aortic dissection describes a tear in the wall of the aorta that results in blood leakage between the layers of the vessel wall as the tear extends along the aorta (Brewster, Cronenwett et al. 2003).

Rupture and dissection risks increase as a function of aneurysm size and rate of enlargement (Kent, Zwolak et al. 2010). Autopsy studies have indicated that aneurysm diameter is an important determinant of rupture, with larger aneurysms having the greatest risk (Brown, Powell et al. 1999, Gibbons CP 2008). A previous study that used medical records of 718 patients with AAA demonstrated an exponential relationship between AAA size, growth rate and rupture risk (Bernstein and Chan 1984). However, although the prevailing standard for defining rupture risk is based on maximum diameter, small AAAs below the threshold
(diameter <55 mm) may also rupture, while large AAAs (diameter >55 mm) may remain stable (Brady, Fowkes et al. 2000). A study that assessed the relationship between aneurysm size and rupture reported that, of 118 cases of rupture, 13% were below the 55 mm threshold (Vorp and Geest 2005). Similarly, an earlier review found that up to 23% of AAAs rupture at a diameter <50 mm (Hall, Busse et al. 2000). Further, results from a retrospective study suggested that peak wall stress is a better predictor of rupture than aneurysm size (Fillinger, Raghavan et al. 2002). Although larger aneurysms are more prone to rupture, there is a small risk of rupture that is independent of size.

2.6.2. Cardiovascular disease mortality risk

Cardiovascular disease is considered the main cause of death in patients with AAA, with evidence demonstrating that mortality due to other cardiovascular diseases is higher than aneurysm-related mortality. More specifically, in a cohort of 2323 patients with AAA, mortality rate due to cardiovascular disease was 16%, while mortality rate from AAA rupture was only 1.5% (Bath, Gokani et al. 2015). In addition, demographic data demonstrated that almost 40% of unexpected AAA ruptures occurred in patients diagnosed with cardiovascular co-morbidities (Vänni, Turtiainen et al. 2016). These findings suggest that the presence of cardiovascular co-morbidities presents a unique and elevated risk in patients with AAA due to both cardiovascular-related and AAA-related death.

Further evidence supports that even patients with small AAA have a significant risk of cardiovascular death, because they exhibit higher levels of biomarkers for cardiovascular disease than levels in healthy controls, such as, high sensitivity c-reactive protein [hs-CRP; mean 2·8 mg/L (95% CI 1·2–6·0) vs 1·3 mg/L (0·5–3·5)] and heart-type fatty acid binding protein [H-FABP; mean 4·6 μg/L (95% CI 3·5–6·0) vs 4·0 μg/L (3·3–5·1)] (Sohrabi, Wheatcroft et al. 2014). Additionally, it was recently demonstrated that cardiovascular risk factors such as hypertension (15%) and hypercholesterolemia (21%) are higher in patients with AAA compared with healthy adults (Bath, Saratzis et al. 2017). Therefore, reducing cardiovascular risk should be considered as a primary treatment target for patients with AAA.

2.6.3. AAA treatment

Surgical treatment is currently the only effective treatment to prevent AAA rupture and aneurysm-related death but is generally only considered for patients with large AAA (>55mm in diameter) (Lederle, Wilson et al. 2002). Surgery is normally avoided in patients with a small AAA (30-55mm in diameter), as evidence support that the rupture risk is lower than the surgery

2.6.3.1. Surgical treatment
To date, there are two different types of surgical AAA repair used: open repair and endovascular repair (EVAR). Open repair of an AAA involves incision of the abdomen to visualise and then repair the AAA with the use of a stent graft and closure of the aorta around the graft (Salartash, Sternbergh et al. 2001). EVAR is a minimally invasive method requiring a small incision in the groin and advancing a stent graft through the femoral artery and into the aorta to the site of the aneurysm (Kodama, Saito et al. 2009). Compared with open surgical repair, EVAR has a significantly lower 30-day postoperative mortality (∼1–2%), (Dangas, O'Connor et al. 2012, Edwards, Schermerhorn et al. 2014). However, the longevity of EVAR success has been challenged as the patient requires recurrent clinical surveillance in order to prevent endo-graft leaks that can prove fatal (Blankensteijn, de Jong et al. 2005, Leurs, Buth et al. 2007). Moreover, studies examining a longer postoperative follow-up period (1–3 months) suggest that post-operative mortality is similar between open repair and EVAR (Greenhalgh, Brown et al. 2010).

2.6.3.2. Post-surgical mortality risk
Prospective studies have reported that post-operative mortality for patients with AAA ranges from 8% to 12% (Carlisle and Swart 2007, Thompson, Peters et al. 2011). Possible contributing factors include older age and aneurysm size (Ouriel, Srivastava et al. 2003, Peppelenbosch, Buth et al. 2004); cardiac complications such as myocardial infarction, dysrhythmia and congestive heart failure (Johnston 1989, Steyerberg, Kievit et al. 1995, Beck, Goodney et al. 2009); and poor preoperative lung and renal function (Brady, Fowkes et al. 2000). Variable postoperative mortality may be due to the multifactorial nature of the AAA disease (Blanchard 1999) with further studies needed to consolidate the impact of surgery on AAA survival.

2.6.3.3. Relationship of cardiorespiratory fitness and post-surgical mortality
A growing body of evidence demonstrates that low cardiorespiratory fitness is strongly associated with postoperative death in patients with AAA (Thompson, Peters et al. 2011, Prentis, Trenell et al. 2012, Barakat, Shahin et al. 2015, Carlisle, Danjoux et al. 2015). AAA surgical repair places substantial metabolic demand on patients during the perioperative period (Salartash, Sternbergh et al. 2001). This is due to a strong inflammatory response that leads to an increase in basal oxygen demand of 110–170 mL.min⁻¹ during the postoperative period.
(Older and Smith 1988). Increased oxygen is required for wound healing, ventilation and substantial haemodynamic intraoperative fluctuations (Davies and Wilson 2004). Failure of the cardiorespiratory system to meet these increased metabolic requirements contributes to postoperative complications and mortality in patients with AAA (Struthers, Erasmus et al. 2008). For example, in a group of 415 patients with AAA (269 undergoing EVAR), patients with low cardiorespiratory fitness (peak oxygen consumption, $\dot{V}O_2\text{peak} < 15 \text{mL.kg}^{-1}.\text{min}^{-1}$) had increased risk of postoperative mortality at 30 and 90 days (Hartley, Pichel et al. 2012). Similarly, in a recent retrospective study of 1096 patients with AAA (483 undergoing EVAR), $\dot{V}O_2\text{peak}$ was significantly correlated with midterm survival (hazard ratio 0.95)(Carlisle, Danjoux et al. 2015). These studies show that poor preoperative cardiorespiratory fitness is associated with a greater risk of postoperative mortality after open and endovascular AAA repair.

Studies that measured cardiorespiratory fitness levels in patients with AAA have reported that their $\dot{V}O_2\text{peak}$ ranged between 13.3 and 20.0 mL.kg$^{-1}$.min$^{-1}$(Kothmann, Batterham et al. 2009, Myers, Powell et al. 2011, Tew, Moss et al. 2012, Barakat, Shahin et al. 2015, Carlisle, Danjoux et al. 2015). Given that a $\dot{V}O_2\text{peak} < 15 \text{mL.kg}^{-1}.\text{min}^{-1}$ is associated with reduced functional capacity and severe cardiovascular disease (Kodama, Saito et al. 2009), and based on the age-matched published normative data from the American College of Sports Medicine, the cardiorespiratory fitness levels of patients with AAA can characterised as poor to very poor (Garber, Blissmer et al. 2011). Taken together, these findings suggest that improvement of cardiorespiratory fitness should be considered as a treatment target in patients with AAA.

2.6.3.4. Management of small AAA

Existing guidelines for aneurysm management in patients with small AAA suggest ultrasound surveillance of the aneurysm diameter every 6 or 12 months until the aneurysm diameter exceeds 55 mm (Silaghi, Branchereau et al. 2007). Patients with small AAA often remain under surveillance for several years, as the annual aneurysm growth rate ranges between 2.0 mm to 5.0 mm (Thompson, Brown et al. 2013). These patients must live with the awareness that they have a potentially life-threatening illness that carries a small risk of sudden death in the months or years before eligibility for surgical repair (Carlisle and Swart 2007, Martin, O’Doherty et al. 2012).

Besides the risk of rupture as aneurysm diameter increases, these patients also have a higher risk of cardiovascular complications and mortality than healthy older adults (Bath, Saratzis et
To reduce cardiovascular risk factors associated with AAA, drug therapy that includes the prescription of antihypertensive medications such as statins and beta-blockers are usual in this population. However, a meta-analysis reported that statin therapy was not able to reduce aneurysm growth (Twine and Williams 2011). Patients with small AAA are also offered lifestyle advice. However, strategies to reduce cardiovascular risk factors, such as smoking cessation, eating a healthy diet and reducing alcohol consumption have proven unsuccessful in slowing AAA growth and reducing cardiovascular-related mortality (Brady, Thompson et al. 2004). To date, there are no effective treatment options for patients with small AAA (30–55 mm); thus, there is a strong need to develop therapies that target AAA progression and overall cardiovascular disease risk and mortality. In this regard, exercise training has led to a number of beneficial outcomes in patients with AAA.

2.6.4. Exercise training in patients with AAA
Previous studies showed that exercise training is a potent treatment for several chronic diseases related with aging (Thompson, Buchner et al. 2003, Taylor, Brown et al. 2004, Davies, Moxham et al. 2010). Despite these findings, limited evidence exists regarding the effect of exercise training on cardiorespiratory fitness and cardiovascular risk in patients with AAA. This is partly due to a previous notion that exercise could be harmful for patients with AAA (Bean, Vora et al. 2004). However, evidence to date reporting a low risk of adverse events in response to (sub)maximal exercise (Myers, Powell et al. 2011, Tew, Batterham et al. 2017) in patients with AAA.

2.6.4.1. Exercise training improves cardiorespiratory fitness in patients with AAA
Several short-term training studies have demonstrated significant improvements in cardiorespiratory fitness in patients with small and large AAA. An early study demonstrated that patients with small AAA increased their ventilatory threshold by 1.1 ±0.8 mL.kg⁻¹.min⁻¹ compared with usual care controls after 6 weeks of cycle ergometer training (Kothmann, Batterham et al. 2009). Similarly, a mean improvement of 2.5 ± 2.9 mL.kg⁻¹.min⁻¹ was reported in the ventilatory threshold of patients with small AAA that underwent 12 weeks of aerobic training compared with baseline values (Tew, Moss et al. 2012). A recent study also reported improvements in ventilatory threshold from a mean of 13.3 ± 3.3 to 15.0 ± 3.4 in patients with small AAA after a 3 months of exercise training (Lima, Vainshelboim et al. 2017). Lastly, an increase in $\dot{V}O_2\text{peak}$ from a mean of 18.4 ± 3.2 to 20.0 ± 4.1 mL.kg⁻¹.min⁻¹ and in ventilatory threshold from 12.0 ± 1.3 to 13.9 ± 2.1 mL.kg⁻¹.min⁻¹ was observed after 6 weeks of aerobic
exercise training in patients with large AAA (Barakat, Shahin et al. 2016). Collectively, these results suggest that aneurysm presence and size does not limit the trainability of these patients.

A longer-term exercise training intervention was also effective in improving cardiorespiratory fitness in patients with small AAA (Myers, McElrath et al. 2014). The exercise group demonstrated significant increases in mean $\dot{V}O_2$ peak after both 3 months (19.5 ± 5.8 to 20.4 ± 6.3 mL.kg⁻¹.min⁻¹) and 12 months (19.6 ± 6.0 to 20.9 ± 5.9 mL.kg⁻¹.min⁻¹) of aerobic exercise. Whilst improvements in cardiorespiratory fitness compared with baseline were no longer evident after 24 and 36 months of exercise training, the exercise benefits were still evident as $\dot{V}O_2$ peak was higher compared with the no exercise group.

2.6.4.2. Exercise training improves postoperative outcomes in patients with AAA

Increasing cardiorespiratory fitness via an exercise training intervention has been used as a tool for improving post-operative mortality in clinical populations (Valkenet, van de Port et al. 2010). To date, only two studies have assessed the effect of exercise training on post-operative outcomes in patients with AAA. A previous study reported that an exercise induced improvement on respiratory parameters is associates with reduced post-operative complications (Dronkers, Veldman et al. 2008). Recently, Barakat et al. (2016) reported that patients who underwent a preoperative exercise intervention demonstrated significantly fewer postoperative complications (cardiac: 8.1%; pulmonary: 11.3%; renal: 6.5%), compared with the usual care control group (no exercise)(Barakat, Shahin et al. 2016). These finding indicate the effectiveness of an exercise training intervention on post-operative mortality of patients with small AAA

2.6.4.3. Effect of exercise training on cardiovascular risk

To date, only one training study in patients with small AAA has explored the effect of exercise training on blood pressure and inflammation, factors that are associated with aneurysm progression (Pearce and Shively 2006, Ruegg, Mason et al. 2010, Azevedo, Prado et al. 2014, Bhak, Wininger et al. 2015) and increased cardiovascular-related mortality (Chobanian, Bakris et al. 2003, Golia, Limongelli et al. 2014). Tew et al. (2012) reported a decrease of 10 mmHg in systolic blood pressure after a short-term (12 weeks) exercise intervention in patients with small AAA (Tew, Moss et al. 2012). Given that a reduction of 7 mmHg in systolic blood pressure is considered clinically significant (Fagard 2006), these training-induced results are promising for reduced cardiovascular risk of AAA patients. Furthermore, the same study demonstrated an increase in cardiorespiratory fitness that was accompanied by a decrease in
systemic inflammation (Tew, Moss et al. 2012). C-reactive protein (CRP), an inflammatory biomarker and contributor to cardiovascular disease, decreased after training from a mean of 1.4 mg/L to 0.9 mg/L. This reduction was clinically important as it decreased the risk stratification of the group from moderate (1.0–3.0 mg/L) to low (<1.0 mg/L) (Ridker, Bassuk et al. 2003).

2.6.4.4. Effect of a bout of exercise on AAA haemodynamics

The acute effect of exercise on the haemodynamic parameters of the aorta may be of significance for AAA progression. AAA formation and growth are associated with adverse haemodynamics, including low and oscillatory WSS (Dalman, Tedesco et al. 2006, Humphrey and Holzapfel 2012). Studies using in vitro flow simulations in AAA models have demonstrated that exercise of moderate intensity increases flow rate and WSS and decreases oscillations in flow in the aneurysmal abdominal aorta compared to resting conditions (Tenforde, Cheng et al. 2010, Suh, Les et al. 2011, Arzani, Les et al. 2014). These exercise-induced changes in haemodynamic conditions also inhibit aortic wall stiffness, upregulate endothelium-derived vasodilators and downregulate vasoconstrictors and inflammatory molecules in aneurysmal rodents (Raaz, Zollner et al. 2015). These transient post-exercise haemodynamic changes in the aorta are assumed to lead to long-term vascular adaptations such as increased endothelial function and lower basal aortic stiffness with exercise training (Romero, Minson et al. 2017). These adaptations may contribute to reduced all-cause cardiovascular risk and a reduction in AAA progression. Although these results are promising, in vivo studies exploring vascular changes after acute exercise in patients with AAA are lacking. A better understanding of the acute effect of exercise on the vasculature of patients with AAA would provide insight into the potential use of exercise as a treatment tool.

2.7. Arterial stiffness

Arterial stiffness is an important mechanical property of the arterial system that describes the capability of an artery (e.g. the aorta) to expand and contract in response to pressure changes (Cecelja and Chowienczyk 2012). Arterial stiffness predominantly affects the aorta (referred to as central arterial stiffness) and proximal elastic arteries (referred to as peripheral arterial stiffness) (O'Rourke, Staessen et al. 2002). Increased arterial stiffness is considered an independent risk factor for cardiovascular disease (Laurent, Boutouyrie et al. 2001). Stiffening of the aorta leads to a range of linked pathophysiological changes within the circulation. The aorta is less able to accommodate the volume of blood ejected by the left ventricle, resulting in a greater pressure increment during systole. Exposing the myocardium to higher systolic
pressures leads to left ventricular hypertrophy (Ohtsuka, Kakihana et al. 1994). Furthermore, changes in arterial compliance due to increased arterial stiffness lead to low WSS and high mechanical stress, which may contribute to the development of atheromatous plaques (Zhao, Ariff et al. 2002) and AAA formation (Vande Geest, Sacks et al. 2006, Raaz, Zollner et al. 2015).

2.7.1. Measures of arterial stiffness
During the past 30 years there has been a great interest in the detection of markers of early cardiovascular disease in order to prevent and reduce cardiovascular mortality and morbidity. Consequently, several techniques have been developed to assess arterial stiffness, some of which are more applicable in the clinical setting than others. Methods that measure arterial compliance (the absolute diameter change for a given change in pressure), arterial distensibility (the relative change in diameter for a given pressure change) (Mackenzie, Wilkinson et al. 2002) and elastic modulus (an artery’s resistance to being deformed elastically when a force is applied) (Gosling and Budge 2003) commonly require expensive equipment (e.g. magnetic resonance imaging, computer tomography, ultrasound). Such equipment is also of limited accessibility, needs a high level of operator technical expertise, and has poor temporal resolution. These limitations have led to the development of a number of commercially available devices that assess arterial stiffness non-invasively (Huck, Bronas et al. 2007). These devices are able to measure arterial stiffness indices, such as pulse wave velocity and wave reflection characteristics, which have been validated to represent segmental and systemic arterial stiffness, respectively (Horvath, Nemeth et al. 2010).

2.7.1.1. Pulse wave velocity
Pulse wave velocity (PWV) is the current criterion measure for assessing arterial stiffness non-invasively (Perk, De Backer et al. 2012). PWV is calculated by measuring the velocity at which a forward pressure wave is transmitted from the aorta through the arterial tree and involves taking readings of the pulse wave from two sites simultaneously (Filipovsky, Ticha et al. 2005). An increase in PWV indicates increased arterial stiffness because the propagated waves travels faster through the stiff artery due to decreased arterial compliance (O’Rourke, Staessen et al. 2002, Hwang, Yoo et al. 2014). The relationship between arterial stiffness and PWV is demonstrated by the Moens-Korteweg equation and based on Newton’s second law of motion:

\[
PWV = \sqrt{\frac{E \times h}{2r \rho}}
\]
where PWV is proportional to the square root of the incremental elastic modulus (E) of the vessel wall, given a constant ratio of wall thickness (h) to vessel radius (r) and blood density (ρ) (O'Rourke, Staessen et al. 2002). The carotid and femoral arteries are the most commonly used measurements sites for PWV because they are superficial, easy to access (Laurent, Cockcroft et al. 2006) and encompass the region of the aorta that exhibits the greatest age-related stiffening (Fig 2) (Mitchell, Parise et al. 2004). The validity of this measure was previously established by correlating carotid-femoral PWV, measured with a semiautomatic device (Complior, Colson, Garges les Gonesse, France), and aortic PWV, measured directly during cardiac catheterisation, in 107 patients with coronary artery disease (r = 0.70) (Podolec, Kopec et al. 2007).

The mechanical properties of the arterial walls change along the arterial tree from the large, more elastic arteries (e.g. aorta, carotid, iliac) to the predominantly muscular arteries that are mostly located in the periphery (e.g. femoral, popliteal, posterior tibial) (Tillin, Chambers et al. 2007). Hence, studies have also explored PWV in other arterial sites (carotid-radial and brachial-ankle), which reflect the stiffness of both the aorta and peripheral arteries (Tanaka, Munakata et al. 2009). However, PWV measured over a long segment composed of arteries with different mechanical characteristics may mask variations in biomechanical properties of the smaller segments and thus provide less information on the position of the arterial

![Figure 2. Carotid-femoral pulse wave velocity (PWV) calculated using the equation $PWV = \frac{\Delta L}{\Delta t}$ where $\Delta L$ is the distance between the carotid and femoral arteries and $\Delta t$ is the transit time measured as the time delay between the arrival of the pulse wave at the common carotid artery and the common femoral artery. The units of PWV are m.s$^{-1}$. (Adapted from Ranjith et al. 2014).](image-url)
abnormalities (Yu, Chuang et al. 2007). Hence, carotid-femoral PWV is considered a superior indicator of arterial stiffness than the PWV measured over a longer segment of the arterial tree (e.g. carotid to radial arterial segment) (Bortolotto, Hanon et al. 1999, Lacroix, Willemet et al. 2012).

PWV intrinsically varies with blood pressure, which is demonstrated by the Bramwell-Hill equation:

\[ PWV = \sqrt{\frac{\Delta V}{\Delta P}} \text{ cm}^2/\text{mmHg} \]

where arterial compliance (the inverse of arterial stiffness) decreases with increasing pressure due to the curvilinear relationship between arterial pressure (P) and volume (V) (Spronck, Heusinkveld et al. 2015). This relationship indicates that the PWV of an arterial segment is not constant but depends upon the distending pressure, best represented by the mean arterial pressure (MAP). As MAP rises, the arterial segment experiences increased circumferential pressure with greater recruitment of inelastic collagen fibres, which leads to an increase in stiffness and PWV (O'Rourke, Staessen et al. 2002).

Endothelial function is also considered a determinant of PWV. It has been suggested that changes in smooth muscle tone alter the distribution of forces within the arterial wall, providing functional regulation of arterial stiffness (Duprez 2010). Studies have demonstrated that modulation of nitric oxide (an endothelium-derived vasodilator) is inversely correlated with changes in PWV (r = -0.69) in healthy middle-aged adults (McEniery, Wallace et al. 2006). Additionally, intra-arterial infusion of endothelin-1 (an endothelium-derived vasoconstrictor peptide) significantly increases PWV by 12% (Fig 3) in healthy adults (McEniery, Qasem et al. 2003). Furthermore, flow-mediated dilatation, a widely used method of assessing endothelial function, is a significant and independent determinant of aortic PWV, suggesting that as endothelial function of a conduit artery declines, the aortic stiffness increases (McEniery, Wallace et al. 2006).
Figure 3. The effect of intra-arterial infusion of endothelin-1 (ET-1) on aortic wave velocity (PWV) measured via catheter. * = significantly different from saline values (P <0.05); ** = significantly different from saline values. (P <0.001). (Adapted from McEnniery et al. 2003)

2.7.1.2. Wave reflection characteristics

An additional method to assess arterial stiffness non-invasively is by measuring wave reflection characteristics. With each cardiac cycle, the heart generates a forward-travelling pressure wave that reflects in the periphery and returns as a backward-travelling wave towards the heart (Nichols and Edwards 2001). Although the origin of the reflected wave cannot be precisely identified, it has been suggested that the wave is partly reflected at sites of impedance mismatch, such as branch points, and at high-resistance arterioles (O’Rourke, Avolio et al. 2003, Liao, Cheng et al. 2011). Multiple small reflections from the peripheral vascular tree travel back to the proximal aorta and merge into a net reflected wave. Hence, the pressure measured at any location of the arterial tree will consist a pick of the net effect of the forward and backward pressure waves (Nichols, O’Rourke et al. 2011) (Fig 4). Some studies have proposed that the capacitive or reservoir function of the aorta also contributes to the magnitude of the backward pressure wave (Davies, Baksi et al. 2010, Hametner, Wassertheurer et al. 2014). Since the rate of blood entering the aorta exceeds that leaving it, due to the peripheral resistance, there is a net storage of blood during systole, which presumably contributes to the pressure of the backward wave. A compliant aorta will buffer the pulsatile ejection of blood from the heart, converting its potential energy into an even outflow. Hence this function largely depends on the elasticity of the aorta. Early return of the reflected wave (during late systole) due to increased arterial stiffness increases both heart load and central pulse pressure, factors that
increase cardiovascular burden (Haider, Larson et al. 2003). Therefore, analysis and estimation of wave reflection characteristics is deemed of great clinical interest.

**Augmentation index**
The augmentation index (AIx) is the most common measure of wave reflection characteristics and provides an integrated summary of wave reflection timing and amplitude (Nichols and Singh 2002). AIx can be measured non-invasively by converting brachial pressure waveforms to a corresponding aortic waveform via a transfer function; it is defined as the difference between the second and the first systolic peaks as a percentage of pulse pressure (PP) (Mackenzie, Wilkinson et al. 2002). The equation used to calculate AIx is

\[ \frac{AP}{PP} \times 100 \]

where augmentation pressure (AP) is defined by the peak systolic pressure minus the first systolic peak and PP is defined by the difference between systolic and diastolic pressure (Fig 4) (Nichols, O'Rourke et al. 2011). The AIx is influenced by heart rate in an inverse and linear manner (Wilkinson, MacCallum et al. 2000); hence, it is often normalised for a heart rate of 75 bpm (AIx75) (Wilkinson, Mohammad et al. 2002). The AIx is also affected by the amplitude and timing of the reflecting pulse wave (Nichols 2005), the duration of left ventricular ejection (London and Guerin 1999) and the blood pressure (Chen, Nevo et al. 1997).

**Reflection magnitude**
A novel developed approach for estimating wave reflection characteristics is via wave separation analysis in which the aortic pressure wave is separated into its forward and backward components. Westerhof et al. (2006) introduced a method through which the triangular flow wave is approximated from the estimated aortic pressure wave. This approach estimates the forward and the reflected pressure waves by matching the start, peak and end of the assumed flow wave to the timings of the foot, the inflection point (the point where the forward and backward wave meet), and the incisura (returning point of the reflected wave) of the aortic pressure wave, respectively (Fig 4). The reflection magnitude (RM) is calculated as the ratio of the backward (Pb) to the forward pressure (Pf) wave. The method gave promising results as estimation of pressure and flow velocity derived from the aortic pressure wave were strongly correlated \( (r^2 = 0.79) \) with pressure and flow velocity measured via aortic catheterisation (Westerhof, Guelen et al. 2006). The characteristics of the forward pressure wave largely depend upon the elastic properties of the aorta and the PWV (Nichols 2005). The characteristics
of the reflected wave depend on a more complex set of determinants, namely the elastic properties of the entire arterial tree (elastic plus muscular arteries and, to a lesser extent, arterioles) and the distance to the major reflected sites (Nichols, Denardo et al. 2008).

Figure 4. Representation of the central arterial waveform. Using the generated aortic pressure waveform (top figure), the augmentation index (AIx) is calculated by expressing augmentation pressure (AP) as a percentage of the central pulse pressure (PP). Using an assumed flow waveform (middle figure), the aortic wave can be separated (bottom figure) into its component forward (Pf) and reflected (Pb) waves, and reflection magnitude (RM) can be calculated (Pb/Pf). (Adapted from Stoner et al. 2016)

2.7.2. The effect of age on arterial stiffness
Elastic arteries become stiffer with age (Mackenzie, Wilkinson et al. 2002). Aging is associated with alterations in a number of structural and functional properties of the large arteries,
including arterial diameter, wall thickness, wall stiffness and endothelial function (Shibata and Levine 2011). Stiffening has been observed primarily in the proximal aorta and its major branches, whereas changes in stiffness in the peripheral arteries such as the femoral, popliteal and posterior tibial arteries are less marked (Avolio, Deng et al. 1985, Benetos, Laurent et al. 1993). The elastic modulus (a number that measures a substance’s resistance to being deformed elastically) of the aorta and major elastic arteries increases with age due to the progressive degradation of the load-bearing components of the arterial wall, such as elastin and collagen (Wheeler, Mukherjee et al. 2015).

Initial changes in aortic stiffness with aging are related to the fact that proximal elastic arteries dilate by 10% with each beat of the heart, while peripheral muscular arteries dilate by only 2–3% (Benetos, Laurent et al. 1993). Hence, age-related aortic stiffening may be due to material fatigue, which accounts for fracture of physical materials that are exposed to repetitive cycles of bending or stretching (Hassan and Kyriakides 1992, Fung 1997). Elastin, the most inert substance in the arterial wall, has a half-life of decades (Carretta 1998) and, like other materials, is subject to fatigue and fracture. Fracture of elastic lamellae is seen in the aorta with aging, and can account both for wall weakening (Cattell, Anderson et al. 1996) and stiffening (Mitchell, Parise et al. 2004). Endothelial dysfunction is also characteristic of arterial aging (Safar, Levy et al. 2003). Data in both humans and experimental animals indicate that impaired endothelial function with aging is mediated by a decrease in NO bioavailability (Durrant, Seals et al. 2009, Montero, Pierce et al. 2015). When the release of NO or its bioavailability is reduced, endothelium-derived factors that cause vasoconstriction (e.g. endothelin-1) become predominant, contributing to the age-related increase in arterial stiffness (Seals, Jablonski et al. 2011). This age-related increase in arterial stiffening is associated with elevated systolic and pulse pressures and has been established as a risk factor for cardiovascular disease (Laurent 2012). In summary, age-related increases in arterial stiffness depend on both structural and functional changes of the vascular system; therefore, age-related vascular changes in arterial stiffness could represent a potential target for treatment and prevention of cardiovascular disease.

2.7.2.1. The effect of age on PWV

Carotid-femoral PWV increases with age. Specifically, Mitchel et al. (2004) demonstrated a linear increase in carotid-femoral PWV with age in a cohort of 521 normotensive adults aged from 50 to 82 years (Mitchell, Parise et al. 2004). This relationship was confirmed in a later study that included 4001 healthy normotensive adults aged 18 to 90 years, which demonstrated...
an exponential increase in carotid-femoral PWV beyond the age of 60 years (Fig 5) (McEniery, Yasmin et al. 2005). Increased carotid-femoral PWV has traditionally been linked to structural alterations in the vascular media and contributes to the increased pulse pressure in older people (Najjar, Scuteri et al. 2005). In contrast, ankle-brachial PWV changes minimally with age (Mitchell, Parise et al. 2004). This difference might be due to the higher proportion of smooth muscle in the brachiocephalic system than in the aorta (Wilting and Chao 2015), or to differential remodelling between these arteries (Wagenseil and Mecham 2009). Although data from epidemiological studies indicate that increased carotid-femoral PWV occurs in the contexts of atherosclerosis and diabetes (Blacher, Asmar et al. 1999, Dart and Kingwell 2001), recent evidence demonstrates that prominent age-associated increases in PWV occur even with little or no atherosclerosis (AlGhatrif, Strait et al. 2013, Kozakova, Morizzo et al. 2015).

Figure 5. Regression curves representing the effect of age on carotid-femoral pulse wave velocity for males (black squares, solid lines) and females (white squares, dashed lines). Data points are the group means for each decile of age. (Adapted from McEniery et al. 2005)

2.7.2.2. Effect of age on wave reflection characteristics
The positive association between age and AIx has been considered linear (Nichols, O'Rourke et al. 1985); however, studies have challenged this relationship by reporting that there is actually a plateau in AIx after the age of 60 years (Mitchell, Parise et al. 2004, McEniery, Yasmin et al. 2005). Mitchell et al. (2004) were the first to question this view, reporting that AIx changes less with age in older individuals and actually declines after age 60 (Mitchell, Parise et al. 2004). Shortly after, McEniery et al. (2005) demonstrated that the relationship between age and AIx was nonlinear in a cohort of 4001 healthy normotensive adults aged 18 to 90 years (Fig 6) (McEniery, Yasmin et al. 2005). The contributions of the forward and
reflected waves to AIx with ageing, and the equation used for AIx calculation, shed some light on this observation. The dominance of the reflected over the forward wave until the age of 60 years explains the progressive rise in AIx (Mitchell, Parise et al. 2004). In contrast, the exponential increase observed in PWV after the age of 60 years (Fig 5) (McEniery, Yasmin et al. 2005) increases the forward pressure wave and therefore reduces the difference between the forward and reflected waves (Namasivayam, McDonnell et al. 2009). This diminishes the difference between the second and first systolic peak (AP) from which AIx is derived and explains the plateau observed in this index in some studies in individuals over the age of 60 (Fig 6).

In contrast to AIx, evidence demonstrates that the reflected wave is positively associated with age. Liao et al. (2011) demonstrated a significant positive correlation (r = 0.29) between age and the reflected pressure wave among 180 healthy individuals with a mean age of 68 years (Liao, Cheng et al. 2011). Aging may impair the vasomotor function of the small peripheral arteries, which would change the impedance properties and increase the intensity of the reflected wave (Hashimoto and Ito 2009). Liao et al. (2011) further demonstrated that age may contribute more to the transit time-independent reflected wave than to the transit time-dependent AIx (Liao, Cheng et al. 2011). Unlike AIx, the calculation of wave reflection characteristics via wave separation analysis allows the estimation of the amount of reflection (a product of the Pb/Pf ratio) without dependence on the timing of the forward and reflected waves (Westerhof, Guelen et al. 2006). Wave separation analysis may therefore provide a more accurate estimation of systemic arterial stiffness in the elderly. However, to date no studies have assessed whether this index is reliable or the effect of age on RM and its components.

![Figure 6](image-url)

**Figure 6. Regression curves representing the effect of age on augmentation pressure** (open circles/open squares) and augmentation index (closed circles/closed squares) for males (solid lines) and females (dashed lines). Data points are the group means for each decile of age. (Adapted from McEniery et al. 2005)
2.7.3. The effect of sex on arterial stiffness

Sex differences in the biomechanical properties of large arteries occur throughout the lifespan and may be influenced by sex steroids. Females display lower arterial stiffness than males until menopause, when this relationship reverses, likely due to a decline in circulating oestrogen (Ahimastos, Formosa et al. 2003, Rossi, Frances et al. 2011). Furthermore, cross-sectional (Rajkumar, Kingwell et al. 1997, Teede 2007) and longitudinal studies (Vitale, Mammi et al. 2017) have associated oestrogen-containing hormonal therapy with lower arterial stiffness in postmenopausal women (Campisi, Cutolo et al. 1993, Welter, Hansen et al. 2003).

Evidence of the effects of androgens on the physiology of the vessel wall is inconsistent. As male sex is one of the major risk factors for the development of cardiovascular disease, it has been suggested that androgens have a detrimental effect on the arterial wall (Hutchison, Sudhir et al. 1997, Nheu, Nazareth et al. 2011). For example, an elevation in androgen levels associated with puberty has been associated with an increase in vascular tone and may therefore contributed to the observed stiffening of the large vessels (Ahimastos, Formosa et al. 2003, Rossi, Frances et al. 2011). In contrast, testosterone was inversely associated with arterial stiffness (carotid-femoral PWV) in 455 healthy middle-aged and older men (Vlachopoulos, Ioakeimidis et al. 2014). Although the mechanism(s) of testosterone–PWV interaction remain uncertain, testosterone is recognised as a direct-acting vasodilator (Kelly and Jones 2013) and has been inversely associated with inflammation (Vlachopoulos, Dima et al. 2005), factors that contribute to arterial stiffness. Thus, the relationship between androgens and arterial stiffness has not been established yet.

2.7.4. Cardiovascular disease and arterial stiffness

Several studies support the mechanistic role of arterial stiffness as a causal determinant of cardiovascular complications and events (Mitchell, Hwang et al. 2010, Vlachopoulos, Aznaouridis et al. 2010, Cecelja and Chowienczyk 2012) such as hypertension (Mitchell 2014), left ventricular hypertrophy (Yucel, Demir et al. 2015) and coronary artery disease (Luo, Feng et al. 2014). Since the ventricle ejects a pulsatile blood flow into a buffering system of distensible arteries, the vascular afterload has both static (non-pulsatile) and dynamic (pulsatile) components (Nichols, O’Rourke et al. 2011). The static component primarily depends on blood viscosity while the dynamic component depends on the elastic properties of the large arteries (Nichols, O’Rourke et al. 2011). Degradation of the elastic properties of the arteries, and hence an increase in arterial stiffness, causes a premature return of the reflected wave in late systole, increasing central PP and the load on the ventricle (Laurent, Boutouyrie et al. 2001), major risk
factors for developing cardiovascular disease (Schram, Chaturvedi et al. 2003, Steppan, Barodka et al. 2011). The increased cyclic stress applied to the aortic wall due to increased arterial stiffness has been associated with aneurysm formation (Dijk, van der Graaf et al. 2004), progression (Kadoglou, Papadakis et al. 2012, Raaz, Zollner et al. 2015) and rupture (Vorp and Geest 2005). Therefore, arterial stiffness might be considered as a treatment target in order to reduce cardiovascular disease prevalence and mortality.

2.7.4.1. PWV and wave reflection characteristics as predictors of cardiovascular disease mortality

Several large epidemiological studies have established carotid-femoral PWV as a predictor of cardiovascular disease and mortality that is independent of MAP and other risk factors, including sex, age, body mass index, current smoking and alcohol intake (Laurent, Boutouyrie et al. 2001, Sutton-Tyrrell, Najjar et al. 2005, Mattace-Raso F 2010, Mitchell, Hwang et al. 2010). Specifically, a large collaborative study that gathered data from 16,867 individuals suggested that a high carotid-femoral PWV index was positively associated with the incidence of cardiovascular disease (Mattace-Raso F 2010). Furthermore, a meta-analysis of 17 longitudinal studies that evaluated carotid-femoral PWV and followed up 15,877 individuals for a mean of 7.7 years demonstrated that an increase of 1 m.s$^{-1}$ in carotid-femoral PWV was associated with a 15% increase in cardiovascular disease mortality (Vlachopoulos, Kardara et al. 2010). Further studies have established that increased carotid-femoral PWV measured at rest is a significant predictor for end-stage renal disease (≥13 m.s$^{-1}$) (Blacher, Guerin et al. 1999), diabetes (≥13 m.s$^{-1}$) (Cruickshank, Riste et al. 2002) and hypertension (≥12 m.s$^{-1}$) (Blacher, Asmar et al. 1999, Laurent, Boutouyrie et al. 2001). An increased PWV also correlates strongly with the prediction of coronary atherosclerosis (van Popele, Mattace-Raso et al. 2006), coronary heart disease and stroke in a population of older individuals, after adjustment for age, sex and blood pressure (Sutton-Tyrrell, Najjar et al. 2005).

Wave reflection characteristics are also associated with cardiovascular risk and events. An epidemiological study of 216 participants demonstrated a positive association between AIx and the risk of cardiovascular events (Nurnberger, Keflioglu-Scheiber et al. 2002). Moreover, in a study of 297 patients with coronary artery disease, AIx was positively associated with increased cardiovascular mortality (hazard ratio of $1.28 \pm 0.4$) (Chirinos, Zambrano et al. 2005). However, in the Framingham study, consisting of 2232 middle-aged and older healthy participants, AIx did not correlate with the risk of cardiovascular disease outcomes (Mitchell, Hwang et al. 2010). Nevertheless, two large prospective studies reported that wave separation analysis may be
superior to AIx as a subclinical marker of cardiovascular disease; one study reported that Pb better predicts 15-year cardiovascular mortality than AIx does (Wang, Cheng et al. 2010), and the other that RM better predicts cardiovascular events than AIx does (Chirinos, Kips et al. 2012). The different predictive performance of AIx, RM and Pb may be due to the fact that AIx is confounded by many factors (e.g. timing of the reflected wave, blood pressure, ejection fraction) with high inter-individual variability (Segers, Carlier et al. 2000). This variability probably limits the ability of AIx as a predictive marker of cardiovascular disease outcomes. In contrast, RM is less sensitive to such confounding factors (Segers, Carlier et al. 2000) and may therefore be more repeatable.

2.7.5. The importance of arterial stiffness in AAA
AAA formation and progression is associated with substantially increased arterial stiffness. Data from a prospective study of 210 patients with AAA showed a significant inverse association between aortic stiffness (measured via ultrasound) and time to aneurysm rupture that was independent of other risk factors (Wilson, Lee et al. 2003). Specifically, for any given AAA diameter, a 10% increase in arterial stiffness was associated with a 28% increase in rupture risk. Further, data from a recent study with an animal model demonstrated that segmental aortic stiffening generates wall stress that precedes and triggers early AAA growth (Raaz, Zollner et al. 2015). The same study also demonstrated that segmental stiffening (induced with an external cuff around the cyclically pressurised aorta) resulted in significant upregulation of the matrix metalloproteinases MMP-2 and MMP-9. These macromolecules are associated with ECM remodelling and aneurysm progression (Pyo, Lee et al. 2000, Longo, Xiong et al. 2002) and enhanced cytokine production (IL-6), known to be critical in AAA pathogenesis (Jones, Brull et al. 2001, Zampetaki, Zhang et al. 2005).

Indices of arterial stiffness such as carotid-femoral PWV and AIx are also increased in patients with AAA. Data in recent studies demonstrated that, compared with healthy control subjects, patients with large AAA present with significantly higher carotid-femoral PWV (mean of $14.8 \pm 4.9 \text{ m.s}^{-1}$ vs $10.0 \pm 1.7 \text{ m.s}^{-1}$) and AIx75 (mean increase of $33.2\% \pm 8.9$ vs $25.1\% \pm 7.8$) (Durmus, Kazaz et al. 2014). Carotid-femoral PWV was also higher in patients with large AAA than in patients with coronary artery disease (mean of $12.99 \pm 3.8 \text{ m.s}^{-1}$ vs $10.03 \pm 1.6 \text{ m.s}^{-1}$) (Kadoglou, Papadakis et al. 2012). Moreover, after adjustment for age, increased PWV was also positively associated with increased aneurysm progression ($r^2 = 0.5$), (Kadoglou, Papadakis et al. 2012). Despite the risk of aneurysm rupture, cardiovascular mortality remains the main cause of death among patients with AAA (Schlosser, Vaartjes et al. 2010). As noted
in Section 2.3.4, increased PWV and increased AIX are associated with increased risk of cardiovascular events and mortality (Chirinos, Zambrano et al. 2005, Mitchell, Hwang et al. 2010). Hence, lowering PWV and AIX may reduce both cardiovascular risk and aneurysm progression in patients with AAA.

2.8. Arterial stiffness and exercise

2.8.1. The relationship between cardiorespiratory fitness and arterial stiffness

Cardiorespiratory fitness is inversely associated with PWV and wave reflection characteristics. Vaitkevicious et al. (1993) were the first to demonstrate that older athletes exhibit a lower carotid-femoral PWV and systolic blood pressure than inactive participants of the same age (Vaitkevicius, Fleg et al. 1993). Similar cross-sectional observations have also been reported in middle-aged men: endurance exercise offsets the typical increase in vascular stiffness associated with aging (Laurent, Marenco et al. 2011). Furthermore, a recent longitudinal study reported that cardiorespiratory fitness was inversely related with PWV in healthy adults. Specifically, over 2 years, PWV in the low-fitness tertile increased more (1.4 ± 0.7 m·s⁻¹) than in the high-fitness group (Gando, Murakami et al. 2016). Likewise, Ramos et al. (2016) were the first to demonstrate that wave reflection characteristics (AIX, RM and Pb) are inversely associated with cardiorespiratory fitness (independent of body fat and other confounding factors) in middle-aged patients with metabolic syndrome (Ramos, Ramos et al. 2016). Together, these studies indicate that high cardiorespiratory fitness may attenuate the age-related increase in arterial stiffness.

A higher cardiorespiratory fitness may protect against age-related increases in arterial stiffness. In this regard, in a preclinical rodent model, the endurance-trained state is associated with a higher overall content of elastin (Nosaka, Tanaka et al. 2003) and collagen cross-linking in the arterial wall, which maintains a more compliant vascular system (Fleenor, Marshall et al. 2010). Additionally, aerobic training in rodents completely reversed smooth muscle α-actin (a marker of collagen-synthesising phenotype) and artery calcification (Fleenor, Marshall et al. 2010). Furthermore, regular aerobic exercise may protect against the age-related increases in arterial stiffness due to its ability to improve traditional risk factors associated with arterial wall degeneration, such as blood pressure, plasma lipids and blood glucose (Mora, Cook et al. 2007, Hasegawa, Fujie et al. 2016). Specifically, in a recent cross-sectional study, cardiorespiratory fitness was inversely associated with plasma lipids and PWV in healthy older individuals (Hasegawa, Fujie et al. 2016). Collectively, data from rodent models and human studies
indicate that exercise may minimise the age-related increases in arterial stiffness by normalising structural factors within the arterial wall.

2.8.2. Aerobic exercise training and arterial stiffness
The effect of aerobic exercise training on reducing arterial stiffness has been investigated in various populations. A recent meta-analysis assessed data from 21 randomised control trials and demonstrated a significant decrease in PWV of $-0.52$ m.s$^{-1}$ (95% CI $-0.76$ to $-0.27$) after 6–24 weeks of aerobic training in 752 (healthy and diseased) adults between the ages of 19 and 71 years (Montero, Vinet et al. 2014). Similarly, a recent meta-analysis assessed data from 27 randomised control trials ($n = 754$) and reported that carotid-femoral PWV and AIx were reduced significantly after aerobic training for 4–52 weeks (Ashor, Lara et al. 2014). This meta-analysis highlighted that the relative and absolute exercise intensity, rather than the volume (frequency $\times$ duration), of the training sessions was associated with reductions in resting AIx. Additionally, longer-term training interventions (>10 weeks) reduced resting PWV and AIx more than short-term interventions (Ashor, Lara et al. 2014). Additionally, a subgroup analysis derived from the same study demonstrated a greater reduction in PWV after the aerobic exercise intervention in participants with stiffer arteries (PWV >8 m.s$^{-1}$). This finding suggests that chronic aerobic exercise can significantly reduce measures of arterial stiffness with greater benefit for individuals with high resting arterial stiffness and, therefore, high cardiovascular risk.

2.8.3. Aerobic exercise training and arterial stiffness in older adults
Several studies have demonstrated that moderate and low intensity aerobic exercise interventions of 8 to 24 weeks can significantly reduce carotid-femoral PWV in older normotensive individuals (Sugawara, Inoue et al. 2004, Miura, Nakagawa et al. 2008, Fantin, Rossi et al. 2012, Hasegawa, Fujie et al. 2016, Kim, Hwang et al. 2016). In contrast, Oudegeest et al. (2013), after conducting 24 months of moderate-intensity aerobic training in older sedentary adults, reported no significant change in carotid-femoral PWV (Oudegeest-Sander, Olde Rikkert et al. 2013). Interestingly, Shibata et al. (2012) demonstrated significant increments in systemic arterial compliance (inverse to arterial stiffness) in older master athletes, but not in sedentary older adults, after a year of moderate to vigorous exercise training (Shibata and Levine 2012). These findings indicate that increased cardiorespiratory fitness may render the older vascular system more responsive to an exercise intervention of the same relative intensity.
Findings in younger individuals demonstrate that training of increased exercise intensity is associated with greater decrements in carotid-femoral PWV (Ciolac, Bocchi et al. 2010). In contrast, Kim et al. (2016) found that moderate-intensity but not high-intensity training led to a decrease in carotid-femoral PWV in older healthy adults (Kim, Hwang et al. 2016). Specifically, participants underwent either moderate continuous-intensity training, performed for 47 min at 70% of heart rate (HR) peak, or high-intensity interval training performed for 40 min of 4×4 min at 90% of HRpeak, alternating with 3×3 min of active recovery at 70% of HRpeak (Kim, Hwang et al. 2016). The duration of the moderate-intensity training per session was longer in order to match the higher caloric expenditure of high-intensity training, which may have provided a greater exercise-induced stimulus that led to the observed decrements in PWV. No other data exist regarding the training effect of exercise intensity on arterial stiffness in older adults; therefore, further research in this area is needed.

2.8.4. Aerobic exercise training and arterial stiffness in patients with cardiovascular disease
The effect of exercise training on arterial stiffness in patients with cardiovascular diseases has been explored in several studies. Short-duration (4–12 weeks) aerobic exercise training of moderate and higher intensity resulted in significant reductions in arterial stiffness in individuals with established cardiovascular disease. Decreases in PWV after moderate-intensity exercise training have been observed in patients with myocardial infarction (Trzos, Kurpesa et al. 2007) and chronic kidney disease (Greenwood, Koufaki et al. 2015), and after high-intensity training in patients with chronic heart failure (Chrysohoou, Angelis et al. 2015). The training-induced decreases in carotid-femoral PWV in these studies ranged from 0.8 to 1.8 m.s\(^{-1}\); importantly the increase in carotid-femoral PWV in the control (no exercise) group ranged from 1.0 to 2.1 m.s\(^{-1}\). Given that 1 m.s\(^{-1}\) increase in this index has been associated with a 15% increase in cardiovascular events (Vlachopoulos, Aznaouridis et al. 2010), short-term aerobic training can evoke beneficial adaptations in the structure of the vascular system that may substantially reduce cardiovascular risk in patients suffering from cardiovascular disease.

2.8.5. Aerobic exercise training and arterial stiffness in patients with hypertension
The effect of moderate-intensity aerobic exercise training on arterial stiffness in patients with hypertension has been well documented; however, results were inconsistent. Some studies demonstrated improvements in arterial stiffness after an exercise intervention (Aizawa and Petrella 2008, Collier, Kanaley et al. 2008, Madden, Lockhart et al. 2013) while others did not (Ferrier, Waddell et al. 2001, Fantin, Rossi et al. 2012). A recent meta-analysis examined data
from 14 trials evaluating the effects of aerobic exercise training on PWV in a total of 472 patients with hypertension (Montero, Roche et al. 2014). After data pooling, the mean change in resting PWV ($-0.19 \text{ m.s}^{-1}$) for aerobic exercise–trained hypertensive patients was non-statistically significant. Post-intervention arterial stiffness was also similar in the aerobic exercise–trained and control patients with hypertension ($-0.10 \text{ m.s}^{-1} [95\% \text{ CI } -0.34 \text{ to } 0.14]$). The authors postulated that the plasticity of the arterial structure and therefore the ability to adapt to exercise training diminished as arteries were chronically exposed to elevated blood pressure (Payne, Wilkinson et al. 2010). Furthermore, it was speculated that stiffer arteries and the impaired sensitivity of the baroreflex described in hypertensive adults (Dauphinot, Kossovsky et al. 2013) led to higher exercise-induced aortic wall stress than in normotensive peers, which could plausibly exceed a potential threshold for beneficial vascular adaptation.

A subgroup meta-regression analysis of the same study (Montero, Roche et al. 2014) revealed that PWV was significantly decreased in aerobic exercise–trained hypertensive adults who also showed a reduction in systolic blood pressure below the median value of 7.6 mmHg. Conversely, no change in PWV was observed in hypertensive adults with a reduction in systolic blood pressure above the median value. The inconsistent effect of aerobic exercise on sympathetic activity in hypertensive individuals (Brown, Dengel et al. 2002) could explain, to some extent, the variable responses of blood pressure and arterial stiffness to aerobic training in this population. However, high-intensity interval exercise training of 16 weeks – 40 min on a treadmill with intensity alternating between 50% (2 min) and 80% (1 min) of HR reserve – significantly reduced PWV in hypertensive individuals compared to the effect of moderate-intensity exercise training (40 min on a treadmill at 60% of HR reserve), (Guimaraes, Ciolac et al. 2010). The fact that high-intensity training has been associated with increased sympathetic activity and greater reductions in blood pressure (Ciolac, Bocchi et al. 2010) may explain this observation. These results suggest that only high-intensity exercise is effective in reducing arterial stiffness in adults with hypertension.

### 2.9. Acute arterial stiffness response to a single bout of exercise

Although exercise is a critical stress that drives the beneficial cardiovascular adaptations associated with exercise training, it is during the recovery period when these adaptations take place (Romero, Minson et al. 2017). Changes in arterial stiffness observed in recovery from exercise are suggested to reveal important insights into a period of cardiovascular benefit or vulnerability (Luttrell and Halliwill 2015) and may be necessary for long-term adaptation (Green and Smith 2017). However, the arterial stiffness response to individual bouts of exercise
have yes to be investigated in patients with cardiovascular disease, including patients with AAA. Hence, exploring the effect of a short bout of exercise on arterial stiffness during recovery would provide insight into the potential efficacy of exercise training in reducing arterial stiffness in this population.

2.9.1. Arterial stiffness response to a single bout of exercise in healthy young adults
Arterial stiffness indices increase from baseline immediately after (i.e. 0–5 min) a bout of aerobic exercise in healthy young adults (Mutter, Cooke et al. 2017). Studies assessing carotid-femoral PWV demonstrate a significant increase until 5 min after maximal exercise (Doonan, Mutter et al. 2013) and also after moderate-intensity exercise (Hull, Ansley et al. 2011, Milatz, Ketelhut et al. 2015) in young adults. Similarly, Alx75 is elevated in the first 5 min after exercise in healthy young adults (Disch, Engelberger et al. 2011, Doonan, Scheffler et al. 2011, Nieman, Dew et al. 2013). As time from exercise cessation increases, there is a consistent trend of a decrease in indices of arterial stiffness towards or below baseline values. Studies have found carotid-femoral PWV to be reduced for up to 60 min after moderate intensity exercise compared to baseline values in young healthy adults (Kingwell, Berry et al. 1997, Heffernan, Collier et al. 2007, Milatz, Ketelhut et al. 2015, Müller, Wilms et al. 2015). Similarly, Alx75 decreased until 60 min after aerobic cycling and treadmill exercise of moderate and high intensity in healthy young adults (Munir, Jiang et al. 2008, Hanssen, Nussbaumer et al. 2015).

The underlying mechanisms of the transient reductions in arterial stiffness following exercise are complex. Short-term reductions in arterial stiffness during the recovery from exercise are unlikely attributed to changes in aortic structure (Whyte and Laughlin 2010), but to improvements in cardiovascular function including changes in the arterial tone (Fok, Jiang et al. 2012), reductions in sympathetic nervous activity (Halliwill, Buck et al. 2013) and anti-inflammatory cytokines (Jae, Yoon et al. 2013) and increases in endothelial function (Munir, Jiang et al. 2008). In more detail, exercise-induced reductions in PWV and Alx75 are accompanied by an increase in blood flow (Kingwell, Berry et al. 1997, Sugawara, Otsuki et al. 2003, Munir, Jiang et al. 2008). The associated increase in blood flow and resultant shear stress on the vessel wall causes vasodilation that may lead to the observed reduction in arterial stiffness indices. In addition, stretching carotid and aortic baroreceptors during exercise inhibits sympathetic activity, which may also result in vasodilation and reduced arterial stiffness during exercise cessation (Halliwill, Buck et al. 2013). Specifically, baroreflex sensitivity was significantly decreased 30 min after cycling exercise in the presence of reduced PWV.
Further research is needed to fully explain the physiological mechanisms of the arterial stiffness response to exercise.

2.9.2. Acute arterial stiffness response to exercise and cardiovascular risk
Individuals with high cardiovascular risk, for example, obese and hypertensive individuals, show an exaggerated and prolonged post-exercise increase in PWV (Keith, Rattigan et al. 2013, Gkaliagkousi, Gavrilaki et al. 2014, Bunsawat, Ranadive et al. 2017). This exaggerated response is associated with an increased risk of exercise-induced cardiovascular events (Eijsvogels, Fernandez et al. 2016, Goodman, Burr et al. 2016). Such a response could trigger several mechanisms that could lead to a cardiovascular event. An abnormal increase in blood pressure, increases sympathetic outflow and circulating catecholamine levels creating a cardiovascular-risk environment that is associated with ischemia, myocardial irritability and thrombotic occlusion (Thompson, Franklin et al. 2007, Goodman, Burr et al. 2016).

An exaggerated and prolonged increase in carotid-femoral PWV response after exercise has also been linked to the future development of cardiovascular disease. A previous study found no associations between resting PWV and markers of end-organ damage in healthy middle-aged adults. However, an abnormal increase in post-moderate intensity exercise PWV was significantly related to end-organ damage estimated by glomerular filtration rate (an index of kidney function) (Keith, Rattigan et al. 2013). Inappropriate aortic stiffening during haemodynamic perturbations, such as that induced during exercise, may represent early vascular dysfunction that contributes to later development of cardiovascular disease. Hence, exploring the immediate response of arterial stiffness to exercise might provide a basis for assessing exercise-induced cardiovascular risk. However, to date studies exploring the post-exercise arterial stiffness response in a population of high cardiovascular risk, such as older adults and patients with cardiovascular disease, such as patients with AAA, are lacking.

2.9.3. Reliability of arterial stiffness indices in response to exercise
As discussed in Sections 2.9.1 and 2.9.2, the response of arterial stiffness to exercise holds significant clinical value compare to resting measurements alone; hence, reliable measurement of indices of arterial stiffness after a bout of exercise is important. To date, the reliability of measuring PWV immediately after exercise has been reported to be excellent in both young and middle-aged adults (intra-class correlation coefficient, ICC 0.94) (Keith, Rattigan et al. 2013), while the post-exercise changes in AIx (ICC 0.69) and AIx75 (ICC 0.82) have only been reported in younger adults (Lim, Faulkner et al. 2016). However, the reliability of measuring post-exercise changes in arterial stiffness in older adults is unknown.
2.9.4. Characteristics and factors that affect the arterial stiffness response to a single bout of exercise

The response of arterial stiffness to exercise is dependent on individual characteristics such as age, sex, fitness level and health status, and on other factors such as exercise intensity and mode. The following section reviews the effect of these determinants on the acute arterial stiffness response to a bout of exercise.

2.9.4.1. The effect of aging on the acute arterial stiffness response to aerobic exercise

As discussed in Section 2.3.2, arterial stiffness at rest increases with age (Mitchell, Parise et al. 2004, McEniery, Yasmin et al. 2005). However, the effect of aging on the arterial-stiffness response to exercise is still poorly understood. To date, only one study has directly compared the effect of aging on post-exercise arterial stiffness. Sugawara et al. (2010) measured femoral-ankle PWV after an acute bout of moderate-intensity, single-leg cycling in old and young participants (Sugawara, Otsuki et al. 2010). A significant decrease in PWV was observed after exercise only in the younger participants. These findings are supported by those of Akazawa et al. (2015), who reported no changes in carotid-femoral PWV after cycling exercise of moderate intensity in healthy, untrained older women (Akazawa, Ra et al. 2015). Thus, although moderate-intensity exercise significantly reduces arterial stiffness in young adults (Kingwell, Berry et al. 1997, Heffernan, Collier et al. 2007, Milatz, Ketelhut et al. 2015), this exercise intensity may be an inadequate stimulus for reducing arterial stiffness in older adults. Age-related alterations in arterial structure and function render the aortic wall stiffer (Mitchell, Parise et al. 2004) and possibly less responsive to exercise-induced vascular alterations. However, to date findings are limited, hence further research that directly compares the effect of aging on post-exercise arterial stiffness is needed.

2.9.4.2. The effect of sex on the acute arterial stiffness response to aerobic exercise

To date, only two studies have directly compared the effect of sex on the acute arterial stiffness response to aerobic exercise. Compared to healthy males, healthy females demonstrated lower carotid-femoral PWV after maximal treadmill exercise (Doonan, Mutter et al. 2013) and lower Alx75 and carotid-femoral PWV after 2 h of treadmill exercise at 75% of VO2peak (Nieman, Dew et al. 2013). In both studies, the younger females demonstrated lower resting arterial stiffness than that of younger males. Therefore, it is plausible that a more compliant vascular system is more responsive to a bout of exercise than a vascular system with greater arterial stiffness (Silver, Snowhill et al. 2003). The authors attributed this difference in resting and
post-exercise arterial stiffness to differences in sex hormones as oestrogen is known to reduce arterial stiffness (Rossi, Frances et al. 2011) whilst androgens have the opposite effect (Pearson, Yandle et al. 2008).

2.9.4.3. The role of cardiorespiratory fitness on the acute arterial stiffness response to aerobic exercise

The effect of cardiorespiratory fitness on the acute arterial stiffness response to exercise has not been directly explored. In younger adults, a greater increase in post-exercise PWV was found after maximal exercise in males of lower cardiorespiratory fitness and higher PWV at rest compared with adults of higher cardiorespiratory fitness and lower resting PWV (Moore, Berrones et al. 2016). These results indicate that post-maximal exercise stress may be less tolerable in individuals with low cardiorespiratory fitness and high arterial stiffness at rest. Furthermore, greater cardiorespiratory fitness protects against inflammation-induced arterial stiffening at rest (Jae, Yoon et al. 2013) and against endothelial dysfunction following higher-intensity exercise in older adults (Bailey, Perissiou et al. 2017). Therefore, greater cardiorespiratory fitness may also protect against prolonged increases in arterial stiffness following exercise by inducing a greater decrease in post-exercise arterial stiffness. To date, the only study to investigate the effect of cardiorespiratory fitness on the arterial stiffness response after submaximal exercise was in older postmenopausal females (Akazawa, Ra et al. 2015). Akazawa et al. (2015) demonstrated a transient post-exercise decrease in AIx but not in PWV after training-induced increases in cardiorespiratory fitness. However, the fitness of the females in this study was considered poor based on normative data (Stensvold, Sandbakk et al. 2017). Higher levels of fitness may lead to greater reductions in post-exercise arterial stiffness. To date, there have been no direct comparisons of the post-exercise arterial stiffness response between older adults with lower and higher levels of cardiorespiratory fitness.

2.9.4.4. The effect of cardiovascular disease on the acute arterial stiffness response to aerobic exercise

In order to understand the effect of AAA on post-exercise arterial stiffness response, the effect of other cardiovascular diseases on this response must be considered. Only a small number of studies have directly compared the acute arterial stiffness response to exercise in patients with established cardiovascular disease, or in those with high cardiovascular risk, with the response in healthy age-matched controls. In a recent study, younger obese adults had a greater increase in post-exercise PWV following maximal exercise (Bunsawat, Ranadive et al. 2017) compared with age-matched normal-weight adults. Similarly, carotid-femoral PWV after maximal exercise increased above baseline for a longer period in patients with uncontrolled hypertension.
than in normotensive adults (Gkaliagkousi, Gavriilaki et al. 2014). However, Millen et al. (2016) demonstrated decreases below baseline in RM and Pb after moderate-intensity exercise in middle-aged adults with pre-hypertension or hypertension (Millen, Woodiwiss et al. 2016). The latter results suggest that moderate-intensity exercise can transiently reduce wave reflection characteristics, even in a clinical population with increased arterial stiffness at rest, such as those with hypertension. Most of the previous studies have explored the post-maximal exercise arterial stiffness response. Submaximal exercise intensities are more commonly adopted for exercise training interventions in clinical populations. However, the effect of cardiovascular diseases on the acute arterial stiffness response to submaximal exercise has yet to be investigated.

2.9.4.5. The effect of exercise intensity on the acute arterial stiffness response to aerobic exercise

Currently there are only two studies that have directly assessed the effect of exercise intensity on the arterial stiffness response to exercise. Tordi et al. (2010) demonstrated greater reductions in carotid-ankle PWV after high-intensity interval cycling exercise (6×4 min at 65% HRmax, 1 min at 85% HRmax) than after moderate-intensity, continuous cycling exercise (30 min at 70% HRmax) in healthy young adults (Tordi, Mourot et al. 2010). Similarly, a greater post-exercise decrease in AIx was observed after high-intensity, interval treadmill exercise (4×4 min at 90-95% HRmax, 3 min active recovery at 70% HRmax) than after moderate-intensity, continuous treadmill exercise (20 min at 80% HRmax) in healthy young adults (Hanssen, Nussbaumer et al. 2015). High-intensity exercise is associated with greater post-exercise reductions in baroreflex sensitivity and greater vasodilation compared with moderate-intensity exercise (Reynolds, De Ste Croix et al. 2017), which may explain the greater decreases in PWV and AIx. Although evidence from these studies highlights the apparent superiority of high-intensity exercise to moderate-intensity exercise in transiently reducing arterial stiffness, these observations have been made only in healthy young adults. In contrast, excessive increases in PWV are observed after a bout of high-intensity exercise in younger adults with cardiovascular complications, including hypertension and obesity (Shim, Yang et al. 2011, Gkaliagkousi, Gavriilaki et al. 2014, Bunsawat, Ranadive et al. 2017). An adverse increase in arterial stiffness in the period following exercise may exacerbate cardiovascular risk and related events (Schultz, La Gerche et al. 2017), and would be a concern for a high risk population such as older individuals and patients with AAA. To date, the effect of exercise intensity on the transient arterial stiffness response to exercise has yet to be explored in older adults and in cardiovascular disease populations such as patients with AAA.
2.9.4.6. The effect of interval exercise on the acute response of arterial stiffness to aerobic exercise

Currently, only three studies have assessed the effect of interval exercise on the acute arterial stiffness response to aerobic exercise. The studies were conducted in young adults using the cardio-ankle vascular index (CAVI), an index of systemic arterial stiffness. CAVI is obtained by recording the distance from the level of the aortic valve (i.e. brachial level) to the measuring point (i.e. the ankle) and the time delay between the closing of the aortic valve to the detected change in the arterial pressure wave at the set point (Sun 2013). Results demonstrated that low-to moderate-intensity (35–50% of HR reserve) interval exercise elicits greater reductions in post-exercise CAVI than does continuous exercise of the same intensity and workload (Wang, Zhang et al. 2014, Zheng, Zhang et al. 2014, Zhou, He et al. 2015). In addition, longer rest intervals did not attenuate the superior effect of interval exercise in reducing post-exercise arterial stiffness (Zhou, He et al. 2015). It has been speculated that atrial natriuretic peptide (ANP), a potent vasodilator, is released when atrial myocytes are distended (Schmitt, Qasem et al. 2004). Thus, during interval exercise, the repeated increases in blood flow may result in larger ANP release than during continuous exercise, leading to greater vasodilation and reductions in arterial stiffness. As noted above, these studies were conducted in young adults; The prescription of interval exercise is becoming popular in adults with cardiovascular disease as it allows for an overall higher exercise intensity stimulus and has potential for additional cardiovascular benefit compared with moderate-intensity continuous exercise (Astorino, Allen et al. 2012, Guiraud, Nigam et al. 2012). However, the effect of this type of exercise on post-exercise arterial stiffness is unknown in older and clinical population.

2.10. Summary

Abdominal aortic aneurysm (AAA) is defined as the localised dilatation of the abdominal aorta, surpassing the normal diameter by 50% and is responsible for ~2% of all deaths worldwide. Surgical aneurysm repair is currently the only effective treatment to prevent AAA rupture and aneurysm-related death but is generally only considered for patients with large AAA. Deteriorations in arterial structure and compliance due to remodelling of the vessel wall increase arterial stiffness in patients with AAA, a factor associated with aneurysm progression, and increased cardiovascular risk. In spite of being under AAA rupture risk and a high risk of cardiovascular morbidity and mortality, treatment options for patients with AAA are currently lacking. Exercise training has been widely reported to reduce resting arterial stiffness, in part due to transient decreases after a bout of exercise. However, little is known about the haemodynamic benefits and safety of acute exercise in patients with AAA. The recovery of
arterial stiffness from a single bout of exercise is suggested to reveal important insights into a period of cardiovascular benefit or vulnerability and may depend on factors such as the intensity of exercise and the fitness level of the individual. Understanding the acute arterial stiffness response to exercise in patients with AAA and older healthy adults may provide an insight into the potential efficacy of exercise training in reducing cardiovascular risk in these populations.
Chapter 3 includes the following manuscript which is currently under review:

Reliability of arterial stiffness indices at rest and following a single-bout of moderate-intensity exercise in older adults

Maria Perissiou¹, Tom G. Bailey¹, Mark Windsor¹, Anthony S. Leicht², Jonathan Golledge³,⁴, Christopher D. Askew¹

1. VasoActive Research Group, School of Health and Sport Sciences, University of the Sunshine Coast, Queensland, Australia.
2. Sport and Exercise Science, James Cook University, Townsville, Queensland, Australia.
3. Queensland Research Centre for Peripheral Vascular Disease, James Cook University, Townsville, Queensland, Australia.
4. Department of Vascular and Endovascular Surgery, The Townsville Hospital, Townsville, Queensland, Australia.

Journal: Clinical Physiology and Functional Imaging (Clin Physiol Funct Imaging)

Author Contributions

Student contribution to work: Involved in the conception of the study, collected all data, analysed and interpreted data, was responsible for writing all drafts of the manuscript including preparation of all figures and tables and modified drafts following co-author recommendations.

Conceived and designed the experiment: MP, TB, CA

Performed the experiment: MP, MW

Analysed the data: MP, TB, CA

Wrote/reviewed the paper: MP, TB, MW, AL, JG, CA
3.1. Introduction
Arterial stiffness increases with age (McEniery, Yasmin et al. 2005) and is one of the earliest detectable manifestations of adverse structural and functional vascular changes, contributing to the pathogenesis of cardiovascular disease (Steppan, Barodka et al. 2011) and end-organ damage (Lee et al. 2010). Observational studies suggest that monitoring arterial stiffness may provide a reliable prognostic marker in the early detection of cardiovascular disease risk (Mackenzie, Wilkinson et al. 2002). Furthermore, treatments that decrease arterial stiffness may be effective in reducing cardiovascular disease risk and mortality in older adults (Chen, Shen et al. 2017).

Increased arterial stiffness is characterised by amplified velocity of the pressure wave along the arterial tree. Carotid to femoral pulse wave velocity (PWV) is the criterion measure for the non-invasive assessment of central aortic arterial stiffness (Cecelja and Chowienczyk 2012), the vascular region which exhibits the greatest age-related stiffening (Mitchell, Parise et al. 2004, Wilkinson, McEniery et al. 2010, Lee Stoner 2012). Additionally, increased systemic arterial stiffness leads to an early return of the reflected pulse pressure wave, causing adverse increases in central pulse pressure (Nichols 2005), contributing to elevated systolic pressure. The augmentation index (AIx) provides an integrated summary of wave reflection timing and amplitude (Nichols and Singh 2002), and is defined as the central augmentation pressure as a percentage of pulse pressure. Pulse wave characteristics can be further interrogated using wave separation analysis to differentiate forward (Pf) and reflected (backward, Pb) pressure waves, independent of transit time (Westerhof, Guelen et al. 2006). Reflection magnitude (RM) expresses the ratio of Pb to Pf travelling pressure waves (Westerhof, Guelen et al. 2006), and may be more sensitive than AIx in predicting cardiovascular disease risk and mortality (Wang, Cheng et al. 2010).

Arterial stiffness increases immediately after exercise before returning to, or below, resting levels during a period of recovery in healthy, younger adults (Mutter, Cooke et al. 2017). This post-exercise decrease in arterial stiffness has been suggested to provide short-term benefits, including a reduction in central blood pressure (Millen, Woodiwiss et al. 2016). However, the increase in arterial stiffness after exercise has been shown to be exaggerated, and remain above baseline levels during recovery in adults with known cardiovascular risk factors, such as obese individuals (Shim, Yang et al. 2011, Bunsawat, Ranadive et al. 2017) and adults with untreated hypertension (Gkaliagkousi, Gavriliaki et al. 2014). Increases in sympathetic nerve activity (Smith, Buffington et al. 2015), oxidative stress (Kawamoto, Ninomiya et al. 2016) and
endothelial dysfunction (Duprez 2010) following exercise are implicated in arterial stiffening, and it is also these underlying mechanisms that contribute to the long-term development of cardiovascular disease and mortality (Dhalla, Temsah et al. 2000, Widmer and Lerman 2014, La Rovere and Christensen 2015). Sustained elevations in arterial stiffness expose the brain and kidneys to high pulsatile central blood pressure and microcirculatory dysfunction (Lee and Oh 2010), which are linked to future risk of stroke and hypertension. For example, an increase in PWV after exercise was strongly associated with abnormal markers of kidney function in older adults (Keith, Rattigan et al. 2013), and therefore monitoring changes in arterial stiffness and central blood pressure during the short-term recovery period after exercise may provide important prognostic information and assist to identify at-risk older adults.

Non-invasive, oscillometric technology (Sphygmocor Xcel; Atcor Medical, Sydney Australia) allows the estimation of PWV and wave reflection characteristics and is increasingly being used in research (Williams, Cockcroft et al. 2014), has FDA clearance, and with its observer-independent application, is well suited for clinical practice (Shoji, Nakagomi et al. 2017). The reliability of resting PWV (ICC: 0.99) and Alx75 (ICC: 0.98) is well established in middle aged adults (Hwang, Yoo et al. 2014). To support the use of post-exercise measures of arterial stiffness in clinical research and practice, the test reliability (precision) needs to be established across repeated assessments. This would enable clinically and statically meaningful post-exercise changes to be established, and therefore support the use of these assessments to assess intervention outcomes, and for prognosis. The reliability of PWV immediately after exercise has been reported in young and middle-aged adults (ICC: 0.94) (Keith, Rattigan et al. 2013) whilst reliability of the changes in Alx (ICC: 0.69) and Alx75 (ICC: 0.82) have only been reported during 30 min of post-exercise recovery in younger adults (Lim, Faulkner et al. 2016). The reliability of post-exercise changes in arterial stiffness in older adults is unknown. Therefore, by conducting three repeated assessments that were each separated by one week, this study aimed to determine the reliability of PWV, Alx, and RM at rest, immediately after, and during post-exercise recovery following a bout of moderate-intensity cycling exercise in older adults.

3.2. Methods

3.2.1. Participants
Ten healthy older participants (71 ± 5 years) were recruited through local community announcements and a University alumni group. Participants were included if they were aged 60-86 years, non-smokers, BMI<39, and had no musculoskeletal problems that limited exercise
participation. Participants were excluded if they had a history of cardiovascular, metabolic or renal disease. Participants with medically untreated hypertension (defined as an average SBP $\geq 140$ mmHg and/or an average DBP $\geq 90$ mmHg) were also excluded. All participants were fully informed of the study procedures and provided written informed consent prior to participation. The study conformed to the Declaration of Helsinki and was approved by the institutional ethics committees.

3.2.2. Experimental Overview
Participants visited the exercise physiology laboratory at the University of the Sunshine Coast on 4 occasions, each separated by at least 7 days. During the baseline visit, anthropometric data were collected, including %body fat composition measurement using bioelectrical impedance analysis (BC 545N, Tanita, Australia), before undertaking a maximal incremental cycling test for the determination of peak power output (PPO) and peak cardiorespiratory fitness ($V\dot{O}_2$peak). Each participant then underwent three repeat experimental visits at the same time of the day in order to reduce the potential confounding influence of diurnal variation on cardiovascular function (Dhaun, Moorhouse et al. 2014). Each experimental visit (visits 1-3) consisted of a bout of moderate-intensity, aerobic cycling exercise. Measures of arterial stiffness (PWV, RM, AIx) were made while participants were supine: 1) at rest before exercise, 2) immediately after exercise (0-5 min), and 3) during 60 min of recovery. Prior to attending the laboratory on each occasion, participants were instructed to abstain from exercise for 24h, caffeine and alcohol for 12h, and to be fasted for 3h.

3.2.3. Maximal incremental cycling test
An incremental cycling test was conducted to determine peak oxygen uptake ($V\dot{O}_2$peak). The test was performed on an upright cycle ergometer (Lode Corival, Lode B.V., Groningen, Netherlands) until volitional exhaustion (McDermott, Liu et al. 1998). Oxygen uptake was measured using a Parvo Medics TrueOne 2400 metabolic cart and software (Parvo Medics, East Sandy UT, USA). Following a 3-min warm up at 0 W, the test commenced at 20 W and increased by 10 W per min until volitional exhaustion. Heart rate (HR) via 12-lead ECG (Mortara Inc., WI, USA) and rating of perceived exertion (RPE), using the 0-10 Borg scale (Pfeiffer, Pivarnik et al. 2002), were measured every 60s during the test. Maximal effort during the test was confirmed based on the presence of the following: heart rate $>90\%$ of age-predicted maximum; respiratory exchange ratio $>1.15$; RPE $>9$; a consistent fall in pedal cadence ($>10$ revolutions per minute, RPM). $V\dot{O}_2$peak was determined as the highest 15s average over the last 60s of peak exercise. From this, peak power output (PPO) at $V\dot{O}_2$peak was used to calculate the
workload for the moderate-intensity exercise session in the experimental visits (Ferguson 2014).

3.2.4. Moderate-intensity continuous exercise (visits 1-3)
Participants performed a bout of cycling exercise consisting of a 3-min standardised warm-up at 0W, followed by 24 min of moderate-intensity continuous cycling (40% PPO) at a cadence of 60 RPM (Lode Corival, Lode B.V., Groningen, Netherlands). This protocol was designed to align with current exercise session recommendations for older adults (20 to 30 min at 40 to 60% of heart rate reserve or 40 to 60% PPO) (Garber, Blissmer et al. 2011). During exercise, HR (using 12-lead ECG) and RPE were measured every 60s. Brachial blood pressure was measured using a manual sphygmomanometer every 6 min.

3.2.5. Arterial stiffness measurements:
Indices of arterial stiffness (PWV, RM and AIx) were measured at baseline after 15 min of quiet rest in the supine position, using the SphygmoCor XCEL device (AtCor Medical, West Ryde, NSW, Australia). Measurements were repeated immediately following exercise (0-5 min) and at 20, 40 and 60 min during exercise recovery. Participants were instructed to remain quiet for 5 min before and during each arterial stiffness measurement. The measurement of wave reflection characteristics (AIx and RM) preceded PWV at all time-points, and a complete cycle for all arterial stiffness measurements lasted ~5 min.

3.2.5.1. Wave reflection characteristics
Brachial artery compression waveforms were collected for the measurement of blood pressure and wave reflection characteristics (SphygmoCor Xcel, AtCor Medical, Sydney, Australia). A cuff was placed on the right arm, midway between the shoulder and the elbow, at the level of the heart following standard manufacturer guidelines that have been described previously (Hwang, Yoo et al. 2014) (Fig 7). Prior to the assessment of wave reflection characteristics, blood pressure was assessed three times using an automated cuff (SphygmoCor Xcel, AtCor Medical, Sydney, Australia), with the average of the last two measurements used (Wilkinson, Fuchs et al. 1998). Subsequently, a corresponding aortic pressure waveform was generated by applying a proprietary digital signal processing and transfer function (Fig 8) (Butlin, Qasem et al. 2012), from which central systolic (cSBP), diastolic (cSDP), central pulse pressure (cPP), mean arterial pressure (MAP), augmentation pressure (AP) and AIx, were derived. Central pulse pressure was calculated as the difference between cSBP and cDBP. Augmentation pressure was calculated as the difference between cSBP and the pressure at the inflection point (the merging of forward and reflected waves). AIx is defined as the augmentation pressure...
expressed as a percentage of pulse pressure. As AIx is significantly affected by heart rate, the index was corrected for a heart rate at 75 beats per minute (AIx75). Wave separation analysis was also automatically applied by the SphygmoCor CVMS software, version 9. This method creates an assumed triangular-shaped flow wave by aligning the start, peak, and end of the flow wave with the foot, inflection point, and notch of the aortic pressure wave, respectively (Westerhof, Guelen et al. 2006). Based on the assumed flow wave, the aortic forward (Pf) and backward (Pb) pressure waveforms were calculated. Reflection magnitude (RM) was calculated as the ratio of Pb to Pf magnitude and expressed as a percentage of Pf [RM=(Pb/Pf) *100].

3.2.5.2. Pulse wave velocity (PWV)
To assess carotid-femoral PWV, carotid pulse waves were obtained by applanation tonometry of the right carotid artery, and femoral pulse waves were obtained by sphygmomanometry of the right thigh. The tonometer was positioned at the site of the carotid pulse whilst a femoral cuff was placed at mid-thigh (Fig 9). The distance between the carotid and femoral arteries was measured from the carotid site above the suprasternal notch, to the proximal edge of the thigh cuff. The thigh cuff was positioned midway between the hip and the knee. This distance and the same placement of the thigh cuff was then used for all repeat sessions for each individual. In the supine position, participants were asked to breathe steadily and remain relaxed to facilitate an optimal carotid pulse tonometry measurement. Once a regular carotid
pulse was detected, femoral pulse waves were collected simultaneously by partially inflating the thigh cuff to 80 mmHg. Measurements were based on 10 s pulse wave traces that were free of artefact and met the quality control threshold of the SphygmoCor Xcel device for pulse-to-pulse variability. PWV was then determined by calculating the ratio of the distance between the pulse measuring sites to the time delay between the carotid and femoral pulse waves (Fig 10) (Wilkinson, McEniery et al. 2010)

3.2.6. Statistical Analyses:
Arterial stiffness indices (PWV, AIx75 and RM) were measured at 1) rest (pre-exercise), 2) immediately post-exercise (0 min post) and 3) during 0-60 min of post-exercise recovery, on three separate occasions. Total area under the curve (AUC) of arterial stiffness indices (0-60
min post-exercise) were calculated to quantify total recovery. Data were also calculated as changes from rest (delta) to account for the small, but non-significant day-to-day variance in resting arterial stiffness values. To determine whether there were any significant changes in arterial stiffness indices at rest or following exercise between the three visits we initially included all data in a two-way (visit*time) linear mixed model (LMM). A two-way (visit*time) LMM was also used to detect delta differences in post-exercise arterial stiffness between visits. All analyses were performed using SPSS (version 21; SPSS, Chicago, IL, USA). All data are presented as mean (95% confidence interval; 95% CI), unless otherwise specified, and statistical significance was set at P<0.05. For P values < 0.000, the value is reported as P<0.001.

The reliability of arterial stiffness indices (PWV, AIx75 and RM) at 1) rest 2) immediately post-exercise and 3) the recovery AUC, across the three repeated visits, was initially determined comparing the mean responses across the three visits using a one-way LMM, and was further characterised using the intraclass correlation coefficient (ICC), the coefficient of variation (ratio of standard deviation to the mean, CV %), standard error of the mean (SEM) and the reliability coefficient (RC) (Weir 2005). ICC was used to assess the relative reliability of arterial stiffness indices, as ICC accounts for both the consistency of arterial stiffness from test to re-test (within-participant change), as well as the systemic change in the mean between visits. The ICC was calculated as the ratio of the squared between subject variance to the sum of squares of between and within subject variance [SD_b^2/(SD_b^2 + SD_w^2)]. ICC values above 0.75 were considered to indicate excellent reliability, 0.40–0.74 good reliability, and less than 0.40 suggests poor reliability (Fleiss 1999). The RC was used as a measure of absolute reliability, which assessed the variability in arterial stiffness due to random and systemic measurement error. The RC is a useful index that quantifies absolute reliability measurement error in the same units as the measurement itself. RC was calculated by multiplying the within-participant SEM by 2.77 (√2*1.96) (Weir 2005, Vaz, Falkmer et al. 2013).

3.3. Results

3.3.1. Participant Characteristics
Participant characteristics are presented in Table 1. Peak power output was 140 W (95% CI 100 to 180), and \( \text{VO}_2\text{peak} \) was 24.6 ml.kg\(^{-1}\).min\(^{-1}\) (95%CI 18.9 to 30.3), which can be characterised as fair according to normative age- and sex-specific data (Garber, Blissmer et al. 2011).
3.3.2. Exercise variables

Heart rate increased by 21 bpm (95%CI 18 to 29, P=0.003) during cycling exercise and did not differ between visits (P=0.465). Similarly, MAP increased by 8 mmHg (95%CI 4 to 10, P=0.002) during cycling exercise, with no differences observed between visits (P=0.840). RPE increased by 2 (95%CI 1 to 3, P<0.001) during exercise, and did not differ between visits (P=0.431).

3.3.3. Resting and post-exercise measures of arterial stiffness

Figure 11 shows the mean responses for PWV (Fig 11A), AIx75 (Fig 11B) and RM (Fig 11C) at rest and at each time point after exercise for the three repeat visits. There was no difference in resting values of arterial stiffness indices between visits. PWV increased from baseline to immediately after exercise [mean increase at 0-5min post of 0.6 m.s\(^{-1}\) (95%CI 0.26 to 0.96, P<0.001)], before returning to near baseline levels. The increase in AIx75 was negligible immediately after exercise, before decreasing below baseline levels at 40 and 60 min post-exercise (60 min decrease of 3.5 % (95%CI 1.4 to 5.7, P<0.001). RM decreased immediately after exercise [mean decrease at 0-5 min post of 10.1 % (95%CI 5.3 to 14.8, P<0.001)] before returning to near baseline levels.

A main effect for “visit” was observed for PWV (Fig 11A), where mean PWV (across all time points) during visit 3 was lower compared to visit 1 (P<0.001) and visit 2 (P=0.042).

| Table 1. Participant characteristics and maximal incremental test results. |
|---------------------------------|-----------------|-----------------|
| Variable                        | Mean± SD        |                 |
| Age, years                      | 71 ± 5          |                 |
| Male, %                         | 70              |                 |
| Weight, kg                      | 70 ± 12         |                 |
| Height, cm                      | 173 ± 11        |                 |
| BMI, kg. m\(^2\)                | 24 ± 3          |                 |
| Heart rate, bpm                 | 59 ± 6.5        |                 |
| Systolic BP, mmHg               | 130 ± 16        |                 |
| Diastolic BP, mmHg              | 75 ± 9          |                 |
| Maximal incremental cycling test|                 |                 |
| \(\dot{VO}_{2peak}\), ml.kg\(^{-1}\).min\(^{-1}\) | 24.6 ± 8.0 |                 |
| Peak power output, W            | 140 ± 51        |                 |
| Peak heart rate, bpm            | 144 ± 19        |                 |

BMI: body mass index; BP: blood pressure; VO\(_{2peak}\): Peak oxygen consumption.
Conversely, AIx75 (Fig 11B) was higher during visit 1 compared to visit 2 (P=0.006), but not compared to visit 3 (P=0.084). There were no significant visit*time interactions for PWV, AIx75, and RM (Fig 11A-C), indicating that the response to exercise (time effect) was consistent across visits. This was confirmed when the change in each variable from baseline (delta) was assessed, which showed that there was no difference in the arterial stiffness response across the visits (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 mins</th>
<th>20 mins</th>
<th>40 mins</th>
<th>60 mins</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1</td>
<td>0.9 ± 1.0</td>
<td>0.4 ± 0.6</td>
<td>0.5 ± 0.7</td>
<td>0.6 ± 0.8</td>
<td>Visit :0.241</td>
</tr>
<tr>
<td>∆PWV (m.s⁻¹)</td>
<td>Visit 2</td>
<td>0.5 ± 0.8</td>
<td>0.2 ± 0.7</td>
<td>0.2 ± 1.0</td>
<td>0.4 ± 1.2</td>
</tr>
<tr>
<td>Visit 3</td>
<td>0.4 ± 0.4</td>
<td>0.3 ± 0.8</td>
<td>0.4 ± 0.8</td>
<td>0.4 ± 0.9</td>
<td>Visit time:0.398</td>
</tr>
<tr>
<td>∆AIx75 (%)</td>
<td>Visit 1</td>
<td>-0.1 ± 9</td>
<td>2.6 ± 7</td>
<td>-2.5 ± 5.2</td>
<td>-3.6 ± 4</td>
</tr>
<tr>
<td>Visit 2</td>
<td>2.4 ± 4</td>
<td>1.7 ± 2</td>
<td>-2.9 ± 4</td>
<td>-3.6 ± 4</td>
<td>Time:0.001</td>
</tr>
<tr>
<td>Visit 3</td>
<td>1.0 ± 6</td>
<td>1.2 ± 4.6</td>
<td>-3.7 ± 3</td>
<td>-3.4 ± 5</td>
<td>Visit*time: 0.943</td>
</tr>
<tr>
<td>∆RM (%)</td>
<td>Visit 1</td>
<td>-14.9 ± 15</td>
<td>-3.4 ± 15</td>
<td>-6.1 ± 10</td>
<td>-0.1 ± 13</td>
</tr>
<tr>
<td>Visit 2</td>
<td>-9.1 ± 10</td>
<td>-9.4 ± 10</td>
<td>-3.1 ± 10</td>
<td>-3.3 ± 11</td>
<td>Time: 0.001</td>
</tr>
<tr>
<td>Visit 3</td>
<td>-6.1 ± 8</td>
<td>-2.9 ± 9</td>
<td>1.7 ± 12</td>
<td>-3.4 ± 10</td>
<td>Visit * time:0.398</td>
</tr>
</tbody>
</table>

Table 2. Delta of PWV, AIx75 and RM from baseline at 0, 20, 40 and 60 min post exercise.

Alx75, augmentation index normalised to a heart rate of 75 bpm; RM, reflection magnitude; PWV, pulse wave velocity, ∆ denotes delta.

3.3.4. Reliability of arterial stiffness at rest, immediately post-exercise, and during recovery

Measures of reliability and the corresponding mean data for the indices of arterial stiffness at rest, immediately post-exercise and during recovery, across the three visits are shown in Table 3. Reliability of PWV at rest was excellent (ICC>.75), and more reliable than the other resting measures of vascular stiffness, particularly RM for which the reliability was borderline good-poor (ICC: 0.40). Reliability of the post-exercise measures of vascular stiffness was similar to that observed at rest for PWV and AIx75; whereas reliability was slightly improved for RM with an increase in the ICC (0.40 to 0.59) and a reduction in the reliability coefficient (11 to 9%) post-exercise compared with rest. Recovery of each vascular stiffness measure during the 60-min period after exercise (area under the curve) did not change across the three visits. The reliability of these recovery measures was similar to that observed at rest, as indicated by the comparable ICC and CV% values (Table 3).
Table 3. Reliability of absolute arterial stiffness indices at rest, immediately post exercise and during exercise recovery (AUC of 0 to 60 min post exercise).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>ICC</th>
<th>CV%</th>
<th>SEM</th>
<th>RC</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV (m.s⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>11.2 ± 2.4</td>
<td>11.2 ± 2.1</td>
<td>10.7 ± 2</td>
<td>0.91</td>
<td>5.0</td>
<td>0.7</td>
<td>1.9</td>
<td>0.84</td>
</tr>
<tr>
<td>Immediately post</td>
<td>12.2 ± 2</td>
<td>11.79 ± 2.4</td>
<td>11.31 ± 2</td>
<td>0.87</td>
<td>6.5</td>
<td>0.7</td>
<td>1.9</td>
<td>0.26</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>721 ± 150</td>
<td>692 ± 133</td>
<td>677 ± 129</td>
<td>0.94</td>
<td>4.9</td>
<td>56</td>
<td>154</td>
<td>0.78</td>
</tr>
<tr>
<td>Alx75 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>25 ± 9</td>
<td>22 ± 8</td>
<td>23 ± 8</td>
<td>0.72</td>
<td>14.0</td>
<td>3</td>
<td>7</td>
<td>0.24</td>
</tr>
<tr>
<td>Immediately post</td>
<td>25 ± 10</td>
<td>24 ± 8</td>
<td>24 ± 5</td>
<td>0.67</td>
<td>16.7</td>
<td>2</td>
<td>7</td>
<td>0.99</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>1441 ± 365</td>
<td>1284 ± 416</td>
<td>1330 ± 400</td>
<td>0.84</td>
<td>11.7</td>
<td>166</td>
<td>462</td>
<td>0.68</td>
</tr>
<tr>
<td>Pf (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>24.5 ± 7</td>
<td>23.7 ± 6</td>
<td>23.3 ± 5</td>
<td>0.71</td>
<td>9.4</td>
<td>1.8</td>
<td>4.8</td>
<td>0.12</td>
</tr>
<tr>
<td>Immediately post</td>
<td>31.2 ± 8</td>
<td>28.4 ± 6</td>
<td>27.3 ± 6</td>
<td>0.54</td>
<td>13.2</td>
<td>2.0</td>
<td>5.6</td>
<td>0.38</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>1586 ± 276</td>
<td>1570 ± 311</td>
<td>1414 ± 349</td>
<td>0.68</td>
<td>8.9</td>
<td>131</td>
<td>364</td>
<td>0.44</td>
</tr>
<tr>
<td>Pb (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>18.2 ± 3</td>
<td>17.4 ± 2</td>
<td>17.8 ± 3</td>
<td>0.54</td>
<td>9.0</td>
<td>0.8</td>
<td>2.2</td>
<td>0.34</td>
</tr>
<tr>
<td>Immediately post</td>
<td>19.4 ± 4</td>
<td>18.9 ± 4</td>
<td>19.5 ± 5</td>
<td>0.73</td>
<td>10.5</td>
<td>1.3</td>
<td>3.6</td>
<td>0.94</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>1093 ± 187</td>
<td>1072 ± 141</td>
<td>1052 ± 242</td>
<td>0.75</td>
<td>7.6</td>
<td>73</td>
<td>202</td>
<td>0.90</td>
</tr>
<tr>
<td>RM (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>75.5 ± 13</td>
<td>74.6 ± 13</td>
<td>74.5 ± 11</td>
<td>0.40</td>
<td>11.5</td>
<td>4</td>
<td>11</td>
<td>0.98</td>
</tr>
<tr>
<td>Immediately post</td>
<td>61 ± 11</td>
<td>66 ± 11</td>
<td>68 ± 10</td>
<td>0.59</td>
<td>9.8</td>
<td>3</td>
<td>9</td>
<td>0.26</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>4190 ± 432</td>
<td>4102 ± 532</td>
<td>4195 ± 399</td>
<td>0.38</td>
<td>9.1</td>
<td>180</td>
<td>500</td>
<td>0.89</td>
</tr>
<tr>
<td>HR (b.min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>60 ± 8</td>
<td>60 ± 8</td>
<td>59 ± 7</td>
<td>0.70</td>
<td>4.9</td>
<td>3</td>
<td>7</td>
<td>0.78</td>
</tr>
<tr>
<td>Immediately post</td>
<td>70 ± 8</td>
<td>69 ± 10</td>
<td>68 ±10</td>
<td>0.62</td>
<td>5.7</td>
<td>3</td>
<td>8</td>
<td>0.81</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>534 ± 170</td>
<td>590 ± 190</td>
<td>580 ± 185</td>
<td>0.77</td>
<td>4.8</td>
<td>170</td>
<td>502</td>
<td>0.62</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>127 ± 18</td>
<td>120 ± 13</td>
<td>118 ± 12</td>
<td>0.80</td>
<td>5.0</td>
<td>5</td>
<td>12</td>
<td>0.78</td>
</tr>
<tr>
<td>Immediately post</td>
<td>134 ± 17</td>
<td>130 ± 18</td>
<td>128 ± 13</td>
<td>0.81</td>
<td>5.8</td>
<td>5</td>
<td>14</td>
<td>0.71</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>10220 ± 1250</td>
<td>10025 ± 1180</td>
<td>9800 ± 930</td>
<td>0.85</td>
<td>3.5</td>
<td>355</td>
<td>984</td>
<td>0.65</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>75 ± 13</td>
<td>70 ± 9</td>
<td>71 ± 10</td>
<td>0.76</td>
<td>5.3</td>
<td>3</td>
<td>9</td>
<td>0.53</td>
</tr>
<tr>
<td>Immediately post</td>
<td>78 ± 9</td>
<td>76 ± 12</td>
<td>75 ± 10</td>
<td>0.89</td>
<td>4.7</td>
<td>3</td>
<td>8</td>
<td>0.64</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>6090 ± 780</td>
<td>5900 ± 780</td>
<td>5740 ± 730</td>
<td>0.91</td>
<td>3.5</td>
<td>242</td>
<td>650</td>
<td>0.57</td>
</tr>
</tbody>
</table>
Figure 11. Absolute PWV (A), Alx75 (B) and RM (C) at rest and post-exercise during visit 1 (white circles), visit 2 (white triangles) and visit 3 (white squares). Data are presented as mean and SD.*indicates significant change across time compared to baseline; post hoc analysis of visit effect revealed mean PWV was lower during visit 3 compared to visit 1 (p<0.001) and 2 (p=0.042), and mean Alx75 was higher in visit 1 compared to visit 2 (p=0.006);
3.4. Discussion
The present study examined the test-retest reliability of exercise-induced, short-term changes in arterial stiffness in healthy older adults. The main findings were that carotid-femoral PWV and wave reflection characteristics (AIx75 and RM) can be measured reliably in healthy older individuals at rest, immediately after, and during one hour of recovery from moderate-intensity cycling exercise across three separate occasions. These findings support the future use of post-exercise arterial stiffness measures for research and in clinical practice.

3.4.1. Reliability of resting arterial stiffness indices
This chapter shows that the test-retest relative reliability of resting carotid–femoral PWV was excellent over three repeat visits in older healthy individuals (ICC: 0.91), consistent with a recent study using the same device in young individuals (ICC: 0.98) (Hwang, Yoo et al. 2014). Furthermore, the ICC values (0.72-0.57) for AIx75, Pf and Pb at rest indicated good reliability and were consistent with recent studies (ICC, AIx75:0.70; Pf: 0.66; Pb: 0.76) also using the same device in young individuals (Lim, Faulkner et al. 2016, Stoner, Credeur et al. 2016). However, despite good reliability of the components of reflection magnitude at rest i.e. the forward and backward pressure wave, the reliability of RM at rest was poor. Similarly, Stoner et al., (2016) reported poor reliability for this index at rest in younger individuals (ICC: 0.48) (Stoner, Credeur et al. 2016). As the RM is the product of a ratio (Pb/Pf) that ranges from 0 to 1, this index is sensitive to small changes (Weir 2005), and may therefore be more vulnerable to signal noise (variance) than PWV and AIx75, as shown in the current study.

This strong relative reliability of PWV at rest corresponded with an absolute reliability (reliability coefficient: 1.9 m.s\(^{-1}\)) similar to that reported by Keith (1.5 m.s\(^{-1}\)). Based on a previous meta-regression analysis, an increase in PWV of 1.0 m.s\(^{-1}\) (or 1 SD) is associated with a 10% increase in cardiovascular risk (Vlachopoulos, Aznaouridis et al. 2010). As such, the absolute reliability of PWV reported in this study likely represents a clinically meaningful threshold. Absolute reliability of the RM (11%) and AIx75 (7%) in this study is also likely to be clinically relevant, given that a 10% change in these indices has been equated with a 10-34% increase in the incidence of cardiovascular events in middle-aged and older adults (Chirinos, Kips et al. 2012). These findings will help inform future interventional studies that aim to detect changes in resting measures of arterial stiffness across time, and provide new evidence to support the prognostic sensitivity of these measures.
3.4.2. Time-course of the changes in arterial stiffness after exercise

PWV increased immediately after exercise, followed by a transient return to baseline. We also observed a transient decrease in AIx75 and reflection magnitude to below resting levels after exercise. These observations are the first in older individuals and are similar to the responses recently reviewed in healthy younger adults (Mutter, Cooke et al. 2017). While resting measures of arterial stiffness reflect structural and functional mechanisms, beneficial reductions in arterial stiffness during recovery following acute exercise in healthy adults are suggested to be functional and are likely mediated by reductions in central blood pressure (Millen, Woodiwiss et al. 2016), vasoconstrictors (endothelin-1) (Schreuder, van Lotringen et al. 2014) and sympathetic nerve activity (Heffernan, Collier et al. 2007), alongside increases in nitric-oxide bioavailability and endothelial function (Campbell, Fisher et al. 2011). Elevations in arterial stiffness following exercise in obese and hypertensive adults (Shim, Yang et al. 2011, Gkaliagkousi, Gavriilaki et al. 2014, Moon, Moon et al. 2015, Bunsawat, Ranadive et al. 2017) are potentially detrimental, and may be due to underlying dysfunction of these mechanisms. Indeed, there is evidence of altered sympathetic baroreflex sensitivity that is exacerbated with exercise (Okada, Galbreath et al. 2013), revealing underlying dysfunction that is not evident at rest (Shim, Yang et al. 2011). As wave reflection characteristics are reported to represent reflected pressure waves from peripheral arteries and are influenced by changes in downstream artery function, it is likely that the decreases we observed in wave reflection (AIx75 and reflection magnitude) following exercise, in contrast to the negligible changes in PWV, are primarily due to peripheral vasodilation and the reduced vascular resistance within the exercising limbs. Exercise intensity could also be a factor contributing to the absence of a post-exercise decrease in PWV, as it has been previously demonstrated that higher intensity exercise results in greater post-exercise attenuations in PWV compared with moderate intensity exercise (Tordi, Mourot et al. 2010).

3.4.3. Reliability of arterial stiffness immediately after exercise

The increase in PWV immediately after exercise (0-5 min post) demonstrated excellent test-retest reliability (ICC: 0.87) over 3 consecutive visits. Similarly, wave reflection characteristics (AIx75, RM, Pf, and Pb) immediately after exercise demonstrated good reliability (ICC: 0.54 to 0.73). These findings are consistent with the reliability of the change in PWV (ICC 0.94) measured immediately after moderate-intensity cycling exercise in younger adults (30 to 54 yrs), where PWV was recorded with participants in the upright position seated on the cycle ergometer (Keith, Rattigan et al. 2013). Although posture affects haemodynamic regulation, PWV is not different between supine and seated positions at rest (Nurnberger, Michalski et al.
2011), and this Chapter’s results suggest the test-retest reliability of the immediate changes in PWV and wave reflection indices following exercise are also similarly reproducible in the supine position, compared to upright (Keith, Rattigan et al. 2013).

3.4.4. Reliability of arterial stiffness indices during exercise recovery

The reliability of post-exercise recovery measures of arterial stiffness was similar to that observed at rest. Reliability of the recovery of arterial stiffness following exercise was excellent for PWV and AIx75. Our results for AIx75 were in agreement with a recent study in younger adults that assessed the reliability of the changes in AIx75 during 30 min of recovery after incremental cycling exercise (ICC: 0.82) (Lim, Faulkner et al. 2016). It is important to note that PWV and AIx75 were significantly lower between visit one and two (at rest and post-exercise). However, this thesis reports no differences in the total post-exercise recovery of all arterial stiffness indices (AUC) across the three repeat visits. When the overall magnitude of the changes in arterial stiffness from resting values (delta) was assessed, any visit effect for PWV and AIx was abolished, indicting there was no familiarisation effect on the relative changes in post-exercise arterial stiffness.

The reliability of RM during exercise recovery was poor despite good reliability of its determinants (Pf: ICC 0.68, Pb: ICC 0.75). An earlier return of the reflected wave has been observed in older adults (Namasivayam, McDonnell et al. 2009), and likely causes the movement of the inflection point (the point where the forward and backward wave meet (Westerhof, Guelen et al. 2006)) into late systole that makes these indices less distinguishable (O’Rourke and Nichols 2005, Westerhof and Westerhof 2012). In addition, our assessment of the AUC of the complete recovery period likely increases the variability of this index compared to measurements at a single time point. In combination with the low ICC values for RM at rest, RM may not be sensitive to meaningful post-exercise changes in older individuals. This chapter shows that the post-exercise response of PWV and AIx75, but not RM, have strong reliability in older adults.

3.4.5. Clinical significance

The clinical significance of changes in arterial stiffness after exercise is not yet established, and this study provides the basis for future research in this area. Given the excellent reliability of the PWV response to exercise in older adults, the utility of this measurement as a prognostic marker should be considered. The reliability coefficient, or smallest detectable change, in the immediate response of PWV after exercise (1.9 m.s\(^{-1}\)) was similar to the change in PWV previously observed after exercise (increase of ~2.0 m.s\(^{-1}\)) in adults with untreated grade I
hypertension (Gkaliagkousi, Gavriilaki et al. 2014). Conversely, an increase in PWV of 1.3 m.s\(^{-1}\) following exercise has been associated with abnormal kidney function in middle-aged adults (Keith, Rattigan et al. 2013), however our data suggests that this is below the smallest detectable change in older adults. For future studies exploring the utility of the immediate change in PWV following exercise, we estimate that a cohort of 8 (assuming a sample SD of 1 m.s\(^{-1}\)) to 17 (SD of 2 m.s\(^{-1}\)) participants per group (with >80% power) would be required to reveal significant differences that correspond with the smallest detectable change (1.9 m.s\(^{-1}\)) in the PWV response.

3.4.6. Limitations
While novel results were presented, this study also had some limitations. The results of this study are not transferable to older individuals with cardiovascular disease, for which these measurements would be of particular interest. Furthermore, future studies may wish to confirm the thesis findings using high-intensity or maximal exercise as this is associated with the greatest exercise-induced risk (Rognmo, Moholdt et al. 2012). Nonetheless, moderate-intensity exercise in this study is recommended for older-adults in line with exercise guidelines for health in older population (Garber, Blissmer et al. 2011).

3.4.7. Conclusion
This study established the reliability of arterial stiffness indices at rest, immediately after exercise and during supine recovery from a bout of moderate-intensity aerobic exercise in healthy older adults. This study demonstrates that post-exercise measures of arterial stiffness are as reliable as measurements under resting conditions. These findings provide the basis for further use and investigation of the post-exercise measures of vascular stiffness, which may provide an insight into the risks and potential benefits of exercise, and may also be a more sensitive prognostic indicator of future cardiovascular risk than resting-measures.
4. Effects of Exercise Intensity and Cardiorespiratory Fitness on the Acute Response of Arterial Stiffness to Exercise in Older Adults.

Chapter 4 includes the following manuscript:

Maria Perissiou¹, Tom G. Bailey¹, Mark Windsor¹, Anthony S. Leicht², Jonathan Golledge³, Jonathan Golledge³, Christopher D. Askew¹

1. Vasactive Research Group, School of Health and Sport Sciences, University of the Sunshine Coast, Queensland, Australia.
2. Sport and Exercise Science, James Cook University, Townsville, Queensland, Australia.
3. Queensland Research Centre for Peripheral Vascular Disease, James Cook University, Townsville, Queensland, Australia.
4. Department of Vascular and Endovascular Surgery, The Townsville Hospital, Townsville, Queensland, Australia.

Author Contributions

Student contribution to work: Involved in the conception of the study, collected all data, analysed and interpreted data, was responsible for writing all drafts of the manuscript including preparation of all figures and tables and modified drafts following co-author recommendations.

Conceived and designed the experiment: MP, MW, TB, CA

Performed the experiment: MP, MW, TB

Analysed the data: MP, TB, CA

Wrote/reviewed the paper: MP, TB, MW, AL, JG, CA
4.1. Introduction
Arterial stiffness increases with age primarily due to increases in artery wall thickness (Tsao, Pencina et al. 2014), and a reduction in endothelial and autonomic function (Thijssen, Carter et al. 2016). An increase in arterial stiffness is one of the earliest detectable manifestations of these adverse structural and functional changes to the vasculature, and contributes to the incidence of hypertension and the development of atherosclerosis (McEniery, Yasmin et al. 2005, Cecelja and Chowienczyk 2012). Carotid to femoral pulse wave velocity (PWV) is the criterion index representing central arterial stiffness (Van Bortel, Laurent et al. 2012). Stiffening of the peripheral arteries also contributes to an early return of the reflected pressure wave, which contributes to an increase in central pulse pressure (Nichols, O’Rourke et al. 2011). Wave reflection characteristics are represented by the augmentation index (AIx) and reflection magnitude (RM) (Nichols and Singh 2002, Westerhof, Guelen et al. 2006). Increases in PWV, AIx and RM at rest are independently associated with the future risk of cardiovascular events and mortality (Mitchell, Hwang et al. 2010, Chirinos, Kips et al. 2012);

Arterial stiffness is reduced with regular physical activity (Gando, Murakami et al. 2016) and aerobic-exercise training in older adults (Fujie, Sato et al. 2014, Fujie, Hasegawa et al. 2015). The benefits of regular exercise on vascular structure and function are commonly attributed to the repeated, transient, hemodynamic perturbations observed in response to a single bout of exercise (Green and Smith 2017, Romero, Minson et al. 2017). Following a bout of aerobic exercise in healthy individuals, arterial stiffness indices have been reported to increase (e.g. 0-5 min post-exercise) before decreasing to, or below, resting levels during a period of recovery (Mutter, Cooke et al. 2017). These transient reductions in arterial stiffness during exercise recovery are suggested to provide a window of benefit (Luttrell and Halliwill 2015) including a transient reduction in central blood pressure (Millen, Woodiwiss et al. 2016). On the other hand, pronounced increases in central blood pressure and PWV have been reported in younger adults who are obese (Bunsawat, Ranadive et al. 2017) or have untreated hypertension (Gkaliagkousi, Gavriilaki et al. 2014). These increases in central blood pressure and PWV may be indicative of exercise-related risk in adults with established cardiovascular disease risk factors (Goodman, Burr et al. 2016).

While higher levels of cardiorespiratory fitness are associated with lower basal (i.e. resting) arterial stiffness in older adults (Gando, Murakami et al. 2016), the influence of cardiorespiratory fitness on the post-exercise arterial stiffness response is not well defined. In younger adults, post-exercise PWV is elevated in those with lower compared to higher
Higher-intensity interval exercise is increasingly recommended for older adults and clinical populations as it enables individuals to exercise at an intensity that may not be sustained with continuous exercise (Francois and Little 2015). Acute higher-intensity interval exercise is reported to induce a greater reduction in PWV and Alx compared to a bout of moderate intensity exercise in younger adults (Tordi, Mourot et al. 2010, Hanssen, Nussbaumer et al. 2015); however, the short-term responses of arterial stiffness to a single bout of exercise have not yet been clearly established in older adults. A better understanding of these responses, and how they are influenced by exercise intensity and cardiorespiratory fitness, would provide a greater insight into the potential risks and benefits of exercise among older adults. Therefore, this study aimed to compare the effect of moderate-intensity continuous and higher-intensity interval exercise on indices of post-exercise arterial stiffness, including PWV and wave reflection characteristics (Alx and RM) in older adults with low, mid and higher levels of cardiorespiratory fitness.

4.2. Methods

4.2.1. Experimental Overview
Participants underwent four laboratory visits, each following an overnight fast, refraining from alcohol and exercise for 24h, and caffeine for 12h, before each visit. Participants were required to consume a standardised meal consisting of 4 oat breakfast biscuits (20g carbohydrate, 8g fat) 3h prior to attending the laboratory. The baseline visit included an incremental maximal cycling test for the determination of cardiorespiratory fitness ($\dot{V}O_{2peak}$) and peak power output. Three experimental visits were then conducted in a cross-over, counter-balanced randomised order, and consisted of a no-exercise control, a moderate-intensity and a higher-intensity exercise protocol. Arterial stiffness was measured at baseline and during 90 min of supine recovery (0, 20, 40, 60 and 90 min). Lab conditions were standardised for each session in a climate controlled room (23 ± 1 °C). To control for the diurnal variation of blood pressure and arterial stiffness, each individual performed their three visits at the same time of day, separated by 7 days (Li, Cordes et al. 2014).

4.2.2. Participants
Fifty-one healthy older males and females aged 71 ±5 y were recruited from a university alumni cohort and local advertisements. Participants were included if they were aged 60-86 y, able to
complete cycling exercise and were non-smokers (>12 months of no smoking history). Participants were excluded if they had a known chronic metabolic or cardiovascular condition, or uncontrolled hypertension (average SBP ≥140 mmHg and/or an average DBP ≥90 mmHg). Participants were informed of the methods and study design verbally and in writing before providing written informed consent. The study conformed to the Declaration of Helsinki and was approved by the institutional ethics committees.

4.2.3. Maximal incremental cycling test
The maximal cardiorespiratory cycling test protocol is described in the Methods section of Chapter 3.2.3 of this thesis. The peak power output achieved during this test was then used to establish the exercise workload in the subsequent experimental visits.

4.2.4. Experimental exercise and control protocols
Following pre-test measurements of arterial stiffness and blood pressure, participants performed a 3-min warm up at 0 watts followed by continuous (24 min moderate-intensity at 40% PPO) or high-intensity interval (12 x 60 s bouts at 70% PPO, separated by 12 x 60 s bouts at 10% PPO) cycling exercise. This design ensured the continuous and interval cycling exercise protocols were duration- and work-matched. The control protocol consisted of duration-matched period of seated rest. Heart rate and RPE were recorded every 2 min during each protocol. Brachial blood pressure was measured using a manual sphygmomanometer every 6 min. Immediately following each protocol, participants were moved back to the supine position for post-protocol measurements of arterial stiffness.

4.2.5. Arterial stiffness measurements
Measures of arterial stiffness (PWV, RM, AIx) were made while participants were supine; using the SphygmoCor XCEL device (AtCor Medical, West Ryde, NSW, Australia), at baseline (after 15 min of quiet rest), and for 0-90 min post-exercise/control. Participants were asked to remain quiet and still, before and during each measurement. Wave reflection characteristics preceded PWV measurements, and these procedures are described in detail in the Methods section of Chapter 3.2.5 of this thesis.

4.2.6. Statistical analyses
Based on previous literature (Doonan, Mutter et al. 2013) who reported a post-exercise difference of the change in PWV of 1.2 ± 2.0 m s\(^{-1}\) between males and females, our power calculation revealed that a cohort of 17 participants per group (assuming a between group post-exercise difference in PWV of 1.2 m s\(^{-1}\) with a SD of 2 m s\(^{-1}\) and > 80% power) would be required to reveal significant difference in the post-exercise PWV response between fitness...
groups. To differentiate the cohort on the basis of cardiorespiratory fitness, participants were stratified into tertiles based on their $\dot{V}O_2^{\text{peak}}$. Each tertile corresponded to low- mid and high fitness levels based on the age and gender related normative data of ACSM. Linear mixed model (LMM) was used to compare anthropometric characteristics and a Pearson’s chi squared test was used to compare categorical data between the three fitness groups. A two-way (group*protocol) LMM was used to compare baseline arterial stiffness indices [PWV (m.s$^{-1}$), AIX75 (%) and RM (%)] across the study visits. A three-way (group*protocol*time) LMM was used to compare measurements of arterial stiffness indices and central blood pressure among fitness groups (low-, mid- and high-fit group), across “time” (baseline, 0-, 20-, 40-, 60-, and 90-min post) and between each protocol (control, moderate- and higher-intensity exercise). Data were also analysed as changes from baseline (delta) to account for any baseline variance. Three-way LMM analysis was also used to detect any differences in heart rate, blood pressure and perceived exertion in response to the protocols among the fitness groups, across time (at 2 min intervals for HR and RPE, and at 6 min intervals for BP) during each protocol. Statistically significant interactions were further investigated with multiple comparisons using the least significant difference approach (Rothman 1990). Analyses were conducted using the Statistical Package for Social Sciences (Version 22; IBM SPSS Inc., Chicago, IL). Statistical significance was set at $P<0.05$ and exact $P$ values are cited ($P$ values of 0.000 are reported as $<0.001$). Data are presented in the text as mean and 95% confidence interval (95%CI), unless otherwise stated.

4.3. Results

4.3.1. Participant characteristics
Participant characteristics of the complete cohort, and comparisons between the low, mid and high fitness groups, are shown in Table 4. Cardiorespiratory fitness, measured as $\dot{V}O_2^{\text{peak}}$ was higher in the high-fit group compared to mid-fit ($P<0.001$) and lower-fit group ($P<0.001$) (Table 4).
Table 4. Participant characteristics.

<table>
<thead>
<tr>
<th></th>
<th>All (n=51)</th>
<th>Low fit (n=17)</th>
<th>Mid fit (n=17)</th>
<th>High fit (n=17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>59</td>
<td>83</td>
<td>83</td>
<td>95</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>72 ± 5</td>
<td>74 ± 5</td>
<td>69 ± 4</td>
<td>70 ± 6</td>
<td>0.801</td>
</tr>
<tr>
<td>Hypertensive (%)</td>
<td>33</td>
<td>30</td>
<td>30</td>
<td>35</td>
<td>0.913</td>
</tr>
<tr>
<td><strong>Anthropometric measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174 ± 8</td>
<td>173 ± 8</td>
<td>172 ± 7</td>
<td>176 ± 9</td>
<td>0.223</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 ± 12</td>
<td>75 ± 12</td>
<td>79 ± 13</td>
<td>76 ± 10</td>
<td>0.545</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>25 ± 3</td>
<td>25 ± 3</td>
<td>26 ± 4</td>
<td>25 ± 3</td>
<td>0.426</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>28 ± 8</td>
<td>27 ± 8</td>
<td>27 ± 5</td>
<td>22 ± 5</td>
<td>0.917</td>
</tr>
<tr>
<td>Waist:Hip ratio</td>
<td>0.88 ± 0.1</td>
<td>1 ± 0.1</td>
<td>1 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.738</td>
</tr>
<tr>
<td><strong>Resting heart rate and blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>56 ± 8</td>
<td>59 ± 8</td>
<td>57 ± 6</td>
<td>51 ± 6</td>
<td>0.100</td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>131 ± 18</td>
<td>122 ± 14</td>
<td>124 ± 10</td>
<td>127 ± 10</td>
<td>0.471</td>
</tr>
<tr>
<td>Brachial DBP (mmHg)</td>
<td>76 ± 9</td>
<td>72 ± 9</td>
<td>72 ± 7</td>
<td>72.8 ± 6</td>
<td>0.682</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>115 ± 6</td>
<td>113 ± 13</td>
<td>114 ± 9</td>
<td>119 ± 7</td>
<td>0.185</td>
</tr>
<tr>
<td>Central DBP (mmHg)</td>
<td>73 ± 7</td>
<td>73 ± 9</td>
<td>73 ± 6</td>
<td>74 ± 6</td>
<td>0.530</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>42 ± 8</td>
<td>40 ± 7</td>
<td>42 ± 7</td>
<td>46 ± 9</td>
<td>0.162</td>
</tr>
<tr>
<td><strong>Medication use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARB / ACE inhibitors (%)</td>
<td>22</td>
<td>12</td>
<td>6</td>
<td>24</td>
<td>0.314</td>
</tr>
<tr>
<td>Antiplatelets (%)</td>
<td>9</td>
<td>18</td>
<td>6</td>
<td>6</td>
<td>0.412</td>
</tr>
<tr>
<td>Beta-blockers (%)</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0.125</td>
</tr>
<tr>
<td>Calcium channel blockers (%)</td>
<td>10</td>
<td>6</td>
<td>12</td>
<td>6</td>
<td>0.762</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>27</td>
<td>30</td>
<td>35</td>
<td>18</td>
<td>0.662</td>
</tr>
<tr>
<td><strong>Maximal incremental cycling test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V}O_{2\text{peak}} ) (L.min⁻¹)</td>
<td>2.2 ± 0.6</td>
<td>1.7 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>2.8 ± 5.6</td>
<td>0.001</td>
</tr>
<tr>
<td>( \dot{V}O_{2\text{peak}} ) (mL.kg⁻¹.min⁻¹)</td>
<td>28.9 ± 7</td>
<td>22.3 ± 3</td>
<td>27.5 ± 2</td>
<td>36.3 ± 6</td>
<td>0.001</td>
</tr>
<tr>
<td>Peak heart rate (bpm)</td>
<td>151 ± 15</td>
<td>142 ± 15</td>
<td>157 ± 14</td>
<td>156 ± 10</td>
<td>0.010</td>
</tr>
<tr>
<td>Peak RER (AU)</td>
<td>1.17 ± 0.1</td>
<td>1.23 ± 0.1</td>
<td>1.13 ± 0.1</td>
<td>1.14 ± 0.09</td>
<td>0.020</td>
</tr>
<tr>
<td>Peak Power (Watts)</td>
<td>163 ± 40</td>
<td>130 ± 27</td>
<td>160 ± 27</td>
<td>198 ± 34</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean and ±SD or number (%). P <0.05 indicates significant difference between fitness groups. SBP, systolic pressure; DBP, diastolic pressure; cPP, central pulse pressure; BMI, body mass index; ARB, Angiotensin II receptor blockers; ACE, angiotensin converting enzyme; \( \dot{V}O_{2\text{peak}} \), peak oxygen uptake; RER, respiratory exchange ratio.

4.3.2. Heart rate, mean arterial pressure and perceived exertion during the experimental protocols

Mean power output (W) during exercise was greater in the higher-fit group [moderate-intensity continuous: mean = 80W, (95% CI, 71 to 91); higher-intensity intervals: mean = 140W (95%
CI, 133 to 152)] compared to the mid [moderate-intensity continuous: mean = 64W, (95% CI, 48 to 74); higher-intensity intervals: mean = 112W (95% CI, 91 to 123), P<0.001] and low-fit group [moderate-intensity continuous: mean = 52W, (95% CI, 45 to 64); higher-intensity intervals: mean = 91W (95% CI, 82 to 103), P<0.001]. No differences were observed among the three fitness groups during exercise for mean heart rate (P=0.12), mean arterial pressure (P=0.94) and RPE (P=0.29). Mean heart rate was greater during higher-intensity interval exercise [mean heart rate 109 b·min⁻¹ (95% CI, 98 to 115)] compared to moderate-intensity continuous exercise [mean heart rate 91 b·min⁻¹ (95% CI, 78 to 100, P<0.001)], and was higher during both exercise protocols compared to control (P<0.05). MAP was greater during higher-intensity [mean MAP 108 mmHg (95% CI, 98 to 112)] compared to moderate-intensity exercise [mean MAP 103 mmHg (95% CI, 89 to 106), P=0.002] and was also higher during both exercise protocols than during control (P<0.05). Mean RPE was 4 (95% CI, 3 to 4) during higher-intensity interval exercise compared to 3 (95% CI, 2 to 3, P<0.001) during moderate-intensity continuous exercise.

4.3.3. Arterial stiffness and hemodynamic indices at baseline and in response to exercise
Arterial stiffness and hemodynamic indices at baseline and during recovery (0 to 90 min post) after exercise/control protocols for the three fitness groups (low-mid and high-fit) are shown in Tables 5 and 6. The relative change (delta) from baseline in PWV, AIx75 and RM among the three fitness groups for each protocol are shown in Figures 12-14. Findings are summarised below.

4.3.4. Baseline arterial stiffness indices
Arterial stiffness indices at baseline were similar across the three separate testing days (pre-exercise/control). AIx75 at baseline was 9.4% (95%CI 2.3 to 16, P=0.010) higher in the lower-fit compared to the higher-fit group (Table 5). There were no differences in PWV and RM between fitness groups.

4.3.5. Post-exercise arterial stiffness and hemodynamic indices
We did not observe a three-way (protocol*group*time) interaction for any of the arterial stiffness and hemodynamic indices (Tables 5 and 6).

4.3.5.1. The effect of exercise intensity and cardiorespiratory fitness on PWV
In control, PWV increased from baseline at all time-points whereas after the exercise protocols PWV increased from baseline at 90 min only (Table 5). A group*protocol interaction (P<0.001)
revealed that delta PWV was lower after both exercise protocols in the mid- and high-fit groups, compared with delta PWV in control (Figure 12). Delta PWV was lower after higher-intensity interval exercise compared to moderate intensity continuous exercise in the mid- and low-fit groups (Figure 12 B and C). Conversely, delta PWV was similar after moderate-intensity continuous exercise compared to control in the low-fit group, but was significantly lower after higher-intensity interval exercise compared to control (Figure 12).

4.3.5.2. The effect of exercise intensity and cardiorespiratory fitness on wave reflection characteristics
AIx75 decreased and remained below baseline for 90 min after control; and AIx75 was also significantly lower than baseline 40 to 90 min after both exercise protocols (Table 5). Delta AIx75 was significantly lower after control compared to both exercise protocols in all fitness groups (Figure 13). RM was unchanged after the control protocol, and decreased to below baseline levels for 20 min after moderate-intensity continuous exercise and for 90 min after higher-intensity interval exercise in all fitness groups (Table 5). A protocol effect (P<0.001) revealed that delta RM was significantly lower after both exercise protocols compared with control, in all the fitness groups (Figure 14). Further, a group*protocol interaction (P=0.009) revealed that delta RM was lower after higher-intensity interval exercise compared to moderate intensity continuous exercise in the in the mid- and low-fit groups, but not in the high-fit group (Fig 14 B and C).

4.3.5.3. The effect of exercise intensity and cardiorespiratory fitness on hemodynamic indices
Heart rate and central blood pressure indices are displayed in Table 6. After control, heart rate decreased below baseline for 90 min in all fitness groups. After both exercise protocols heart rate increased above baseline levels in all fitness groups, before returning to baseline by 60 min post. In all fitness groups, central blood pressure indices increased above baseline for 90 min after control. After both exercise protocols in all fitness groups, cPP increased immediately after exercise (0 min post), and decreased below baseline levels up to 60 min post-exercise.
Table 5. Arterial stiffness at baseline and after control, moderate continuous- and high-intensity interval exercise in low-, mid- and high-fit groups.

<table>
<thead>
<tr>
<th>Fitness groups</th>
<th>Protocol</th>
<th>Baseline</th>
<th>P values</th>
<th>Protocols*Time:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>CON</td>
<td>11.1 ± 2.2</td>
<td>12.0 ± 2.1*</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>11.5 ± 2.0</td>
<td>12.1 ± 2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>12.3 ± 2.3</td>
<td>12.1 ± 2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>11.2 ± 2.0</td>
<td>11.9 ± 2.0*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>11.3 ± 2.1</td>
<td>12.0 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>11.4 ± 1.9</td>
<td>11.9 ± 1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>11.2 ± 1.4</td>
<td>12.0 ± 1.9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>11.3 ± 1.8</td>
<td>11.7 ± 1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>11.3 ± 2.0</td>
<td>11.5 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>CON</td>
<td>24.7 ± 11</td>
<td>22.9 ± 1.8*</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>26.5 ± 11</td>
<td>21.5 ± 1.1*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>24.9 ± 11</td>
<td>24.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>23.8 ± 9</td>
<td>17.0 ± 1.7*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>22.7 ± 9</td>
<td>21.5 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>25.0 ± 9</td>
<td>23.2 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>14.7 ± 10</td>
<td>10.7 ± 8*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>17.5 ± 9</td>
<td>15.5 ± 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>16.3 ± 7</td>
<td>17.8 ± 8</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>CON</td>
<td>73.0 ± 16</td>
<td>77.7 ± 12</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>80.4 ± 13</td>
<td>62.0 ± 1.3*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>82.7 ± 13</td>
<td>57.3 ± 14*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>76.2 ± 11</td>
<td>71.7 ± 10</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>75.0 ± 10</td>
<td>63.2 ± 1.1*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>78.0 ± 9</td>
<td>60.3 ± 9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>78.5 ± 13</td>
<td>73.9 ± 11</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>77.1 ± 12</td>
<td>70.7 ± 11*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>79.8 ± 10</td>
<td>65.0 ± 12*</td>
<td></td>
</tr>
<tr>
<td>PWV (m.s⁻¹)</td>
<td>CON</td>
<td>120.0 ± 2.1</td>
<td>122.2 ± 2.5*</td>
<td>0.948</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>121.9 ± 2.0</td>
<td>119.2 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>121.9 ± 2.4</td>
<td>121.9 ± 2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>120.1 ± 2.0</td>
<td>120.2 ± 2.3*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>119.7 ± 2.0</td>
<td>117.2 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>117.2 ± 2.1</td>
<td>117.2 ± 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>120.1 ± 2.0</td>
<td>120.2 ± 2.0*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>119.7 ± 2.0</td>
<td>117.2 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>117.2 ± 2.1</td>
<td>117.2 ± 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>120.1 ± 2.0</td>
<td>120.2 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>119.7 ± 2.0</td>
<td>117.2 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>117.2 ± 2.1</td>
<td>117.2 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>RM (%)</td>
<td>CON</td>
<td>70.0 ± 13</td>
<td>79.0 ± 13</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>84.9 ± 10</td>
<td>77.6 ± 13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>79.7 ± 15*</td>
<td>79.4 ± 9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>78.7 ± 13</td>
<td>77.2 ± 12</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>70.6 ± 9*</td>
<td>72.8 ± 10</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>69.3 ± 12*</td>
<td>69.3 ± 12*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>74.3 ± 10</td>
<td>71.8 ± 11</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>75.3 ± 11*</td>
<td>78.0 ± 11</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>79.8 ± 12*</td>
<td>78.9 ± 12*</td>
<td></td>
</tr>
<tr>
<td>Pf (mmHg)</td>
<td>CON</td>
<td>16.7 ± 3.1</td>
<td>18.9 ± 4.1*</td>
<td>0.533</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>18.6 ± 3.6</td>
<td>18.3 ± 3.3*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>18.2 ± 3.9</td>
<td>16.8 ± 3.0*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>19.4 ± 3.3</td>
<td>18.5 ± 3.3*</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>18.0 ± 2.8</td>
<td>19.2 ± 4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>18.5 ± 2.4</td>
<td>18.5 ± 4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>17.8 ± 4.1</td>
<td>18.7 ± 4.4*</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>18.7 ± 4.2</td>
<td>19.9 ± 3.9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>19.0 ± 3.8</td>
<td>19.4 ± 3.9*</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD; CON, no-exercise control; MOD, moderate intensity exercise; HIGH, higher-intensity exercise; Alx75, augmentation index normalised to a heart rate of 75 bpm; RM, reflection magnitude; Pf, forward pressure wave; Pb, backward pressure wave; PWV, pulse wave velocity. *Significantly different to baseline based on protocol*time post-hoc comparisons.
Table 6. Heart rate and blood pressure indices at baseline and after control, moderate continuous- and high-intensity interval exercise in low, mid and high fit groups.

<table>
<thead>
<tr>
<th>Fitness groups</th>
<th>Protocol</th>
<th>Time point (min)</th>
<th>P-Value</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0 post</td>
<td>20 post</td>
</tr>
<tr>
<td>Low</td>
<td>CON</td>
<td>60 ± 10</td>
<td>56 ± 9*</td>
<td>54 ± 8*</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>59 ± 7</td>
<td>67 ± 9*</td>
<td>62 ± 8*</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>58 ± 8</td>
<td>71 ± 12*</td>
<td>63 ± 10*</td>
</tr>
<tr>
<td>Mid</td>
<td>CON</td>
<td>58 ± 8</td>
<td>54 ± 6*</td>
<td>52 ± 4*</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>56 ± 6</td>
<td>67 ± 8*</td>
<td>62 ± 7*</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>56 ± 6</td>
<td>67 ± 8*</td>
<td>62 ± 7*</td>
</tr>
<tr>
<td>High</td>
<td>CON</td>
<td>52 ± 6</td>
<td>59 ± 7*</td>
<td>55 ± 8*</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>51 ± 6</td>
<td>59 ± 7*</td>
<td>55 ± 8*</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>51 ± 6</td>
<td>64 ± 7*</td>
<td>59 ± 7*</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td></td>
<td>Baseline</td>
<td>6 ± 9</td>
<td>6 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 post</td>
<td>6 ± 9</td>
<td>6 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 post</td>
<td>6 ± 9</td>
<td>6 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 post</td>
<td>6 ± 9</td>
<td>6 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 post</td>
<td>6 ± 9</td>
<td>6 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90 post</td>
<td>6 ± 9</td>
<td>6 ± 9</td>
</tr>
<tr>
<td>cSBP (mmHg)</td>
<td></td>
<td>Baseline</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>cDBP (mmHg)</td>
<td></td>
<td>Baseline</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>cPP (mmHg)</td>
<td></td>
<td>Baseline</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90 post</td>
<td>78 ± 6</td>
<td>78 ± 6</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD; CON, no-exercise control; MOD, moderate-intensity exercise; HIGH, higher-intensity exercise; SBP systolic blood pressure; SDP, diastolic blood pressure; cPP, central pulse pressure. *Significantly different to baseline based on protocol*time post-hoc comparisons.
Figure 12. Delta PWV, response of low-fit (circles), mid-fit (squares) and high-fit (triangles) to control (A), moderate continuous (B) and high-intensity interval (C) exercise. Data are mean and error bars represent mean ± SEM. #Significantly different to control; *high-intensity protocol significantly different to moderate-intensity protocol.
Figure 13. Delta AIx75, response of low-fit (circles), mid-fit (squares) and high-fit (triangles) to control (A), moderate continuous (B) and high-intensity interval (C) exercise. Data are mean and error bars represent mean ± SEM. #Significantly different to control; *high-intensity protocol significantly different to moderate-intensity protocol.
Figure 14. Delta RM, response of low-fit (circles), mid-fit (squares) and high-fit (triangles) to control (A), moderate continuous (B) and high-intensity interval (C) exercise. Data are mean and error bars represent mean ± SEM. *Significantly different to control; *high-intensity protocol significantly different to moderate-intensity protocol.
4.4. Discussion
This study demonstrates that the post-exercise arterial stiffness response is dependent on both the intensity of exercise and the level of cardiorespiratory fitness in older adults. PWV and RM were lower after higher-intensity interval exercise compared to control in older adults of all fitness levels. Following moderate-intensity continuous exercise, this relative reduction in PWV was diminished in low-fit, but maintained in mid-and higher-fit older adults.

4.4.1. Arterial stiffness at rest
It has previously been suggested that high levels of cardiorespiratory fitness suppress the age-related increases in resting arterial stiffness (Gando, Murakami et al. 2016). In the current cohort of older adults, it was found that resting Alx75 was lowest in those with the highest cardiorespiratory fitness, which is consistent with previous reports (Binder, Bailey et al. 2006, Denham, Brown et al. 2016, Ramos, Ramos et al. 2016). In contrast, this study did not observe differences in the resting measures of PWV between the fitness groups. There have only been limited studies of the effect of fitness on PWV in older adults, showing that PWV is lower in masters athletes (Vaitkevicius, Fleg et al. 1993) and high-fit post-menopausal females (Gando, Kawano et al. 2010) when compared to low-fit control groups. The inconsistency between studies could be attributed to differences in the cardiorespiratory fitness levels of the high-fit group between this study (36 ml.kg⁻¹.min⁻¹) and the study of Vaitkevicius et al. (45 ml.kg⁻¹.min⁻¹). Due to increased age-related aortic stiffening (Mitchell, Parise et al. 2004), a higher cardiorespiratory fitness (than the reported in this Chapter) is potentially required in order to achieve lower resting carotid to femoral PWV compared to lower-fit individuals. Indeed, this fitness effect was not seen in a similar cohort (high-fit group at a similar level to this study (33 vs 36 ml.kg⁻¹.min⁻¹)) when PWV was assessed at the carotid-to-femoral segment (Ramos, Ramos et al. 2016), which is consistent with the finding reported in this Chapter, in older adults. Furthermore, a recent study suggested that the differences observed in PWV among individuals with differing levels of cardiorespiratory fitness is due to body fat differences and not fitness per se (Moore, Berrones et al. 2016). These findings may justify further our results as we observed no differences in %body fat and BMI between the three fitness groups in our study.

The conflicting effects of fitness of resting measures of Alx75 and PWV highlights crucial differences between the determinants of these indices of arterial stiffness. There is a progressive stiffening of the arterial tree from the ascending aorta and large elastic arteries towards the peripheral muscular conduit arteries (London and Pannier 2010). Carotid-to-femoral PWV quantifies the velocity of the pulse wave through the aorta, which undergoes the
greatest stiffening with age (Mitchell, Parise et al. 2004). Augmentation index (AIx75) characterizes the magnitude of the reflected pressure wave as it contributes to systolic blood pressure, and has therefore been reported to reflect peripheral arterial stiffness (Nichols 2005). Recent studies have challenged this view, suggesting that augmentation index is largely determined by aortic reservoir pressure (Wang, O'Brien et al. 2003). Aortic reservoir pressure increases with age probably as a result of impaired aortic compliance and the limited ability to buffer increases in aortic blood volume during cardiac ejection (Davies, Baksi et al. 2010). Aortic reservoir pressure has recently been shown to be lower in middle-aged adults with higher cardiorespiratory fitness (Ramos, 2016), and therefore may contribute to the lower AIx75 of the higher-fit older adults in the present study. Reflected pressure from the peripheral arteries is reported to be better represented by the reflection magnitude (RM, ratio of forward to backward pressure waves) (Hughes, Park et al. 2013, Millen, Woodiwiss et al. 2016). The finding of this study that RM did not differ between the fitness groups further supports the interpretation that differences in resting AIx75 between fitness groups are not likely to be caused by alterations in peripheral reflected wave function.

4.4.2. Post-exercise arterial stiffness
During recovery from a bout of aerobic exercise, indices of arterial stiffness have been reported to increase (Hull, Ansley et al. 2011, Gkaliagkousi, Gavriilaki et al. 2014), decrease (Kingwell, Berry et al. 1997, Heffernan, Collier et al. 2007, Lane, Ranadive et al. 2013), or remain unchanged (McCLean, Clegg et al. 2011, Akazawa, Ra et al. 2015) compared with baseline levels. This variance in the post-exercise arterial stiffness response is suggested to be due to the anatomical segment being assessed, the frequency of measures, as well as the age and health status of study participants (Mutter, Cooke et al. 2017). In the current cohort of older healthy adults, decreases in wave reflection characteristics following exercise were observed, but there were negligible changes in carotid-femoral PWV. Carotid-femoral PWV has been shown to be less susceptible to acute post-exercise changes when compared with peripheral PWV, i.e. changes in femoral–dorsalis pedis PWV (Heffernan, Collier et al. 2007, Munir, Jiang et al. 2008). The RM is reported to represent the reflected pressure from the peripheral arteries (Hughes, Park et al. 2013, Millen, Woodiwiss et al. 2016), which is dependent on changes in downstream arterial function, including any changes as a result of local muscle activity (Hickson, Nichols et al. 2016). Hence, it is likely that the reductions in wave reflection characteristics observed in the present study relate to peripheral vasodilation in the lower limbs during the period immediately following exercise.
The use of the no-exercise control condition, allowed this study to control for the influence of time and supine rest on changes in arterial stiffness. Indeed, a progressive rise in PWV was observed, which may reflect an increase in vascular tone (Fok, Jiang et al. 2012), and there was a reduction in AIx75, which may be due to a redistribution of blood volume and an altered cardiac preload (van de Velde, Eeftinck Schattenkerk et al. 2017) during the supine control condition. Compared to control, post-exercise PWV and RM were lower following a bout of higher-intensity interval exercise, while post-exercise AIx75 was higher as compared with control. These findings challenge previous studies that did not use a time-adjusted control comparison and reported post-exercise reductions in AIx75 (Lane, Ranadive et al. 2013, Hanssen, Nussbaumer et al. 2015, Millen, Woodiwiss et al. 2016). Our finding of a higher AIx75 after exercise as compared to after a period of rest further supports the notion that the augmentation index is largely determined by aortic reservoir pressure, as discussed earlier, which increases with exercise (Climie, Srikanth et al. 2015). McClean et al. (2011) previously observed no differences in post-exercise PWV as compared to control in younger adults; however, the authors only measured PWV immediately following exercise (McClean, Clegg et al. 2011). Similar to the present findings, Wang et al. (2014) reported a lower arterial stiffness (cardio-ankle vascular index; CAVI) after both continuous and interval moderate-intensity exercise as compared to control when measured during 40 min of recovery (Wang, Zhang et al. 2014), although this study also observed a decrease in arterial stiffness from baseline after exercise. This is the first study in healthy older adults to report a lower post-exercise arterial stiffness, where the response is attenuated as compared with control, (PWV and RM) following a bout of higher-intensity exercise.

4.4.2.1. The effect of exercise intensity on the arterial stiffness response to exercise

Compared with higher-intensity exercise, the response of post-exercise PWV and RM to moderate intensity continuous exercise was less pronounced in both the low and mid-fit groups. This is consistent with the augmented effect of higher-intensity compared to moderate-intensity exercise on lowering post-exercise arterial stiffness in some (Tordi, Mourot et al. 2010, Hanssen, Nussbaumer et al. 2015) but not all studies (Siasos, Athanasiou et al. 2016) in younger adults. Intensity-dependent changes in post-exercise arterial stiffness are complex, and may be due to increases in vessel tone and reductions in central pressure with higher intensity exercise (Millen, Woodiwiss et al. 2016). Transient reductions in cPP following exercise were observed in this study, and whilst this has previously been associated with corresponding changes in PWV (Lim, Pearman et al. 2016), there was not an intensity (protocol) effect for cPP, and thus
it is unlikely to explain these findings. Blood flow and shear stress are augmented with increased exercise intensity (Santana, Moreira et al. 2013, Bond, Hind et al. 2015) and may partly explain these findings. Shear-mediated increases in endothelial function (Munir, Jiang et al. 2008) and the activation of vasodilators e.g. nitric-oxide and prostaglandins (Poveda, Berrazueta et al. 1998), and inhibition of vasoconstrictors e.g. endothelin-1 expression (Di Francescomarino, Sciartilli et al. 2009) have all been associated with transient decreases in arterial stiffness after exercise. Further, exercise-induced suppression of pro-inflammatory cytokines (Jablonski, Donato et al. 2015) and intensity-dependant decreases in post-exercise sympathetic nerve activity (Heffernan, Collier et al. 2007) may also contribute to the lower arterial stiffness following higher-intensity exercise.

4.4.2.2. The effect of cardiorespiratory fitness on the arterial stiffness response to exercise

Post-exercise arterial stiffness has previously been reported to be lower in younger adults with high, compared with low, levels of cardiorespiratory fitness (Moore, Berrones et al. 2016). A similar trend was observed in the current study following moderate-intensity exercise, where the response of the low-fit group (delta PWV) did not differ to the rises observed during control, whereas participants in the high-fit group demonstrated an attenuation of the PWV response after both exercise intensities as compared to control. This suggests that higher levels of cardiorespiratory fitness may improve the sensitivity or responsiveness of the vasculature to submaximal exercise in older adults, and this may relate to the improved endothelial function and vascular tone seen with training and increased fitness in older adults (DeSouza, Shapiro et al. 2000, Pialoux, Brown et al. 2009). It is also possible that the absolute exercise intensity, which was greatest in the high-fit group, may have a generated a greater stimulus for post-exercise changes in arterial stiffness and contributed to our observations. However, it is important to note that there were no differences in heart rate, blood pressure or perceived exertion between the groups during exercise.

The significant differences found in post-exercise PWV and RM between the fitness groups, also support the recent suggestion that subtle underlying differences in arterial stiffness that are undetectable at rest become more pronounced with acute exercise (Keith, Rattigan et al. 2013, Schultz, La Gerche et al. 2017). It has also been reported that the arterial stiffness response to maximal exercise may be a useful tool to detect small but clinically relevant changes in vascular health (Shim, Yang et al. 2011, Bunsawat, Ranadive et al. 2017) and this paper extends this suggestion to now show that submaximal exercise may also reveal important
differences in arterial stiffness between adults with higher and lower levels of cardiorespiratory fitness.

4.4.2.3. Clinical implications
As increased PWV and RM reflect increased cardiovascular risk (Mitchell, Hwang et al. 2010, Chirinos, Kips et al. 2012), the lowering in arterial stiffness with exercise may reflect a transient cardiovascular benefit, and a reduction in cardiovascular risk (Millen, Woodiwiss et al. 2016). Similar to how post-exercise hypotension may predict chronic blood pressure reductions with repeated exercise sessions (Kiviniemi, Hautala et al. 2014), the lowering of arterial stiffness may also contribute to the decrease in resting arterial stiffness after exercise training in older adults (Kim, Hwang et al. 2016). Whether the acute influence of exercise intensity or cardiorespiratory fitness seen in the present study has any implications for the effect of exercise training on arterial stiffness in older adults remains to be determined.

4.4.2.4. Limitations
This study included healthy older male and female adults and the fact that sex-related differences in arterial stiffness (Coutinho, Borlaug et al. 2013) may limit some comparisons in the present study is acknowledged. However, it should be noted that the proportion of males and females was similar in each fitness group in this study, and thus is unlikely to have substantially influenced these findings. The inclusion of participants with controlled hypertension (n=16) may have also influenced these findings, but again the proportion of those with hypertension was similar between groups (30-35%). Anti-hypertensive medication has been reported to lower PWV at rest (Mahmud and Feely 2008), and may have confounded the differences in arterial stiffness across fitness groups in this study. Finally, this thesis did not include measures of potential mechanisms involved in the changes in arterial stiffness with exercise, and this should be the focus of future research.

4.4.3. Conclusions
In conclusion, the present study suggests that the post-exercise arterial stiffness response is dependent on both the intensity of exercise and the level of cardiorespiratory fitness in healthy older adults. PWV increases during prolonged seated rest in older adults. Moderate-intensity continuous exercise has a positive attenuation on PWV as compared with seated-rest control in those with higher, but not lower, levels of fitness. PWV and RM are lower after higher-intensity interval exercise as compared with control in older adults of all levels of cardiorespiratory fitness. Submaximal exercise reveals differences in arterial stiffness responses between adults with higher and lower levels of cardiorespiratory fitness.
5. Arterial Stiffness Response Following a Bout of Exercise in People with and without Abdominal Aortic Aneurysm

Chapter 4 includes the following manuscript:

Maria Perissiou¹, Tom G. Bailey¹-², Mark Windsor¹, Kim Greaves¹-³, Jill O’Donnell³, Karl Schulze⁴, Anthony S. Leicht⁵, Jonathan Golledge⁶, Christopher D. Askew¹

1. VasoActive Research Group, School of Health and Sport Sciences, University of the Sunshine Coast, Queensland, Australia.
2. Centre for Research on Exercise, Physical Activity and Health, School of Human Movement and Nutrition Sciences, University of Queensland
3. Sunshine Coast Hospital and Health Service, Queensland, Australia.⁴Sunshine Vascular Private Clinic, Queensland, Australia.
4. Sport and Exercise Science, James Cook University, Townsville, Queensland, Australia.
5. Queensland Research Centre for Peripheral Vascular Disease, James Cook University and the Townsville Hospital

Author Contributions

Student contribution to work: Involved in the conception of the study, collected all data, analysed and interpreted data, was responsible for writing all drafts of the manuscript including preparation of all figures and tables and modified drafts following co-author recommendations.

Conceived and designed the experiment: MP, MW, TB, CA
Performed the experiment: MP, MW, TB
Analysed the data: MP, TB, CA
Wrote/reviewed the paper: MP, TB, MW, KG, JO, KS, AL, JG, CA
5.1 Introduction
Abdominal aortic aneurysm (AAA) occurs in up to 10% of males aged over 65 (Sakalihasan, Limet et al. 2005) and is responsible for ~2% of all deaths worldwide (Nordon, Hinchliffe et al. 2011). For patients with a small AAA (aortic diameter 30-55 mm), there is often no survival benefit of surgery (Lederle, Wilson et al. 2002), with patients placed under clinical surveillance with no treatment options. Increased aortic stiffness has been shown to contribute to the development and progression of AAA in animal-models (Raaz, Zollner et al. 2015) and in humans (Hoegh and Lindholt 2009). Carotid-femoral pulse wave velocity (PWV), which is the gold-standard non-invasive measure of aortic stiffness (Van Bortel, Laurent et al. 2012), is elevated in adults with AAA (Durmus, Kazaz et al. 2014) and is associated with both AAA growth and rupture (Kadoglou, Papadakis et al. 2012, Raaz, Zollner et al. 2015). Wave reflection characteristics such as the augmentation index (AIx) and reflection magnitude (RM) are used represent systemic arterial stiffness (Nichols and Edwards 2001). Collectively, these measures of aortic and systemic arterial stiffness are strongly associated with the risk of cardiovascular events and mortality (Mitchell, Hwang et al. 2010, Chirinos, Kips et al. 2012) and likely contribute to the elevated cardiovascular risk of patients with AAA (Bath, Saratzis et al. 2017). Therefore, treatments that reduce arterial stiffness may help mitigate AAA growth and cardiovascular risk in patients with AAA.

Exercise training has been proposed as a potential adjunct treatment option for patients with AAA (Kothmann, Batterham et al. 2009, Tew, Moss et al. 2012), with evidence to date reporting a low risk of adverse events in response to maximal and submaximal exercise (Myers, Powell et al. 2011, Tew, Batterham et al. 2017). Many of the cardiovascular benefits associated with regular exercise are attributed to the frequent, repetitive haemodynamic perturbations associated with each bout of exercise (Green, Hopman et al. 2017). A single bout of exercise transiently lowers arterial stiffness in healthy younger (Kingwell, Berry et al. 1997, Mutter, Cooke et al. 2017) and older adults (Chapter 4), and is commonly accompanied by short-term improvements in arterial function and central blood pressure (Millen, Woodiwiss et al. 2016). This reduction in arterial stiffness with submaximal exercise may also be more pronounced and beneficial in individuals who exhibit higher levels of resting arterial stiffness (Ashor, Lara et al. 2014, Millen, Woodiwiss et al. 2016), such as patients with AAA.
Higher-intensity exercise is suggested to induce greater acute reductions in PWV and AIx compared with moderate intensity exercise in healthy young (Tordi, Mourot et al. 2010, Hanssen, Nussbaumer et al. 2015), which was also observed in older healthy adults in Chapter 4. Conversely, in adults with established cardiovascular risk factors including untreated hypertension (Gkaliagkousi, Gavriilaki et al. 2014) and obesity (Shim, Yang et al. 2011, Bunsawat, Ranadive et al. 2017), arterial stiffness is reported to be elevated during recovery following a bout of high-intensity exercise. An adverse increase in arterial stiffness during the recovery from exercise may exacerbate cardiovascular risk and related events (Schultz, La Gerche et al. 2017), and would be a concern in high-risk patients with AAA. Interval-based exercise consisting of intermittent bouts of high-intensity exercise interspersed with periods of low-intensity recovery has recently been proposed as a feasible exercise mode for patients with large AAA awaiting surgery (Tew, Batterham et al. 2017), but the effect of this type of exercise on post-exercise arterial stiffness is unknown.

Determining the post-exercise arterial stiffness response will add to our understanding of the short-term risks and benefits of exercise in patients with AAA, and will provide an insight into the potential efficacy of interval exercise training in reducing cardiovascular risk in this patient group. Therefore, this study aimed to compare the effect of a single bout of moderate-intensity continuous and higher-intensity interval exercise on the post-exercise arterial stiffness response between patients with AAA and healthy older adults.

5.2 Methods
5.2.1 Participant recruitment
Twenty-two male participants with small AAA (30-45 mm diameter) and 22 healthy males (control) were included. Patients with AAA were recruited through local public and private vascular clinics and had a confirmed diagnosis via duplex ultrasound within the 6 months prior to study entry. Healthy participants were recruited through local advertisement and a university alumni group. Participants were included if they were 60-86 years of age and were able to exercise; and were excluded if they had uncontrolled hypertension (systolic pressure; >140mmHg, diastolic pressure>90 mmHg) or were deemed unsuitable for exercise by a cardiologist because of reversible ischemia during exercise or uncontrolled cardiac arrhythmia with recurrent episodes or symptoms on exertion. Exclusion criteria also included heart failure, critical aortic stenosis, ankylosing spondylitis, chronic obstructive pulmonary disease, peripheral neuropathy, limiting venous insufficiency, or any other diagnosed vascular disease (e.g. Raynaud’s or vasculitis). Healthy control participants were excluded if they had a
family history of AAA or aneurysmal disease. All participants provided written informed consent to participate in the study, which conformed to the Declaration of Helsinki. The study was approved by the human research ethics committees of the University of the Sunshine Coast and the Prince Charles Hospital, Brisbane.

5.2.2 Maximal incremental cycling test
The maximal cardiorespiratory cycling test protocol is described in the Methods section of Chapter 3.2.3 of this thesis. The peak power output achieved during this test was then used to establish the exercise workload in the subsequent experimental visits.

5.2.3 Experimental exercise and control protocols
Following pre-test measurements of arterial stiffness and blood pressure, participants performed a 3-min warm up at 0 watts followed by continuous (24 min moderate-intensity at 40% PPO) or high-intensity interval (12 x 60 s bouts at 70% PPO, separated by 12 x 60 s bouts at 10% PPO) cycling exercise. This design ensured the continuous and interval cycling exercise protocols were duration- and work-matched. The control protocol consisted of duration-matched period of seated rest. Heart rate and RPE were recorded every 2 min during each protocol. Brachial blood pressure was measured using a manual sphygmomanometer every 6 min. Immediately following each protocol, participants were moved back to the supine position for post-protocol measurements of arterial stiffness.

5.2.4 Arterial stiffness measurements
Indices of arterial stiffness (PWV, AIx and RM) were measured in the supine position using the SphygmoCor XCEL device (AtCor Medical, West Ryde, NSW, Australia), at baseline (after 15 min of quiet rest), and for 0-90 min post-exercise/control. Participants were asked to remain quiet and still, before and during each measurement. Wave reflection characteristics preceded PWV measurements, and these procedures are described in detail in the Methods section of Chapter 3.2.5 of this thesis.

5.2.5 Statistical analysis
Based on previous literature (Gkaliagkousi, Gavriilaki et al. 2014) who reported a post-exercise difference of the change in PWV of 1.2 ± 0.9 m s⁻¹ between healthy and hypertensive individuals, our power calculation revealed that a cohort of 18 participants per group (assuming a between group post-exercise difference in PWV of 1.2 m s⁻¹ with a SD of 2 m s⁻¹ and > 80% power) would be required to reveal significant difference in the post-exercise PWV response between a healthy and a clinical group. A one-way linear mixed model (LMM) was used to compare anthropometric characteristics and cardiorespiratory fitness between patients with
AAA and healthy adults. Pearson’s chi squared test was used to compare categorical data between patients with AAA and healthy adults. A two-way (group*protocol) LMM was used to compare baseline arterial stiffness indices [PWV, AIx75 and RM] between patients with AAA and healthy adults across the study visits. A three-way (group*protocol*time) LMM was used to compare measurements of arterial stiffness indices (PWV, AIx75 and RM) and central blood pressure between the two groups (AAA and healthy), across “time” (baseline, 0-, 20-, 40-, 60-, and 90-min post) and between each protocol (control, moderate- and higher-intensity exercise). Data were also analysed as changes from baseline (delta) in order to account for individual day to day baseline variance and between-group variance. Based on their effect on the arterial stiffness response to exercise cardiorespiratory fitness and %body fat were used as co-variates in this analyses. (Moore, Berrones et al. 2016, Perissiou, Bailey et al. 2018).

Three-way LMM analysis was also used to detect any differences in heart rate, blood pressure and perceived exertion in response to the acute protocols between the two groups (AAA and healthy adults), across time (at 2 and 6 min intervals for HR/RPE and BP, respectively) during each protocol (control, moderate- and higher-intensity exercise). Statistically significant interactions were further investigated with multiple comparisons using Fisher’s least significant difference approach (Rothman 1990). Analyses were conducted using the Statistical Package for Social Sciences (Version 22; IBM SPSS Inc., Chicago, IL). Statistical significance was set at P≤0.05 and exact P values are cited (P values of 0.000 are reported as <0.001). Data are presented in the text as mean and 95% confidence interval (95%CI) unless otherwise stated.

5.3. Results

5.3.1. Participant characteristics
Participant characteristics and comparisons between patients with AAA and healthy adults are shown in Table 7. Cardiorespiratory fitness, measured as \( \dot{V}O_2\)peak, and heart rate at peak exercise during the maximal incremental cycling test were lower in patients with AAA compared with healthy adults (Table 7).
Table 7. Characteristics of AAA patients and healthy adults.

<table>
<thead>
<tr>
<th></th>
<th>AAA (n=22)</th>
<th>Healthy (n=22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>74 ± 6</td>
<td>72 ± 5</td>
<td>0.202</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.1</td>
<td>1.75 ± 6.6</td>
<td>0.161</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.1 ± 16</td>
<td>80 ± 12</td>
<td>0.283</td>
</tr>
<tr>
<td>BMI (kg.m(^{-2}))</td>
<td>28 ± 9.1</td>
<td>26 ± 3.6</td>
<td>0.072</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>28.6 ± 5.7</td>
<td>25 ± 5.2</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>Clinical information</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum AAA diameter (mm)</td>
<td>36 ± 0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hypertension, N (%)</td>
<td>15 (68)</td>
<td>5 (22)</td>
<td>0.006</td>
</tr>
<tr>
<td>Dyslipidaemia, N (%)</td>
<td>18 (82)</td>
<td>8 (36)</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes, N (%)</td>
<td>2 (9)</td>
<td>0 (0)</td>
<td>0.478</td>
</tr>
<tr>
<td>Smoking - current, N (%)</td>
<td>2 (9)</td>
<td>1 (5)</td>
<td>0.697</td>
</tr>
<tr>
<td>Smoking - previous, N (%)</td>
<td>12 (55)</td>
<td>11 (50)</td>
<td>0.701</td>
</tr>
<tr>
<td>Previous stroke, N (%)</td>
<td>2 (9)</td>
<td>0 (0)</td>
<td>0.488</td>
</tr>
<tr>
<td>Previous MI, N (%)</td>
<td>6 (27)</td>
<td>1 (5)</td>
<td>0.021</td>
</tr>
<tr>
<td>Previous CABG, N (%)</td>
<td>11 (50)</td>
<td>1 (5)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Medication use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARB/ACE inhibitors, N (%)</td>
<td>9 (40)</td>
<td>4 (18)</td>
<td>0.140</td>
</tr>
<tr>
<td>Anti-platelet, N (%)</td>
<td>13 (60)</td>
<td>2 (9)</td>
<td>0.003</td>
</tr>
<tr>
<td>Beta-blockers, N (%)</td>
<td>9 (40)</td>
<td>2 (9)</td>
<td>0.034</td>
</tr>
<tr>
<td>Calcium channel blockers, N (%)</td>
<td>4 (18)</td>
<td>1 (5)</td>
<td>0.345</td>
</tr>
<tr>
<td>Statins, N (%)</td>
<td>20 (90)</td>
<td>9 (40)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Hemodynamic variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting heart rate (bpm)</td>
<td>59 ± 9</td>
<td>59 ± 7</td>
<td>0.824</td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>129 ± 13</td>
<td>124 ± 13</td>
<td>0.258</td>
</tr>
<tr>
<td>Brachial DBP (mmHg)</td>
<td>73 ± 7</td>
<td>74 ± 9</td>
<td>0.785</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>118 ± 12</td>
<td>115 ± 12</td>
<td>0.469</td>
</tr>
<tr>
<td>Central DBP (mmHg)</td>
<td>74 ± 6</td>
<td>74 ± 9</td>
<td>0.775</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>44 ± 7</td>
<td>40 ± 7</td>
<td>0.137</td>
</tr>
<tr>
<td><strong>Maximal incremental cycling test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute $\dot{V}O_2$ peak, L.min(^{-1})</td>
<td>1.58 ± 0.36</td>
<td>1.94 ± 0.35</td>
<td>0.002</td>
</tr>
<tr>
<td>Relative $\dot{V}O_2$ peak, mL.kg(^{-1}.min(^{-1})</td>
<td>19.03 ± 3.54</td>
<td>24.47 ± 2.78</td>
<td>0.001</td>
</tr>
<tr>
<td>Peak heart rate, bpm</td>
<td>126 ± 15</td>
<td>148 ± 16</td>
<td>0.001</td>
</tr>
<tr>
<td>Peak heart rate, age-predicted %</td>
<td>86 ± 10</td>
<td>97 ± 11</td>
<td>0.001</td>
</tr>
<tr>
<td>Peak RER, AU</td>
<td>1.17 ± 0.10</td>
<td>1.19 ± 0.11</td>
<td>0.575</td>
</tr>
<tr>
<td>Peak power output, W</td>
<td>120 ± 20</td>
<td>150 ± 30</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ±SD or percentage (%). BMI, body mass index; AAA, abdominal aortic aneurysm; MI, myocardial infarction; CABG, coronary artery bypass graft; ARB, Angiotensin II receptor blockers; ACE, angiotensin converting enzyme; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; $\dot{V}O_2$ peak, peak oxygen uptake; RER, respiratory exchange ratio.
5.3.2. Heart rate, mean arterial pressure and perceived exertion during the exercise protocols
Mean power output (W) during exercise was greater in healthy adults [moderate-intensity continuous: mean = 58W, (95% CI, 53 to 61); higher-intensity intervals: mean = 100W (95% CI, 93 to 107)] compared to patients with AAA [moderate-intensity continuous: mean = 48W, (95% CI, 43 to 51); higher-intensity intervals: mean = 83W (95% CI, 76 to 90), P<0.001]. No significant differences were observed between patients with AAA and healthy adults in heart rate, blood pressure and RPE throughout each protocol (P>0.05). Mean heart rate was higher during both exercise protocols compared to during control [control mean heart rate 62 b.min\(^{-1}\) (95% CI, 58 to 67, P<0.001)], and was greater during higher-intensity interval exercise [mean heart rate 100 b·min\(^{-1}\) (95% CI, 87 to 96)] compared to moderate-intensity continuous exercise [mean heart rate 92 b·min\(^{-1}\) (95% CI, 95 to 103, P<0.001)]. Overall, MAP was higher during both exercise protocols compared with during control (P<0.05), whilst there was no difference in MAP during moderate-and higher-intensity exercise (P=0.324). Mean RPE was 5 (95% CI, 4 to 6) during higher-intensity interval exercise compared to 3 (95% CI, 2 to 3, P<0.001) during moderate-intensity continuous exercise.

5.3.3. Central blood pressure and arterial stiffness indices at baseline and in response to exercise
Mean central blood pressure and arterial stiffness indices at baseline and during recovery (0 to 90 min post) after exercise/control protocols for AAA and healthy groups are shown in Tables 8 and 9. The relative change (delta) from baseline in PWV, AIx75 and RM between patients with AAA and healthy adults for each protocol are shown in Figures 15-17. Findings are summarised below.

5.3.4. Baseline central blood pressure and arterial stiffness indices
Arterial stiffness indices measured at baseline were similar across the three separate testing days (pre-exercise/control) (P>0.05). PWV measured at baseline was 2.2 m.s\(^{-1}\) (95% CI 0.9 to 3.5, P<0.001) higher in AAA compared with the healthy group (Table 8). There were no differences in AIx75, RM and central blood pressure indices measured at baseline between groups.

5.3.5. Post-exercise central blood pressure and arterial stiffness indices
There was no significant three-way (protocol*group*time) interaction for any of the arterial stiffness and central blood pressure indices (Tables 8 and 9). After control, PWV increased from baseline at all time-points, whereas after the moderate-intensity continuous exercise,
PWV was only elevated from baseline at 90 min. Conversely, PWV immediately decreased compared with baseline after higher-intensity interval exercise (at 0-min post), before increasing above baseline at 90 min (Table 8). After both exercise protocols, delta PWV was lower at all time-points compared to control (Fig 15). In addition, delta PWV was lower after higher-intensity interval exercise at 0 and 40 min post compared with after moderate-intensity exercise (Fig 15B/C).

AIX75 decreased and remained below baseline for 90 min after all three protocols, however, there was no protocol*time interaction (Table 8, Fig 16). After control RM remained unchanged, whereas RM decreased after both exercise protocols compared with baseline (Table 8). After both exercise protocols delta RM was lower at all time-points compared with control, however there was no difference between exercise protocols (Fig 17).

In both groups, heart rate decreased below baseline for 90 min after control and was increased above baseline for 20 min after moderate-intensity continuous exercise, and for 40 min after higher-intensity interval exercise (Table 9). Central blood pressure indices (cSBP, cDBP cPP) increased above baseline for 90 min after control (Table 9). After moderate-intensity exercise cSBP and cPP were lower compared with baseline at 20 min post, whilst after higher-intensity interval exercise cSBP and cPP remained lower compared with baseline from 20 to 60 min post. In addition, cSBP and cPP were lower after higher-intensity interval exercise from 40 to 60 min post compared with after moderate-intensity exercise. No post-exercise changes were observed for cDBP (Table 9).
Table 8. Arterial stiffness at baseline and after no-exercise control, moderate-intensity continuous and higher-intensity interval exercise in patients with AAA and healthy older adults.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Protocol</th>
<th>Baseline</th>
<th>0 post</th>
<th>20 post</th>
<th>40 post</th>
<th>60 post</th>
<th>90 post</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Time point (min)</td>
<td>P-Values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18.3 ± 12*</td>
<td>18.8 ± 12*</td>
<td>23.3 ± 12*</td>
<td>22.2 ± 12*</td>
<td>21.7 ± 12*</td>
</tr>
<tr>
<td>AAA</td>
<td>CON</td>
<td>14.0 ± 2</td>
<td>14.9 ± 2*</td>
<td>14.9 ± 2*</td>
<td>15.1 ± 2*</td>
<td>15.3 ± 2*</td>
<td>15.3 ± 2*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>14.2 ± 2</td>
<td>14.0 ± 2</td>
<td>13.8 ± 2</td>
<td>14.3 ± 2</td>
<td>14.5 ± 2</td>
<td>15.0 ± 2*</td>
<td>0.001 0.001 0.001</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>14.3 ± 2</td>
<td>13.2 ± 2*</td>
<td>13.8 ± 2</td>
<td>13.9 ± 2</td>
<td>14.3 ± 2</td>
<td>15.3 ± 2*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>11.8 ± 2</td>
<td>12.5 ± 2*</td>
<td>12.9 ± 3*</td>
<td>12.6 ± 2*</td>
<td>12.9 ± 2*</td>
<td>13.1 ± 2*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>12.5 ± 2</td>
<td>12.4 ± 2</td>
<td>12.4 ± 2</td>
<td>12.4 ± 2</td>
<td>12.4 ± 2</td>
<td>13.1 ± 2*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>12.5 ± 2</td>
<td>12.4 ± 2*</td>
<td>12.2 ± 2</td>
<td>12.7 ± 2</td>
<td>12.7 ± 2</td>
<td>13.3 ± 2*</td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>CON</td>
<td>20.1 ± 10</td>
<td>17.2 ± 10</td>
<td>18.2 ± 11</td>
<td>15.8 ± 9</td>
<td>16.3 ± 12</td>
<td>15.9 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>22.0 ± 10</td>
<td>20.8 ± 12</td>
<td>20.7 ± 10</td>
<td>19.8 ± 9</td>
<td>18.5 ± 9</td>
<td>19.3 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>22.0 ± 10</td>
<td>21.5 ± 11</td>
<td>22.7 ± 9</td>
<td>18.4 ± 11</td>
<td>18.4 ± 10</td>
<td>20.0 ± 9</td>
<td>0.001 0.001 0.346</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>24.5 ± 10</td>
<td>20.3 ± 8</td>
<td>21.5 ± 8</td>
<td>18.2 ± 9</td>
<td>19.0 ± 11</td>
<td>22.0 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>24.9 ± 10</td>
<td>22.4 ± 11</td>
<td>26.7 ± 11</td>
<td>21.0 ± 10</td>
<td>21.5 ± 11</td>
<td>23.2 ± 12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>24.5 ± 10</td>
<td>22.4 ± 9</td>
<td>25.1 ± 10</td>
<td>21.9 ± 11</td>
<td>20.5 ± 12</td>
<td>20.3 ± 10</td>
<td></td>
</tr>
<tr>
<td>AAA</td>
<td>CON</td>
<td>72 ± 11</td>
<td>74 ± 14</td>
<td>73 ± 10</td>
<td>72 ± 13</td>
<td>69 ± 8</td>
<td>73 ± 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>74 ± 12</td>
<td>63 ± 11*</td>
<td>71 ± 11*</td>
<td>70 ± 9*</td>
<td>70 ± 12*</td>
<td>72 ± 8*</td>
<td>Protocol*Time: 0.001</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>74 ± 11</td>
<td>60 ± 11*</td>
<td>67 ± 14*</td>
<td>68 ± 12*</td>
<td>72 ± 10*</td>
<td>72 ± 12*</td>
<td>0.001 0.001 0.190</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>73 ± 12</td>
<td>75 ± 13</td>
<td>78 ± 14</td>
<td>77 ± 13</td>
<td>79 ± 14</td>
<td>78 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>80 ± 11</td>
<td>63 ± 11*</td>
<td>72 ± 8*</td>
<td>75 ± 12*</td>
<td>76 ± 13*</td>
<td>76 ± 14*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>81 ± 12</td>
<td>58 ± 13*</td>
<td>69 ± 14*</td>
<td>71 ± 10*</td>
<td>72 ± 13*</td>
<td>75 ± 12*</td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>CON</td>
<td>26.5 ± 5</td>
<td>27.6 ± 5</td>
<td>26.9 ± 5</td>
<td>26.8 ± 5</td>
<td>27.5 ± 5</td>
<td>29.1 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>27.1 ± 4</td>
<td>30.1 ± 5*</td>
<td>25.4 ± 4</td>
<td>27.2 ± 4</td>
<td>27.1 ± 5</td>
<td>28.0 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>26.4 ± 5</td>
<td>32.2 ± 6*</td>
<td>26.9 ± 6</td>
<td>26.3 ± 7</td>
<td>25.1 ± 5</td>
<td>28.4 ± 6*</td>
<td>0.001 0.241 0.090</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>24.8 ± 5</td>
<td>25.8 ± 6</td>
<td>24.1 ± 5</td>
<td>23.0 ± 6</td>
<td>24.2 ± 5</td>
<td>25.1 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>23.8 ± 4</td>
<td>30.5 ± 7*</td>
<td>25.1 ± 6</td>
<td>24.0 ± 5</td>
<td>24.8 ± 6</td>
<td>25.4 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>23.3 ± 5</td>
<td>30.0 ± 6*</td>
<td>24.9 ± 5</td>
<td>23.7 ± 5</td>
<td>24.3 ± 5</td>
<td>24.6 ± 5*</td>
<td></td>
</tr>
<tr>
<td>AAA</td>
<td>CON</td>
<td>18.8 ± 3</td>
<td>20.3 ± 4</td>
<td>19.7 ± 4</td>
<td>19.2 ± 4</td>
<td>19.5 ± 5</td>
<td>21.2 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>19.9 ± 3</td>
<td>19.0 ± 4</td>
<td>17.8 ± 2*</td>
<td>19.2 ± 4*</td>
<td>18.7 ± 3</td>
<td>20.1 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>19.5 ± 4</td>
<td>19.1 ± 4*</td>
<td>17.6 ± 3*</td>
<td>17.6 ± 4*</td>
<td>18.0 ± 3*</td>
<td>20.0 ± 3</td>
<td>0.001 0.001 0.266</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>17.4 ± 4</td>
<td>18.9 ± 4</td>
<td>18.4 ± 4</td>
<td>17.7 ± 3</td>
<td>18.8 ± 4</td>
<td>19.2 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>18.8 ± 4</td>
<td>18.6 ± 3</td>
<td>17.9 ± 3*</td>
<td>17.6 ± 3*</td>
<td>18.5 ± 4</td>
<td>18.7 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>18.3 ± 3</td>
<td>16.9 ± 3*</td>
<td>16.7 ± 3*</td>
<td>16.7 ± 3*</td>
<td>17.0 ± 3*</td>
<td>17.9 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ±SD; CON, no-exercise control; MOD, moderate intensity continuous exercise; HIGH, higher- intensity interval exercise; AIx75, augmentation index normalised to a heart rate of 75 bpm; RM, reflection magnitude; PWV, pulse wave velocity. *Significantly different to baseline based on protocol*time post-hoc comparisons.
Table 9. Heart rate and blood pressure indices at baseline and after no-exercise control, moderate-intensity continuous and higher-intensity interval exercise in patients with AAA and healthy older adults.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Protocol</th>
<th>Time point (min)</th>
<th>P-Values</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0 post</td>
<td>20 post</td>
</tr>
<tr>
<td>AAA</td>
<td>CON</td>
<td>59 ± 9</td>
<td>56 ± 8*</td>
<td>56 ± 8*</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>60 ± 9</td>
<td>68 ± 12*</td>
<td>62 ± 9*</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>59 ± 9</td>
<td>69 ± 11*</td>
<td>65 ± 8*</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>60 ± 10</td>
<td>55 ± 9*</td>
<td>54 ± 7*</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>58 ± 7</td>
<td>68 ± 9*</td>
<td>62 ± 8*</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>58 ± 7</td>
<td>71 ± 14*</td>
<td>63 ± 11*</td>
</tr>
<tr>
<td>Healthy</td>
<td>MOD</td>
<td>117 ± 14</td>
<td>120 ± 12</td>
<td>114 ± 11*</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>115 ± 11</td>
<td>117 ± 12</td>
<td>111 ± 12*</td>
</tr>
<tr>
<td>AAA</td>
<td>CON</td>
<td>73 ± 6</td>
<td>77 ± 7*</td>
<td>77 ± 8*</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>74 ± 8</td>
<td>75 ± 7</td>
<td>73 ± 7</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>73 ± 7</td>
<td>74 ± 7</td>
<td>73 ± 7</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>74 ± 9</td>
<td>76 ± 9*</td>
<td>75 ± 10*</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>75 ± 10</td>
<td>77 ± 9</td>
<td>74 ± 10</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>74 ± 10</td>
<td>77 ± 10</td>
<td>74 ± 10</td>
</tr>
<tr>
<td>Healthy</td>
<td>MOD</td>
<td>43 ± 7</td>
<td>47 ± 8*</td>
<td>46 ± 9*</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>45 ± 8</td>
<td>44 ± 9</td>
<td>40 ± 6*</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>41 ± 9</td>
<td>45 ± 8</td>
<td>41 ± 6*</td>
</tr>
<tr>
<td></td>
<td>MOD</td>
<td>42 ± 8</td>
<td>42 ± 7</td>
<td>40 ± 7</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
<td>41 ± 7</td>
<td>40 ± 8</td>
<td>37 ± 6*</td>
</tr>
</tbody>
</table>

Data are presented as mean ±SD; CON, no-exercise control; MOD, moderate intensity continuous exercise; HIGH, higher-intensity interval exercise; AIx75, augmentation index normalised to a heart rate of 75 bpm; RM, reflection magnitude; PWV, pulse wave velocity. *Significantly different to baseline based on protocol*time post-hoc comparisons.
Figure 15. Delta PWV response of AAA (white triangles) and healthy (black circles) groups to no-exercise control (A), moderate-intensity continuous (B) and higher-intensity interval (C) exercise. Data are mean and SEM; PWV, pulse wave velocity. *Significantly different to control; and α significantly different to moderate-intensity based on protocol*time post-hoc comparisons.
Figure 16. Delta AIx75 response of AAA (white triangles) and healthy (black circles) groups to no-exercise control (A), moderate-intensity continuous (B) and high-intensity interval (C) exercise. Data are mean and SEM; AIx75, augmentation index normalised to a heart rate of 75 bpm. *Significantly different to control; and † significantly different to moderate-intensity based on protocol*time post-hoc comparisons.
Figure 16. Delta RM response of AAA (white triangles) and healthy (black circles) groups to no-exercise control (A), moderate-intensity continuous (B) and high-intensity interval (C) exercise. Data are mean and SEM; RM, reflection magnitude. *Significantly different to control; and # significantly different to moderate-intensity based on protocol*time post-hoc comparisons.
5.4. Discussion
This study investigated the effect of a single bout of exercise on post-exercise measures of aortic (PWV) and systemic artery stiffness (RM, AIx) in patients with AAA compared with healthy older adults. The post-exercise PWV and RM responses were attenuated following exercise compared with control. With higher-intensity interval exercise, post-exercise PWV was lower compared with moderate-intensity continuous exercise in patients with AAA and healthy older adults. While PWV was higher in the AAA patients at rest, there was no difference in the post-exercise aortic and systemic arterial stiffness response compared with healthy older adults.

5.4.1. Arterial stiffness at rest
Resting PWV was higher in patients with AAA compared with healthy older adults, which is consistent with most (Kadoglou, Moulakakis et al. 2012, Durmus, Kazaz et al. 2014, Abbas, Cecelja et al. 2015) but not all previous reports (Lee, Sung et al. 2013, Bailey, Davies et al. 2014). Conversely, for resting wave reflection characteristics no differences were observed between groups. Carotid-to-femoral PWV represents a measure of aortic stiffness (Van Bortel, Laurent et al. 2012) and in vivo studies have reported significantly higher aortic stiffness in aneurysmal compared with healthy aortas (Xiong, Wang et al. 2008), attributed to chronic structural changes in the aortic media (Pearce and Shively 2006, Azevedo, Prado et al. 2014). Wave reflection characteristics represent the net reflected wave that travels from the peripheral vascular tree back to the proximal aorta and are largely determined by the vascular properties of the peripheral arterial system (Nichols and Edwards 2001, Nichols 2005), which may not be directly affected by the presence of an aortic aneurysm. Indeed, a previous study reported similar resting AIx75, Pb and brachialANKLE PWV (representing PWV in the periphery) values between patients with AAA and healthy control participants (Lee, Sung et al. 2013). These findings suggest that the presence of AAA is associated with local stiffening of the aorta, but not peripheral artery stiffness relative to healthy older adults. While this may be a manifestation of the localised aneurysm, the effect of other comorbidities cannot be excluded. Hypertension, dyslipidaemia, and adiposity were more prevalent in the AAA patients in the present study, all of which have been shown to influence resting PWV independent of peripheral wave reflection characteristics (Mitchell, Hwang et al. 2010, Mitchell 2014, Moore, Berrones et al. 2016, Ramos, Ramos et al. 2016).
5.4.2. Post-exercise arterial stiffness

Compared to baseline levels, arterial stiffness was previously shown to be decreased (Millen, Woodiwiss et al. 2016) or unaltered (Akazawa, Ra et al. 2015) after a bout of aerobic exercise in middle-aged and older healthy adults. In the current cohort of patients with AAA and healthy older adults, there were transient significant decreases from baseline in RM after both exercise protocols, and in PWV only after higher-intensity interval exercise. Following the no-exercise control period, we observed a progressive rise in PWV, but no change in RM. Compared to the resting control protocol, exercise significantly lowered (attenuated) PWV and RM in both patients with AAA and healthy older adults. This finding is in line with previous studies in younger (Wang, Zhang et al. 2014) and older adults across a broad range of cardiorespiratory fitness levels (as presented in Chapter 4).

The transient attenuation in arterial stiffness and central blood pressure after a single bout of submaximal exercise suggests there is short term cardiovascular benefit and risk reduction for patients with AAA, similar to that for healthy older adults, (Schultz, La Gerche et al. 2017). For patients with AAA, the observed attenuation in aortic stiffness and wave reflection magnitude may contribute to a reduced central blood pressure and alleviate stress on the AAA wall, perhaps limiting growth and rupture risk (Ruegg, Mason et al. 2010, Raaz, Zollner et al. 2015). Recent physical activity guidelines in clinical populations, including patients with AAA, recommended that reducing sitting time is associated with improved functional capacity and reduced postoperative mortality and morbidity (Tew, Ayyash et al. 2018). This Chapter demonstrates that interrupting sitting time with exercise attenuates the increases in aortic stiffness observed with prolonged supine rest. The findings suggest intermittent periods of exercise should be encouraged in patients with small AAAs.

Despite a higher resting PWV in patients with AAA, the changes in PWV during the recovery from exercise were similar compared with healthy older adults. As discussed above, structural changes to the aortic wall are primarily responsible for the higher resting arterial stiffness in patients with AAA compared with healthy adults (Azevedo, Prado et al. 2014). Short-term lowering of arterial stiffness during the recovery from exercise are unlikely attributed to changes in aortic structure (Whyte and Laughlin 2010), but to improvements in cardiovascular function including changes in the arterial tone (Fok, Jiang et al. 2012), reductions in sympathetic nervous activity (Halliwill, Buck et al. 2013) and anti-inflammatory cytokines (Jae, Yoon et al. 2013), and increases in endothelial function (Munir, Jiang et al. 2008). Post-exercise reductions in blood pressure were associated with decreases in arterial stiffness in one (Lim,
Pearman et al. 2016) but not all previous studies (Heffernan, Collier et al. 2007, Tordi, Mourot et al. 2010). Hence, the short-term reductions in central blood pressure following exercise in this study may be partly attributed to the lower PWV and RM after exercise compared with control. Whether the changes in arterial stiffness are attributable to similar exercise-induced mechanisms in patients with AAA compared with older adults remains to be investigated.

5.4.2.1. Clinical implications
Interval exercise is increasingly being tested and recommended in adults with cardiovascular disease as it allows for an overall higher exercise intensity stimulus and has potential for additional cardiovascular benefit compared with moderate-intensity continuous exercise (Weston, Wisløff et al. 2014, Ramos, Dalleck et al. 2017). In this study, there was a tendency for higher-intensity interval exercise to induce a lower post-exercise PWV compared with moderate-intensity continuous exercise in both patients with AAA and healthy older adults. This is consistent with reports in younger (Tordi, Mourot et al. 2010) and older adults (as presented in Chapter 4), and may infer greater post-exercise benefits for cardiovascular function, including intensity-dependent reductions in blood pressure (Quinn 2000) and increases in shear-mediated vasodilation (Green, Maiorana et al. 2004, Santana, Moreira et al. 2013). The majority of exercise training studies in patients with AAA have adopted a conservative prescription of low or moderate-intensity continuous exercise (Kothmann, Batterham et al. 2009, Tew, Moss et al. 2012) due to concerns of an increased risk of cardiovascular events, including aneurysms rupture, with higher-intensity exercise (Bean, Vora et al. 2004). While there are some reports that a single bout of high-intensity continuous exercise may exacerbate arterial stiffness in those with cardiovascular disease (Gkaliagkousi, Gaviilik et al. 2014, Bunsawat, Ranadive et al. 2017), no adverse arterial stiffness increases were observed in the current study in patients with AAA. The findings of this study, and a recent feasibility study reporting no adverse events even in patients with large AAA (Tew, Batterham et al. 2017), support the potential use of interval-based high-intensity exercise as part of the exercise plan for patients with AAA.

5.4.2.2. Limitations
Prescription medications in the current study were more prevalent in patients with AAA than in the healthy group. These include beta blockers, which have previously been reported to improve the PWV response to exercise in hypertensive adults (Gkaliagkousi, Gaviilik et al. 2014); however this study found no differences in arterial stiffness responses between those who were using prescribed medications and those who were not. Furthermore, only patients
with small (<45 mm), asymptomatic AAA who had been cleared to exercise by a cardiologist were recruited and thus our findings may not relate to AAA patients who are deemed high-risk or unable to exercise. In addition, the cohort of this study consisted of only male participants. It has been shown that the post-exercise arterial stiffness response is enhanced in young healthy females compared with males (Doonan, Mutter et al. 2013), and thus findings of this study cannot be extrapolated to older females with and without AAA.

5.4.2. Conclusion
In conclusion, an acute bout of submaximal exercise lowered PWV and RM during the recovery from exercise compared with seated-control, in patients with AAA and healthy older adults. This effect is most marked following higher-intensity interval exercise. The findings of this study suggest that a single bout of exercise provides short-term cardiovascular benefits in patients with AAA and healthy older adults. Whether repeated bouts of activity and exercise training lead to a chronic lowering of arterial stiffness and a reduction in cardiovascular risk in this population warrants future investigation.
6. General discussion

The main aim of this thesis was to assess the acute response of arterial stiffness to exercise in patients with abdominal aortic aneurysm (AAA). This thesis had three primary aims:

1. To establish the reliability of the acute arterial stiffness response to exercise in healthy older adults (Chapter 3).

2. To determine the effect of exercise intensity and cardiorespiratory fitness on the acute arterial stiffness response to exercise in healthy older adults (Chapter 4).

3. To compare the acute arterial stiffness response to exercise in patients with small AAA and healthy adults of a similar age (Chapter 5).

6.1 Arterial stiffness measures are reliable at rest and in response to a bout of exercise in older adults

The test-retest reliability of indices of arterial stiffness at rest and after exercise in healthy older adults was investigated and is reported in Chapter 3. This study demonstrated that arterial stiffness indices can be measured reliably at rest, immediately after and during exercise recovery in healthy older adults, with pulse wave velocity (PWV) having the highest reliability at rest and after exercise. This study further observed that despite good reliability of its components (i.e. the forward (Pf) and backward pressure (Pb) waves), the reliability of reflection magnitude (RM) was poor, at rest and during exercise recovery and good immediately after exercise. Given that RM is calculated as the ratio of (Pb/Pf) that ranges from 0 to 1, this index is sensitive to small changes (Weir 2005), and may therefore be more vulnerable to signal noise (variance) than PWV and AIx75.

The repeatability of arterial stiffness indices at rest were also established in Chapters 4 and 5; and no significant differences were found in PWV, AIx75 and RM among the three visits. The findings in Chapter 5 are important for this thesis findings because the repeatability of PWV and AIx75 has been previously challenged in patients with AAA. Lee et al. (2013) demonstrated that, compared with preoperative values, PWV and AIx75 significantly increased after endovascular repair (EVAR). This finding suggests that the aneurysm itself was affecting the measurement and that EVAR possibly corrects the effect of AAA on PWV and AIx75 (Lee, Sung et al. 2013). However, studies using magnetic resonance angiography demonstrated
decreased aortic compliance at the level of the aneurysm sac after EVAR (van Herwaarden, Muhs et al. 2006, Long, Rouet et al. 2009). Further, a recent study demonstrated that, unlike after EVAR, PWV does not change after AAA open repair (Gray, Goodman et al. 2016). These findings suggest that the stiffness characteristics of the graft used in EVAR may affect PWV and AIx75, not the aneurysm per se. The findings reported in Chapter 5 are the first to demonstrate that measurements of PWV, AIx75 and RM at rest are repeatable in patients with small AAA.

Considering the important role of arterial stiffness as an independent predictor of cardiovascular mortality and morbidity (Mitchell, Hwang et al. 2010, Chirinos, Kips et al. 2012), Chapter 3 provides additional evidence to support the prognostic sensitivity of measures of arterial stiffness in older healthy adults. The excellent relative reliability of PWV at rest corresponded with an absolute reliability (RC: 1.9 m.s\(^{-1}\)) similar to that reported by Keith et al. (2013) (1.5 m.s\(^{-1}\)). While the clinical meaningfulness of this smallest detectable change remains to be fully established in older adults, it has previously been shown that a 1.0 m.s\(^{-1}\) (or 1SD) increase in PWV is associated with a 10% increase in cardiovascular risk (Vlachopoulos, Aznaouridis et al. 2010). However, it has been reported that the predictive value of increased arterial stiffness is greater in patients with cardiovascular disease (Vlachopoulos, Aznaouridis et al. 2010), hence the reported results require confirmation in clinical populations of various ages and health states.

Chapter 3 also provides the basis for further use and investigation of post-exercise measures of arterial stiffness in older adults. The absolute variance of the reported post-exercise change in PWV (1.9 m.s\(^{-1}\)) is similar to the increase in PWV (~2.0 m.s\(^{-1}\)) found after maximal exercise in young adults with untreated Grade I hypertension (Gkaliagkousi, Gavriilaki et al. 2014). Such increases are associated with a transient increase in the risk of adverse cardiovascular events after exercise (Goodman, Burr et al. 2016). An increase in PWV of 1.3 m.s\(^{-1}\) following exercise has been associated with abnormal kidney function in middle-aged adults (Keith, Rattigan et al. 2013), however our data suggests that this is below the smallest detectable change in older adults. For future studies exploring the utility of the immediate change in PWV following exercise, we estimate that a cohort of 8 (assuming a sample SD of 1 m.s-1) to 17 (SD of 2 m.s-1) participants per group (with >80% power) would be required to reveal significant differences that correspond with the smallest detectable change (1.9 m.s-1) in the PWV response.
Although post-exercise changes in arterial stiffness provide a potential basis for assessing exercise-induced cardiovascular risk (Gkaliagkousi, Gavriilaki et al. 2014, Bunsawat, Ranadive et al. 2017) and the future risk of end-organ damage (Keith, Rattigan et al. 2013) that are not apparent from resting measurements alone, these changes are yet to be translated into clinical relevance for healthy older adults. Thus, both the physiological determinants and the clinical implications of the post-exercise changes in arterial stiffness indices in older adults require further investigation. Overall, the findings reported in Chapter 3 provide essential knowledge about the precision of measurements of the short-term response of arterial stiffness following a single bout of moderate intensity exercise in healthy older adults. Further, they support the potential use of measures of post-exercise arterial stiffness for research and in clinical practice.

6.1 Acute response of arterial stiffness to exercise
This thesis identifies arterial stiffness as a potential treatment target due to its association with cardiovascular disease risk in healthy older adults and patients with small AAA (Mackenzie, Wilkinson et al. 2002, O'Rourke, Staessen et al. 2002). A transient decrease in arterial stiffness after exercise may represent a short-term reduction in cardiovascular risk (Millen, Woodiwiss et al. 2016) and a marker for future vascular adaptation with training (Romero, Minson et al. 2017). Studies in younger and middle-aged adults have found carotid-femoral PWV, AIx75, RM and Pb to be reduced below baseline for up to 60 min after exercise of moderate intensity or high intensity in healthy young and middle-aged adults (Kingwell, Berry et al. 1997, Heffernan, Collier et al. 2007, Munir, Jiang et al. 2008, Milatz, Ketelhut et al. 2015, Müller, Wilms et al. 2015, Millen, Woodiwiss et al. 2016). Chapters 4 and 5 add to the existing literature by reporting post-exercise reductions in PWV compared to no-exercise control in healthy older adults and patients with small AAA. Similar reductions in the same populations are reported in post-exercise RM from baseline levels and/or compared to no-exercise control.

The mechanisms responsible for the post-exercise reductions in arterial stiffness are currently not well understood. Short-term reductions in arterial stiffness during the recovery from exercise are unlikely attributed to changes in aortic structure (Whyte and Laughlin 2010), but to improvements in cardiovascular function (Green and Smith 2017). Changes in the arterial tone (Fok, Jiang et al. 2012), reductions in sympathetic nervous activity (Halliwill, Buck et al. 2013) and anti-inflammatory cytokines (Jae, Yoon et al. 2013) have been previously related to post-exercise decreases in arterial stiffness. Furthermore, post-exercise reductions in blood pressure were associated with decreases in arterial stiffness in one (Lim, Pearman et al. 2016)
but not all previous studies (Heffernan, Collier et al. 2007, Tordi, Mourot et al. 2010). Hence, the short-term reductions in central blood pressure following exercise observed in Chapters 4 and 5 may be partly attributed to the lower PWV and RM after exercise compared with control. In addition, shear-mediated increases in endothelial function (Munir, Jiang et al. 2008) and the activation of vasodilators e.g. nitric-oxide and prostaglandins (Poveda, Berrazueta et al. 1998), and inhibition of vasoconstrictors e.g. endothelin-1 expression (Di Francescomarino, Sciartilli et al. 2009) have all been associated with transient decreases in arterial stiffness after exercise. Recent studies from our lab may partly explain our findings as they demonstrated significant improvements in post-exercise endothelial function in healthy older adults (Bailey, Perissiou et al. 2017) and patients with AAA (Bailey, Perissiou et al. 2017). Although current evidence is elucidative, the exact physiological mechanisms of the arterial stiffness response to exercise require further clarification.

A lowering of arterial stiffness during the recovery from each discrete bout of exercise are believed to contribute to the long-term vascular adaptations seen after an exercise training intervention (Romero, Minson et al. 2017). Findings demonstrated a decrease in carotid-femoral PWV 24 h after a 30 min bout of moderate- to vigorous-intensity aerobic exercise in young (Perdomo, Moody et al. 2016) and older (Michaelides, Soulis et al. 2011) adults. Although these studies provide some insight regarding the prolonged effect of exercise on the vasculature, the direct relationship between acute and chronic changes in arterial stiffness has not been established yet. Studies have further investigated this relationship in major determinants of arterial stiffness such as blood pressure and endothelial function. Specifically, the magnitude of change in systolic blood pressure after acute exercise is strongly correlated with the change in resting systolic blood pressure after chronic training in pre-hypertensive individuals (Liu, Goodman et al. 2012) and in patients with coronary artery disease (Kiviniemi, Hautala et al. 2014). However, neither of these studies could identify a common mechanism for the acute and chronic responses in blood pressure. Recurring exercise-induced increases in blood flow and shear stress may also represent a beneficial stimulus contributing to longer term improvements in endothelial function and NO bioavailability (Awolesi, Widmann et al. 1994, Tinken, Thijssen et al. 2010). These improvements are thought to contribute to lower resting arterial stiffness (Duprez 2010). Although the above explanations are plausible, they are speculative. Hence, a study that quantifies the relationship between a decrease in arterial stiffness after acute exercise and the decrease in arterial stiffness at rest after a training intervention is warranted.
Overall, the research reported in chapters 4 and 5 of this thesis demonstrates the acute benefits of aerobic exercise on arterial stiffness in healthy older individuals and in patients with small AAA. The acute reductions observed in post-exercise arterial stiffness may represent a marker for future vascular adaptation with training. Given that this thesis studied a cohort of individuals with increased arterial stiffness and risk of cardiovascular events (Mitchell, Hwang et al. 2010, Bath, Saratzis et al. 2017), the reported transient post-exercise reductions in PWV and RM are promising.

6.2 Interpretation of post-exercise change in arterial stiffness indices and the importance of the control protocol

The novel addition of the control (seated rest) protocol in this thesis provides an important insight into the response of the arterial stiffness indices to exercise. The findings in chapters 4 and 5 demonstrate that PWV and wave reflection characteristics are differentially affected in a time-dependent manner after exercise of both moderate and higher intensity, and after a period of prolonged supine rest.

In chapters 4 and 5, PWV did not decrease below baseline after exercise. This finding is consistent with that of a previous study, which concluded that acute exercise offers no benefit for vascular stiffness in older adults (Akazawa, Ra et al. 2015). However, we observed a progressive rise in PWV during prolonged supine rest. This thesis is the first to report a lower post-exercise PWV response following a bout of moderate or higher-intensity exercise compared to control, in healthy older adults and patients with AAA.

No previous studies have assessed the acute effects of supine rest on PWV. During control the increase in central blood pressure across time corresponded to the observed increases in PWV. Indeed, blood pressure is one of the major determinants of PWV (O'Rourke, Staessen et al. 2002) and may partly reflect these findings. In addition, the progressive rise observed in PWV during the control protocol may reflect an increase in vascular tone (Fok, Jiang et al. 2012). Further, decreases in endothelial function measured using brachial artery FMD have been noted in older adults after prolonged rest (Bailey, Perissiou et al. 2017). Still, it is unclear whether such changes might explain this rise in PWV. Hence, the clinical relevance of acute increases in PWV during supine rest is currently unknown. Based on a previous meta-regression analysis, an increase in PWV of 1.0 m.s⁻¹ (or 1 SD) is associated with a 10% increase in cardiovascular risk (Vlachopoulos, Aznaouridis et al. 2010). However, this is a chronic change and its relevance to acute increases in arterial stiffness is not known. As sitting time increases all-cause
Findings in Chapters 4 and 5 demonstrated negligible changes compared with baseline in AIx75 immediately post-exercise. Recent evidence demonstrated, that following a bout of aerobic exercise, arterial stiffness indices (including AIx75), have been reported to increase (e.g. 0-5 min post-exercise) before decreasing below, resting levels during exercise recovery (Mutter, Cooke et al. 2017). This immediate post-exercise increase has been attributed to exercise induced increases in autonomic function that lead to peripheral vasoconstriction (Fok, Jiang et al. 2012) and a subsequent increase in cardiac preload (Houghton, Jones et al. 2016).

Hence, absence of a post-exercise increase in AIx75 may indicate autonomic dysfunction in older adults and patients with AAA. Interestingly, the change in posture from upright to supine position immediately after exercise may have affected the immediate response to exercise of AIx75. Studies suggest that AIx75 is not only affected by its traditional components (i.e. the reflected wave), but also by aortic reservoir function (Wang, O'Brien et al. 2003, Davies, Baksi et al. 2010, Huijben, Mattace-Raso et al. 2012). As such, a change in posture could lead to a redistribution of blood volume and an altered cardiac-preload (van de Velde, Eeftinck Schattenkerk et al. 2017) and may have therefore attenuated any increases in AIx75 immediately post exercise.

Findings in Chapters 4 and 5 found that decreases in AIx75 during exercise recovery were similar to those observed after the control protocol. These findings challenge those of previous studies that reported post-exercise decrements in AIx75 below baseline (Lane, Ranadive et al. 2013, Hanssen, Nussbaumer et al. 2015, Millen, Woodiwiss et al. 2016, Radhakrishnan, Swaminathan et al. 2016), but did not use a time-adjusted control comparison. Previous studies in young (Jaccoud, Rotaru et al. 2012) and older (Heim, Liaudet et al. 2013) healthy adults demonstrated that AIx75 is significantly lower when measured in the supine than in the seated position. This difference was attributed to a longer transit time of the diastolic reflected wave in the supine position; this parameter is affected by the transmural pressure of conduit arteries, which decreases during supine rest (Rosales-Velderrain, Cardno et al. 2011). Conversely, no changes were observed in RM during the control protocol in the studies reported in Chapters 4 and 5. These findings are consistent with those of a recent study that assessed the effect of posture on RM and its components (Pf and Pb); supine posture did not affect the measurements of this index (Stoner, Credeur et al. 2016). Wave separation analysis, the technique used for the estimation of RM (product of Pb/Pf ratio) allows for its calculation without dependence on
the timing of the forward and reflected waves (Westerhof, Guelen et al. 2006). This may explain the different response observed in this thesis between AIx75 and RM. Further, reflected pressure from the peripheral arteries is reported to be better represented by the RM (Hughes, Park et al. 2013). Hence, RM may provide a clearer insight into the time-dependent response from exercise of the forward and backward pressure waves. However, this index demonstrated poor reliability at rest and in response to exercise in Chapter 3 in older adults. Hence, post-exercise changes in RM in older adults should be interpreted with caution.

An interesting observation reported in Chapters 4 and 5 is the different response in PWV and AIx75 during supine rest in the control protocol. Although both PWV and AIx75 are considered indices of central arterial stiffness and are expected to respond in a linear way to a particular stimulus (Obara, Hayashi et al. 2009), their determinants may differentiate their responses. Carotid-to-femoral PWV quantifies the velocity of the forward pressure wave through the aorta (McEniery, Wallace et al. 2006) and is suggested to be determined by the structure (elastin/collagen content) (Wagenseil and Mecham 2012), and vascular smooth muscle tone of the aortic wall (Fok, Jiang et al. 2012). Conversely, AIx75 characterises the magnitude of the reflected pressure wave as it contributes to systolic blood pressure, and has therefore been reported to mostly reflect peripheral arterial stiffness (Nichols 2005). Rotaru et al. (2015) demonstrated that an increase in transit time of the diastolic reflected wave during the supine position (which would decrease AIx75) is not coupled to a decrease in carotid-femoral PWV. The authors suggested that the diastolic waves, observed on a reconstructed central pulse wave, mostly correspond to reflections generated in the lower limbs; hence, carotid-femoral PWV is not affected (Rotaru, Liaudet et al. 2015). These findings may explain the different responses of PWV and AIx75 observed during the control protocol in this thesis.

Overall, when assessing the acute effect of exercise on arterial stiffness in older people, it is important to consider which arterial stiffness index is used, the determinants of that index, and the response relative to a period of rest.

6.3 Acute response of arterial stiffness to exercise is intensity dependent
The effect of exercise intensity on the acute response of arterial stiffness to exercise was explored in the study reported in Chapter 4, and also explored in the study reported in Chapter 5. The results showed that PWV and RM were lower after short-term exercise than after control protocol (seated rest) in both healthy adults and patients with AAA. PWV and RM were further reduced by increasing the intensity of exercise. Previous findings in younger adults...
demonstrated lower PWV after higher-intensity exercise than after moderate-intensity exercise (Tordi, Mourot et al. 2010). A previous study showed that the exercise-induced reduction in arterial stiffness is accompanied by a dilatation of the arteries and an increase in blood flow (Munir, Jiang et al. 2008). In addition, an increasing dosage of nitroglycerin infusion provoked similar adaptations in arterial stiffness to those induced by increasing exercise intensities (Munir, Jiang et al. 2008). Therefore, dilatation of the arteries induced by nitric oxide through increased blood flow and shear stress is apparently a key mechanism underlying the intensity effect of post-exercise reduction in arterial stiffness reported in Chapters 4 and 5.

Previous studies in individuals with high cardiovascular risk reported a prolonged increase in PWV above baseline after a single bout of high-intensity continuous exercise (Gkaliagkousi, Gavrilaki et al. 2014, Bunsawat, Ranadive et al. 2017). An exaggerated exercise-induced increase in carotid-femoral PWV could trigger several mechanisms that could lead to a cardiovascular event. Among them is an abnormal increase in blood pressure, which in turn increases sympathetic outflow and circulating catecholamine levels, leading to a cardiovascular risk environment associated with ischemia, myocardial irritability and thrombotic occlusion (Thompson, Franklin et al. 2007, Goodman, Burr et al. 2016). Interval exercise is increasingly being tested and recommended in adults with cardiovascular disease as it allows for an overall higher exercise intensity stimulus and has potential for additional cardiovascular benefit compared with moderate-intensity continuous exercise (Weston, Wisløff et al. 2014, Ramos, Dalleck et al. 2017). Hence, in the current thesis interval mode was used in order to safely enable higher intensity exercise in a cohort of high cardiovascular risk. This factor may have contributed to the lack of a prolonged post-exercise increase in PWV. Thus, Chapters 4 and 5 demonstrated that high-intensity interval exercise does not appear to exacerbate arterial stiffness in healthy older adults or patients with AAA.

6.4 Acute response of arterial stiffness to exercise depends on cardiorespiratory fitness

The study reported in Chapter 4 assessed the effect of cardiorespiratory fitness on the acute arterial stiffness response to exercise. The findings suggest that cardiorespiratory fitness affects the post-exercise response of PWV: the PWV of individuals with mid and high levels of cardiorespiratory fitness was lower after moderate-intensity exercise than the PWV of those with low fitness. Further, the results reported in Chapters 4 and 5 demonstrate for the first time that moderate-intensity exercise may not be a sufficient stimulus for transiently decreasing
PWV in individuals with low cardiorespiratory fitness, such as patients with small AAA and older sedentary individuals.

In contrast with previous literature (Vaitkevicius, Fleg et al. 1993), Chapter 4 reports no differences in baseline PWV among older individuals of differing levels of cardiorespiratory fitness. These inconsistent findings might be attributed to differences in the cardiorespiratory fitness levels of the high-fitness group in this study (mean $\dot{V}O_{2peak}$ of 36 ml.kg$^{-1}$.min$^{-1}$) and the earlier study by Vaitkevicius et al. (mean $\dot{V}O_{2peak}$ of 45 ml.kg$^{-1}$.min$^{-1}$). Due to increased arterial stiffening among older adults (Mitchell, Parise et al. 2004), a higher level of cardiorespiratory fitness than that observed in the present study may be required to offset the age-related increase in PWV. Initially, we hypothesised that individuals with higher cardiorespiratory fitness would be more responsive to exercise than their sedentary peers because they should maintain a more compliant vascular system at rest (Gando, Murakami et al. 2016). Despite the fact that Chapter 4 reported no differences in resting PWV, our results demonstrate that higher cardiorespiratory fitness is associated with more favourable effects on post-exercise PWV. These findings suggest that exercise reveals a fitness effect on arterial stiffness that was not apparent from resting measurements alone.

Findings reported in Chapter 4 further demonstrate that the post-exercise response of PWV after moderate-intensity exercise is not different to control in individuals with low cardiorespiratory fitness. Conversely, individuals with mid or high fitness were responsive after both exercise protocols. Similarly, in the study reported in Chapter 5, which included individuals of low cardiorespiratory fitness, post-exercise PWV decreased below baseline levels after high-intensity exercise but not after moderate-intensity exercise. Increasing levels of cardiorespiratory fitness are associated with increased contractility of vascular smooth muscle after exercise (Thijssen, Maiorana et al. 2010), which may explain the observed differences in post-exercise PWV between individuals of low and higher cardiorespiratory fitness. Collectively, the findings presented in Chapters 4 and 5 suggest that, in individuals with low fitness, the post-exercise response of arterial stiffness is blunted after moderate-intensity exercise. Higher-intensity exercise may be required in these individuals in order to evoke beneficial vascular adaptations. Given that reductions in arterial stiffness during exercise recovery may be necessary for the long-term vascular adaptations seen with training (Romero, Minson et al. 2017); the results of this thesis indicate that exercise intensity and cardiorespiratory fitness should be considered when prescribing an exercise intervention in order to improve vascular health in both healthy older adults and patients with AAA.
6.5 Response of arterial stiffness to exercise is similar in patients with AAA and healthy older adults

Findings presented in Chapter 5 provide new knowledge regarding the acute arterial stiffness response to exercise in patients with AAA. Exercise has been widely reported to reduce resting arterial stiffness (Montero, Vinet et al. 2014), which is an independent predictor of cardiovascular disease risk and mortality (Mitchell, Hwang et al. 2010, Vlachopoulos, Aznaouridis et al. 2010). Higher resting arterial stiffness is also associated with increased aneurysm progression (Kadoglou, Papadakis et al. 2012) and rupture risk among AAA patients (Raaz, Zollner et al. 2015). The findings in Chapter 5 suggest that a single bout of exercise provides short-term cardiovascular benefits in patients with AAA and healthy older adults and support the potential use of exercise for reducing cardiovascular risk in patients with AAA.

The findings presented in Chapter 5 show that despite a higher resting PWV in patients with AAA, exercise did not exacerbate aortic stiffness, with the changes in PWV during the recovery from exercise being similar compared with healthy older adults. The transient attenuation in PWV and RM after a single bout of submaximal exercise compared with control, suggests there may be a short-term cardiovascular benefit from exercise for patients with AAA, similar to that reported for healthy older adults (Schultz, La Gerche et al. 2017). For patients with AAA, the observed attenuation in aortic stiffness and wave reflection magnitude may contribute to a reduced central blood pressure and alleviate stress on the AAA wall, perhaps limiting growth and rupture risk (Ruegg, Mason et al. 2010, Raaz, Zollner et al. 2015). Recent physical activity guidelines in clinical populations, including patients with AAA, recommended that reducing sitting time is associated with improved functional capacity and reduced postoperative mortality and morbidity (Tew, Ayyash et al. 2018). This study demonstrates that interrupting sitting time with exercise attenuates the increases in aortic stiffness observed with prolonged supine rest. The findings suggest intermittent periods of exercise should be encouraged in patients with small AAAs.

There was a tendency for higher-intensity interval exercise to induce a lower post-exercise PWV compared with moderate-intensity continuous exercise in both patients with AAA and healthy older adults. This is consistent with reports in younger (Tordi, Mourot et al. 2010) and older adults (as presented in Chapter 4), and may infer greater post-exercise benefits for cardiovascular function, including intensity-dependent reductions in blood pressure (Quinn 2000) and increases in shear-mediated vasodilation (Green, Maiorana et al. 2004, Santana, Moreira et al. 2013). It is important to note that the exercise protocol of this thesis followed
recent exercise guidelines for individuals of high cardiovascular risk (Garber, Blissmer et al. 2011, Seron, Lanas et al. 2014) hence participants of this cohort did not exceed 70% of heart rate max during higher-intensity interval protocol. Therefore, I am unaware if a higher intensity would be beneficial for patients with AAA. As discussed earlier, previous evidence in young adults of high cardiovascular risk (hypertensive and obese) reported a higher PWV after high-intensity continuous exercise when compared with their healthy peers (Gkaliagkousi, Gavriilaki et al. 2014, Moore, Berrones et al. 2016, Bunsawat, Ranadive et al. 2017). In addition, a recent feasibility trial of high-intensity interval training reported that patients with AAA are unable to reach a prescribed intensity at 85% of heart rate max (Tew, Batterham et al. 2017). Therefore, the prescribed intensity in Chapter 5 may be suitable for future prescription in patients with AAA.

Although the results presented in this thesis are encouraging and provide new knowledge regarding the acute vascular response to exercise of patients with AAA, further investigations should examine whether higher-intensity interval exercise leads to chronic reductions in arterial stiffness in these patients. Further, whether these reductions decrease aneurysm progression and reduce the risk of morbidity and mortality from cardiovascular comorbidities requires confirmation. Future studies should explore the effect of high-intensity interval exercise training on indices of arterial stiffness. Hence, a randomised controlled trial comparing two patient groups (exercise training group vs usual care controls) is necessary.

6.6 Methodological considerations and research limitations
The context in which findings of this thesis can be applied is important. Only patients with small, asymptomatic AAA who had been cleared to exercise by a cardiologist were recruited for the current research project and thus our findings may not relate to AAA patients who are deemed high-risk or unable to exercise. In addition, the cohort of Chapter 5 (patients with AAA and the healthy control group) consisted of only male participants. It has been shown that the post-exercise arterial stiffness response is enhanced in young healthy females compared with males (Doonan, Mutter et al. 2013), and thus our findings cannot be extrapolated to older females with and without AAA.

As expected, prescribed pharmacological treatment was more prevalent in patients with AAA than in the healthy group (Table 7, Chapter 5). Medications such as beta-blockers and statins, which were prevalent in patients with AAA have been previously reported to reduce arterial stiffness indices, including PWV and AIx75, at rest (Gkaliagkousi, Gavriilaki et al. 2014,
Sahebkar, Pecin et al. 2016) and in response to exercise (Gkaliagkousi, Gavriilaki et al. 2014). Although the current research found no differences in arterial stiffness responses between those who were using prescribed medications and those who were not (see Chapter 5), such medications could potentially alter the arterial stiffness response to exercise. Lastly, endothelial function is suggested to affect the arterial stiffness response to exercise (McEniery, Qasem et al. 2003). However assessing endothelial function was not one of the aims of this thesis and therefore this thesis was unable to control for this parameter.

6.7 Summary of findings
This thesis provides new knowledge regarding the acute response of arterial stiffness to exercise in patients with AAA and healthy older adults. This thesis findings demonstrate that short bouts of submaximal exercise provide similar short-term cardiovascular benefits between patients with AAA and healthy older adults. Additionally, this thesis provides evidence that higher intensity interval exercise leads to a greater lowering of post-exercise PWV compared with moderate-intensity continuous exercise and control, both in patients with AAA and in healthy older individuals. Furthermore, this thesis demonstrates that interval exercise of higher-intensity may be required in older adults and in patients with AAA with low cardiorespiratory fitness in order to evoke transient post-exercise reductions in arterial stiffness. Based on the notion that acute post-exercise reductions in arterial stiffness contribute to chronic vascular adaptations with training, this thesis suggests that exercise intensity and cardiorespiratory fitness should be considered when prescribing an exercise intervention to improve vascular health in healthy older adults and patients with AAA. Lastly, this thesis provides essential knowledge regarding the precision of measures of the short-term responses in arterial stiffness after a single bout of exercise in healthy older adults and supports the potential use of such measures for research and in clinical practice.
7. References


"Age and cardiorespiratory fitness are associated with arterial stiffening and left ventricular remodelling." J Hum Hypertens 24(3): 197-206.


intramyocellular or extramyocellular lipid content and arterial stiffness." J Hum Hypertens 30(10): 606-612.


interrelationships with endothelial function and arterial morphology measures."
Vascular Health and Risk Management 3(3): 343-349.


aplantation tonometry in pregnant and nonpregnant women." J Hypertens 30(6): 1161-1168.


Physiology - Regulatory Integrative and Comparative Physiology 309(12): R1540-R1545.


8. Appendix

8.1 Published version of Chapter 3

Reliability of arterial stiffness indices at rest and following a single bout of moderate-intensity exercise in older adults

Maria Perissiou1, Tom B. Bailey2, Mark Windsor2, Anthony S. Leicht2, Jonathan Gollidge2,5 and Christopher D. Askew1

1VasoActive Research Group, School of Health and Sport Sciences, University of the Sunshine Coast, Maroochydore, 2Centre for Research in Exercise, Physical Activity and Health, School of Human Movement and Nutrition Sciences, The University of Queensland, Brisbane, Australia, 3Sport and Exercise Science, James Cook University, 4Queensland Research Center for Peripheral Vascular Disease, James Cook University, and 5Department of Vascular and Endovascular Surgery, The Townsville Hospital, Townsville, Queensland, Australia

Summary

Short-term changes in arterial stiffness with exercise are proposed to better reflect vascular impairments than resting measures alone and are suggested as a prognostic indicator of cardiovascular risk in older adults. Arterial stiffness indices are reliable at rest, but the time-course and reliability of postexercise changes in arterial stiffness in older adults are unknown. The precision of postexercise changes in arterial stiffness should be determined prior to their use in large prospective trials. This study assessed the between-day reliability of the changes in pulse wave velocity (PWV), augmentation index (AIx75) and reflection magnitude (RM) following an exercise bout in older adults. Ten older adults (71 ± 5 years) were tested on three separate days, 7 days apart. PWV, AIx75 and RM were assessed at rest, immediately post and at 20, 40 and 60 min during recovery after moderate-intensity cycling. Intraclass correlation coefficient (ICC) and reliability coefficient (RC) were used to assess the relative and absolute reliability of arterial stiffness responses. PWV increased, and RM decreased immediately after exercise (P<0.05), and returned to baseline during recovery. AIx decreased during recovery (P=0.001). Resting ICC values were 0.91 (PWV), 0.72 (AIx75) and 0.80 (RM). Reliability of the immediate changes following exercise was high for PWV (ICC=0.87, RC=1.9 m s⁻¹) and moderate for AIx75 (ICC=0.64, RC=7%) and RM (ICC=0.59, RC=9%). Reliability of the postexercise responses was similar to that at rest for all measures of arterial stiffness. These findings indicate that postexercise changes in arterial stiffness indices are reliable in healthy older adults and supports further investigation of the prognostic value of these responses.

Introduction

Arterial stiffness increases with age (McIntire et al., 2005) and is one of the earliest detectable manifestations of adverse structural and functional vascular changes, contributing to the pathogenesis of cardiovascular disease (Stephan et al., 2011) and end-organ damage (Lee & Oh, 2010). Contrast to noninvasive pulse wave velocity (PWV) is the criterion measure for the non-invasive assessment of central aortic stiffness (Cecelia & Chowtonczyk, 2017), the region which exhibits the greatest age-related stiffening (Mitchell et al., 2004; Wilkinson et al., 2010; Stoner et al., 2012). Augmentation index (AIx) provides an integrated summary of wave reflection timing and amplitude (Nichols & Singh, 2002), and is expressed as a percentage of central pulse pressure. Wave separation analysis enables the calculation of reflection magnitude (RM) expressed as the ratio of reflected (backward, Pr) to forward (P1) pressure waves (Westbroek et al., 2006). While resting indices of arterial stiffness are used to determine the risk of cardiovascular disease development and mortality (Mitchell et al., 2010; Chirinos et al., 2012), the augmented haemodynamic changes with exercise may be more sensitive for revealing vascular abnormalities and cardiovascular risk in healthy adults.

Arterial stiffness increases immediately after exercise before returning to, or below, resting levels during a period of recovery in healthy, younger adults (Mutten et al., 2017). However, the increase in arterial stiffness after exercise has been shown to be exaggerated and remains above baseline levels during recovery in adults with known cardiovascular...
of heart rate reserve or 40–60% PPO (Garber et al., 2011). During exercise, HR (using 12-lead ECG) and RPE were measured every 60s. Brachial blood pressure was measured every 6 min using a manual sphygmomanometer.

Arterial stiffness measurements

Indices of arterial stiffness (PWV, RM and AIX) were measured at baseline after 15 min of quiet rest in the supine position, using the SphygmoCor XCEL device (AtCor Medical, West Ryde, NSW, Australia). Measurements were repeated immediately following exercise (0–5 min) and at 20, 40 and 60 min during exercise recovery. Participants were instructed to remain quiet and still for 5 min before and during each arterial stiffness measurement. The measurement of wave reflection characteristics (AIX and RM) preceded PWV at all time points, and a complete cycle for all arterial stiffness measurements took ~5 min.

Wave reflection characteristics

Brachial artery waveforms were recorded on the right upper arm, following manufacturer guidelines (Hwang et al., 2014) by inflating a brachial cuff to suprapastolic pressure, approximately midway between the shoulder and the elbow. Each measurement cycle commenced with three repeat measurements of brachial blood pressure, and an index of the last two measurements was used. Subsequently, a corresponding aortic pressure waveform was generated by applying proprietary digital signal processing and transfer function (Berlin et al., 2012), from which central systolic (cSBP), diastolic (cSDP), pulse pressure (cPP), augmentation pressure (AP), AIX, RM, Pfr and Pfb were derived. Central pulse pressure was calculated as the difference between cSBP and cSDP. Augmentation pressure is defined as the difference between cSBP and the pressure at the inflection point caused by the merging of forward and reflected pulse wave. AIX was defined as the augmentation pressure expressed as a percentage of pulse pressure. As AIX is significantly affected by heart rate, the index was corrected for a heart rate at 75 beats per minute (AIX75). Wave separation analysis was applied using SphygmoCor CVMS software (AtCor Medical, Sydney, Australia). This method creates an assumed triangular-shaped flow wave by aligning the start, peak and end of the flow wave with the foot, inflection point and notch of the aortic pressure wave, respectively (Wiesenhoff et al., 2006). Based on the assumed flow wave, the aortic forward (Pf) and backward (Pb) pressure waveforms were calculated. Reflection magnitude (RM) was calculated as the ratio of Pb to Pf and expressed as a percentage: RM = Pb/Pf × 100.

Pulse wave velocity (PWV)

To assess carotid-femoral PWV, carotid pulse waves were obtained by application tonometry of the right carotid artery and femoral pulse waves were obtained by sphygmomanometry of the right thigh using a thigh cuff. The tonometer was positioned at the site of the carotid pulse, while a femoral cuff was placed at mid-thigh. Following the operator’s guidelines (SphygmoCor Xcel Vi), the distance between the carotid and femoral arteries was measured from the carotid site above the suprasternal notch to the proximal edge of a thigh cuff over the femoral artery at the leg midway between the hip and the knee. The distance from the inguinal fold to the femoral cuff was standardized at 200 mm. This distance and the same placement of the thigh cuff was then used for all repeat sessions for each individual. In the supine position, participants were asked to breathe steadily and remain relaxed to facilitate an optimal carotid pulse tonometry measurement. Once a regular carotid pulse was detected, femoral pulse waves were collected simultaneously by partially inflating the thigh cuff to 80 mmHg. PWV was then determined by calculating the ratio of the distance between the pulse measuring sites to the time delay between the carotid and femoral pulse waves (Wilkinson et al., 2010).

Statistical analyses

Arterial stiffness indices (PWV, AIX75 and RM) were measured at (i) rest (preexercise), (ii) immediately postexercise (0 min post) and (iii) during 0–60 min of postexercise recovery, on three separate occasions. Using the trapezoidal rule (Tallarida & Murray, 1987), total area under the curve (AUC) of arterial stiffness indices (0–60 min postexercise) was calculated to quantify total recovery. Data were also calculated as changes from rest (delta) to account for the small, but non-significant day-to-day variance in resting arterial stiffness values. All data were initially tested for normality and screened for outliers. To determine whether there were any significant changes in arterial stiffness indices at rest or following exercise between the three visits, we initially included all data in a two-way (visit*time) linear mixed model (LMM). The same analyses were also used to detect differences in postexercise delta measures of arterial stiffness between visits. All analyses were performed using SPSS (version 21; SPSS, Chicago, IL, USA). All data are presented as mean (95% confidence interval; 95% CI), unless otherwise specified, and statistical significance was set at P < 0.05. For P values < 0.000, the value is reported as P < 0.001.

The reliability of arterial stiffness indices (PWV, AIX75 and RM) at (i) rest, (ii) immediately postexercise and (iii) the recovery AUC, across the three repeated visits, was initially determined comparing the mean differences between the three visits using a one-way LMM and was further characterized using the intraclass correlation coefficient (ICC), the coefficient of variation (ratio of standard deviation to the mean, CV%), standard error of the mean (SEM) and the reliability coefficient (RC) (Weir, 2005). ICC was used to assess the relative reliability of arterial stiffness indices, as ICC accounts for both the consistency of arterial stiffness from test to retest (within-participant change), as well as the change in the mean.
between visits. The ICC was calculated as the ratio of the squared between-subject variance to the sum of squares of between- and within-subject variance: $\frac{(SD)_{\text{between}}^2/(SD_{\text{between}}^2 + SD_{\text{within}}^2)}{}$. ICC values above 0.75 were considered to indicate excellent reliability, 0.40-0.74 good reliability, and <0.40 suggests poor reliability (Reiss, 1999). The RC was used as a measure of absolute reliability, which accounts for variability in arterial stiffness due to random and systematic measurement error. The RC quantifies absolute reliability measurement error in the same units as the measurement itself. RC was calculated by multiplying the within-participant SEM by $2.77(\sqrt{2})^{1.96}$ (Weir, 2005; Vaz et al., 2013).

## Results

### Participant characteristics

Participant characteristics are presented in Table 1. $\dot{V}O_{2\text{peak}}$ was 14.6 ml kg$^{-1}$ min$^{-1}$ (95% CI 18.9–30.3), which can be characterized as fair according to normative age- and sex-specific data (Garber et al., 2011).

### Exercise variables

Heart rate increased by 21 bpm (95% CI 18.4–29.7, $P = 0.003$) during cycling exercise and did not differ between visits ($P = 0.465$). MAP increased by 8 mmHg (95% CI 4–10, $P = 0.002$) during cycling exercise, with no differences observed between visits ($P = 0.084$). RPE increased by 2 (95% CI 1–3, $P = 0.001$) during exercise and did not differ between visits ($P = 0.431$).

### Resting and postexercise measures of arterial stiffness

Figure 1 shows the mean responses for PWV (Fig 1a), AIX 75 (Fig 1b) and RM (Fig 1c) at rest and at each time point after exercise for the three repeat visits. There was no difference in resting values of arterial stiffness indices between visits. PWV increased from baseline to immediately after exercise [mean increase at 0–5 min post of 0.6 m s$^{-1}$ (95% CI 0.26–0.96, $P = 0.001$)], before returning to near baseline levels. The increase in AIX 75 was negligible immediately after exercise, before decreasing below baseline levels at 40 and 60 min post-exercise (60 min decrease of 3.5% (95% CI 1.4–5.7, $P = 0.001$), RM decreased immediately after exercise [mean decrease at 0–5 min post of 10.1% (95% CI 5.3–14.8, $P = 0.001$)] before returning to near baseline levels.

A main effect for ‘visit’ was observed for PWV (Fig 1a), where mean PWV (across all time points) during visit 3 was lower compared to visit 1 ($P = 0.001$) and visit 2 ($P = 0.042$). Conversely, AIX 75 (Fig 1b) was higher during visit 1 compared to visit 2 ($P = 0.001$), but not compared to visit 3 ($P = 0.084$).

There were no significant visit*time interactions for PWV, AIX 75 and RM (Fig 1a–c), indicating a consistent response to exercise (time effect) across visits. This was confirmed when the change in each variable from baseline (delta) was assessed, demonstrating no difference in the arterial stiffness response across the visits (Table 2).

### Reliability of arterial stiffness at rest, immediately postexercise and during recovery

Measures of reliability, and the corresponding mean data, for the indices of arterial stiffness at rest, immediately postexercise and during recovery, across the three visits are shown in Table 3. Reliability of PWV at rest was excellent (ICC 0.75) and more reliable than the other resting measures of vascular stiffness, particularly RM for which the reliability was borderline good–poor (ICC 0.40). Reliability of the postexercise measures of vascular stiffness was similar to that observed at rest for PWV and AIX; whereas reliability was slightly improved for RM with an increase in the ICC (0.40–0.59) and a reduction in the reliability coefficient (11–9%) postexercise compared with rest. Recovery of each vascular stiffness measure during the 60-min period after exercise (area under the curve) did not change across the three visits, and the reliability of these recovery measures was similar to that observed at rest, as indicated by the comparable ICC and CV% values (Table 3).

### Discussion

The present study examined the between-day reliability of exercise-induced, short-term changes in arterial stiffness in healthy older adults. Our main findings were that carotid–femoral PWV and AIX 75 can be measured reliably in healthy older individuals at rest, immediately after and during one hour of recovery from moderate-intensity cycling exercise across three separate occasions. While RM demonstrated poor reliability at rest, we demonstrate that this index can be measured reliably immediately after and during recovery from exercise.
Figure 1  Pulse wave velocity (PWV) (a), Axs75 (b) and reflection magnitude (RM) (c) at rest and postexercise during visit 1 (white circles), visit 2 (white triangles) and visit 3 (white squares). Error bars represent SD,* indicates significant change across time compared to baseline; post hoc analysis of visit effect revealed mean PWV was lower during visit 3 compared to visit 1 (P<0.001) and 2 (P = 0.042), and mean Axs75 was higher in visit 1 compared to visit 2 (P = 0.006).

Table 2  Change of PWV, Axs75 and RM from baseline at 0, 30, 40 and 60 min postexercise.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit</th>
<th>0 min post</th>
<th>20 min post</th>
<th>40 min post</th>
<th>60 min post</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔPWV (ms⁻¹)</td>
<td>Visit 1</td>
<td>0.9 ± 0.9</td>
<td>0.4 ± 0.6</td>
<td>0.5 ± 0.7</td>
<td>0.6 ± 0.8</td>
<td>Visit: 0.024</td>
</tr>
<tr>
<td></td>
<td>Visit 2</td>
<td>0.5 ± 0.8</td>
<td>0.2 ± 0.7</td>
<td>0.2 ± 1.0</td>
<td>0.4 ± 1.2</td>
<td>Time: 0.019</td>
</tr>
<tr>
<td></td>
<td>Visit 3</td>
<td>0.4 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td>0.4 ± 0.4</td>
<td>0.4 ± 0.9</td>
<td>Visit*Time: 0.019</td>
</tr>
<tr>
<td>ΔAxs75 (%)</td>
<td>Visit 1</td>
<td>-0.1 ± 0.9</td>
<td>2.6 ± 7</td>
<td>-2.5 ± 5</td>
<td>-3.6 ± 4</td>
<td>Visit: 0.031</td>
</tr>
<tr>
<td></td>
<td>Visit 2</td>
<td>2.4 ± 4</td>
<td>1.7 ± 2</td>
<td>-3.9 ± 4</td>
<td>-3.6 ± 4</td>
<td>Time: 0.001</td>
</tr>
<tr>
<td></td>
<td>Visit 3</td>
<td>1.0 ± 6</td>
<td>1.2 ± 5</td>
<td>-3.7 ± 3</td>
<td>-3.4 ± 5</td>
<td>Visit*Time: 0.943</td>
</tr>
<tr>
<td>ΔRM (%)</td>
<td>Visit 1</td>
<td>-1.4 ± 1.5</td>
<td>-3.4 ± 15</td>
<td>-6.1 ± 10</td>
<td>-3.1 ± 13</td>
<td>Visit: 0.211</td>
</tr>
<tr>
<td></td>
<td>Visit 2</td>
<td>-9.1 ± 10</td>
<td>-9.4 ± 10</td>
<td>-3.1 ± 10</td>
<td>-3.3 ± 11</td>
<td>Time: 0.011</td>
</tr>
<tr>
<td></td>
<td>Visit 3</td>
<td>-6.1 ± 8</td>
<td>-2.9 ± 9</td>
<td>1.7 ± 12</td>
<td>-3.4 ± 10</td>
<td>Visit*Time: 0.943</td>
</tr>
</tbody>
</table>

Axs75, augmentation index normalized to a heart rate of 75 bpm; RM, reflection magnitude; PWV, pulse wave velocity, Δ denotes delta (change).

Reliability of resting arterial stiffness indices

We show that the between-day relative reliability of resting carotid-femoral PWV was excellent in older adults (ICC: 0.91), consistent with a recent study using the same device in young individuals (ICC: 0.98) (Hwang et al., 2014). Furthermore, the ICC values (0.72–0.57) for Axs75, PI and PBs at rest were consistent with recent studies (ICC, Axs75: 0.70–0.75; PI: 0.57, PBs: 0.72–0.78).
Table 3  Reliability of arterial stiffness indices at rest, immediately post exercise and during exercise recovery.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>ICC</th>
<th>CV%</th>
<th>SEM</th>
<th>RC</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV (m s⁻¹)</td>
<td>11.2 ± 2.4</td>
<td>11.2 ± 2.1</td>
<td>10.7 ± 2.0</td>
<td>0.91</td>
<td>5.0</td>
<td>0.7</td>
<td>1.9</td>
<td>0.84</td>
</tr>
<tr>
<td>Immediately post</td>
<td>13.2 ± 2.0</td>
<td>11.79 ± 2.4</td>
<td>11.31 ± 2.0</td>
<td>0.87</td>
<td>6.5</td>
<td>0.7</td>
<td>1.9</td>
<td>0.26</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>712 ± 150</td>
<td>692 ± 133</td>
<td>677 ± 129</td>
<td>0.94</td>
<td>4.9</td>
<td>5.6</td>
<td>15.4</td>
<td>0.78</td>
</tr>
<tr>
<td>Axs75 (%)</td>
<td>Rest</td>
<td>23 ± 9</td>
<td>22 ± 8</td>
<td>23 ± 8</td>
<td>0.72</td>
<td>14.0</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Immediately post</td>
<td>23 ± 10</td>
<td>24 ± 8</td>
<td>24 ± 5</td>
<td>0.67</td>
<td>16.7</td>
<td>2</td>
<td>7</td>
<td>0.99</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>1441 ± 365</td>
<td>1384 ± 416</td>
<td>1330 ± 400</td>
<td>0.84</td>
<td>11.7</td>
<td>162</td>
<td>462</td>
<td>0.68</td>
</tr>
<tr>
<td>PF (mmHg)</td>
<td>Rest</td>
<td>24.5 ± 7</td>
<td>23.7 ± 6</td>
<td>23.3 ± 5</td>
<td>0.71</td>
<td>9.4</td>
<td>1.4</td>
<td>8.4</td>
</tr>
<tr>
<td>Immediately post</td>
<td>31.2 ± 8</td>
<td>28.4 ± 6</td>
<td>27.3 ± 6</td>
<td>0.54</td>
<td>13.2</td>
<td>2.0</td>
<td>5.6</td>
<td>0.38</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>1586 ± 276</td>
<td>1570 ± 311</td>
<td>1414 ± 349</td>
<td>0.68</td>
<td>8.9</td>
<td>131</td>
<td>364</td>
<td>0.44</td>
</tr>
<tr>
<td>Pw (mmHg)</td>
<td>Rest</td>
<td>18.2 ± 3</td>
<td>17.4 ± 2</td>
<td>17.8 ± 3</td>
<td>0.54</td>
<td>9.0</td>
<td>0.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Immediately post</td>
<td>19.4 ± 4</td>
<td>18.9 ± 4</td>
<td>19.5 ± 5</td>
<td>0.73</td>
<td>10.5</td>
<td>1.3</td>
<td>3.6</td>
<td>0.94</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>1093 ± 187</td>
<td>1072 ± 141</td>
<td>1052 ± 242</td>
<td>0.75</td>
<td>7.6</td>
<td>23</td>
<td>262</td>
<td>0.90</td>
</tr>
<tr>
<td>RM (%)</td>
<td>Rest</td>
<td>75.5 ± 13</td>
<td>74.6 ± 13</td>
<td>74.5 ± 11</td>
<td>0.40</td>
<td>11.5</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Immediately post</td>
<td>61 ± 11</td>
<td>66.4 ± 12</td>
<td>68 ± 10</td>
<td>0.59</td>
<td>9.8</td>
<td>3</td>
<td>9</td>
<td>0.26</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>4190 ± 432</td>
<td>4102 ± 532</td>
<td>4195 ± 399</td>
<td>0.38</td>
<td>9.1</td>
<td>180</td>
<td>500</td>
<td>0.89</td>
</tr>
<tr>
<td>HR (b min⁻¹)</td>
<td>Rest</td>
<td>60 ± 8</td>
<td>60 ± 8</td>
<td>59 ± 7</td>
<td>0.70</td>
<td>4.9</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Immediately post</td>
<td>70 ± 8</td>
<td>69 ± 10</td>
<td>68 ± 10</td>
<td>0.62</td>
<td>5.7</td>
<td>3</td>
<td>8</td>
<td>0.81</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>534 ± 170</td>
<td>590 ± 190</td>
<td>580 ± 185</td>
<td>0.72</td>
<td>6.7</td>
<td>170</td>
<td>502</td>
<td>0.62</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>Rest</td>
<td>127 ± 18</td>
<td>120 ± 13</td>
<td>118 ± 12</td>
<td>0.80</td>
<td>5.0</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Immediately post</td>
<td>134 ± 17</td>
<td>130 ± 18</td>
<td>128 ± 13</td>
<td>0.81</td>
<td>5.8</td>
<td>5</td>
<td>14</td>
<td>0.71</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>10220 ± 1530</td>
<td>1025 ± 1180</td>
<td>9800 ± 930</td>
<td>0.85</td>
<td>3.5</td>
<td>355</td>
<td>994</td>
<td>0.65</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>Rest</td>
<td>75 ± 13</td>
<td>70 ± 9</td>
<td>71 ± 10</td>
<td>0.76</td>
<td>5.3</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Immediately post</td>
<td>78 ± 9</td>
<td>76 ± 12</td>
<td>75 ± 10</td>
<td>0.89</td>
<td>4.7</td>
<td>3</td>
<td>8</td>
<td>0.64</td>
</tr>
<tr>
<td>Recovery (AUC)</td>
<td>6698 ± 780</td>
<td>5900 ± 780</td>
<td>5740 ± 730</td>
<td>0.91</td>
<td>3.5</td>
<td>242</td>
<td>650</td>
<td>0.57</td>
</tr>
</tbody>
</table>

P < 0.05 indicates significant difference between visits; data are presented as mean ± SD; ICC, intraclass correlation; Immediately post (0 min); CV%, coefficient of variation; SEM, standard error of the mean; RC, reliability coefficient; Axs75, augmentation index normalised to a heart rate of 75 bpm; PF, forward pressure wave; Pw, backward pressure wave; RM, reflection magnitude; PWV, pulse wave velocity; HR, heart rate; SBP, systolic blood pressure; DBP diastolic blood pressure.

PF: 0.66; Pw: 0.76) in young and older individuals (Stoner et al., 2016; Mitchelmore et al., 2017). Despite good reliability of the forward and backward pressure wave, the reliability of RM at rest was poor, as also observed previously (Stoner et al., 2016). RM is an index that may be sensitive to small changes (Weir, 2005) and vulnerable to signal noise (variance) compared with PWV and Axs75. The excellent relative reliability of PWV at rest corresponded with an absolute reliability (RC: 1.9 m s⁻¹) similar to that reported by Keith et al. (2013) (1.5 m s⁻¹). While the clinical meaningfulness of this smallest detectable change remains to be fully established in older adults, it has previously been shown that a 1.0 m s⁻¹ (or ISD) increase in PWV is associated with a 10% increase in cardiovascular risk (Vlachopoulos et al., 2010).

**Time-course of the changes in arterial stiffness after exercise**

PWV increased immediately after exercise, followed by a transient return to baseline. We also observed a transient decrease in Axs75 and reflection magnitude to below resting levels after exercise. These observations are the first in older individuals and are similar to the responses recently reviewed in healthy younger adults (Müller et al., 2017). While resting measures of arterial stiffness reflect structural and functional mechanisms, beneficial reductions in arterial stiffness during recovery following acute exercise in healthy adults are suggested to be functional and are likely mediated by reductions in central blood pressure (Müller et al., 2016), vasoconstrictors (endothelin-1) (Schreuder et al., 2014) and sympathetic nerve activity (Heffeman et al., 2007), alongside increases in nitric oxide bioavailability and endothelial function (Campbell et al., 2011). Elevations in arterial stiffness following exercise in obese and hypertensive adults (Shin et al., 2011; Gkaliagkousi et al., 2014; Moon et al., 2015; Bunsawan et al., 2017) are potentially detrimental and may be due to underlying dysfunction of these mechanisms. Indeed, there is evidence of altered sympathetic baroreflex sensitivity that is exacerbated with exercise (Okada et al., 2013), revealing underlying dysfunction that is not evident at rest (Shin et al., 2011). As wave reflection
characteristics are reported to represent reflected pressure waves from peripheral arteries and are influenced by changes in downstream artery function, it is likely that the decreases we observed in wave reflection (Aix75 and reflection magnitude) following exercise, in contrast to the negligible changes in PWV, are primarily due to peripheral vasodilation and the reduced vascular resistance within the exercising limbs.

Reliability of arterial stiffness indices during exercise recovery

The reliability of postexercise recovery measures of arterial stiffness was similar to that observed at rest. Reliability of the recovery of arterial stiffness following exercise was excellent for PWV and Aix75, but poor for RM despite good reliability of its determinants (Pa: ICC 0.68, Pb: ICC 0.75). Our results for Aix75 were in agreement with a recent study in younger adults that assessed the reliability of the changes in Aix75 during 30 min of recovery after incremental cycling exercise (ICC: -0.82) (Liu et al., 2016). An earlier return of the reflected wave has been observed in older adults (Namasiavaram et al., 2009) and likely causes the movement of the reflection point (the point where the forward and backward wave meet (Westerhof et al., 2006)) into late systole that makes these indices less distinguishable (O’Rourke & Nichols, 2005; Westerhof & Westerhof, 2012). In addition, our assessment of the AUC of the complete recovery period likely increases the variability of this index compared to measurements at a single time point. In combination with the low ICC values for RM at rest, RM may not be sensitive to meaningful postexercise changes in older individuals. We show that the postexercise response of PWV and Aix75, but not RM, have strong reliability in older adults.

The clinical significance of changes in arterial stiffness after exercise is not yet established, and this study provides the basis for future research in this area. Given the excellent reliability of the PWV response to exercise in older adults, the utility of this measurement as a prognostic marker should be considered. The reliability coefficient, or smallest detectable change, in the immediate response of PWV after exercise (1.9 m s⁻¹) was similar to the change in PWV previously observed after exercise (increase of ~2.0 m s⁻¹) in adults with untreated grade I hypertension (Gkaliagkousi et al., 2014). Conversely, an increase in PWV of 1.3 m s⁻¹ following exercise has been associated with abnormal kidney function in middle-aged adults (Keith et al., 2013); however, our data suggest that this is below the smallest detectable change in older adults. For future studies exploring the utility of the immediate change in PWV following exercise, we estimate that a cohort of 8 (assuming a sample SD of 1 m s⁻¹) to 17 (SD of 2 m s⁻¹) participants per group (with >80% power) would be required to reveal significant differences that correspond with the smallest detectable change (1.9 m s⁻¹) in the PWV response.

A strength of this study is that we report the between-day reliability of resting and postexercise arterial stiffness indices in older healthy individuals, using an observer-independent portable device. Our study also included males and females within the target age range, which helps to generalize our findings to older healthy adults. There are also some limitations. Our results are not necessarily transferable to older individuals with cardiovascular disease, for which these measurements would be of particular interest. Furthermore, future studies may wish to confirm our findings using high-intensity or maximal exercise as this is associated with the greatest exercise-induced risk (Rognum et al., 2012). Nonetheless, moderate-intensity exercise, as used in this study, is recommended for older adults in line with current exercise guidelines for health in older adults (Garber et al., 2011).

Conclusion

This study established the reliability of arterial stiffness indices at rest, immediately after moderate-intensity cycling exercise and during supine recovery in healthy older adults. We demonstrated that postexercise measures of arterial stiffness are as reliable as measurements under resting conditions. These findings provide new information about the smallest detectable changes in arterial stiffness with exercise and the potential use of these measures for the assessment of cardiovascular risk. Further investigation of the physiological determinants and clinical significance of postexercise measures of arterial stiffness is warranted.

Acknowledgements

This research was supported by grants from the National Health and Medical Research Council (1000967, 1022752, 1079369), The Townsville Hospital and the Inflammation and Healing Research Cluster at the University of the Sunshine Coast. Professor Jonathan Goldacre’s work is supported by fellowships from the NHMRC (1117061) and the Queensland Government (Senior Clinical Research Fellowship).

Conflicts of interest

The authors declare no conflict of interest.

References


Butlin M, Queen A, Avolio AP. Estimation of central aortic pressure waveform features derived from the brachial cuff volume.


Effects of exercise intensity and cardiorespiratory fitness on the acute response of arterial stiffness to exercise in older adults

Maria Perissiou1 · Tom G. Bailey2 · Mark Windsor1 · Michael Chi Yuan Nam3 · Kim Greaves1,3 · Anthony S. Leicht4 · Jonathan Gollidge5,6 · Christopher D. Askew1,4

Received: 10 February 2018 / Accepted: 23 May 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract
Purpose Increased arterial stiffness is observed with ageing and in individuals with low cardiorespiratory fitness (\(\dot{V}O_{2\text{peak}}\)), and associated with cardiovascular risk. Following an exercise bout, transient arterial stiffness reductions offer short-term benefit, but may depend on exercise intensity. This study assessed the effects of exercise intensity on post-exercise arterial stiffness in older adults with varying fitness levels.

Methods Fifty-one older adults (72 ± 5 years) were stratified into fitness tertiles (\(\dot{V}O_{2\text{peak}}\): low-, 22.3 ± 3.1; mid-, 27.5 ± 2.4 and high-fit 36.3 ± 6.5 mL kg\(^{-1}\) min\(^{-1}\)). In a randomised order, participants underwent control (no-exercise), moderate-intensity continuous exercise (40% of peak power output; PPO), and higher-intensity interval exercise (70% of PPO) protocols. Pulse wave velocity (PWV), augmentation index (AIx75) and reflection magnitude (RM) were assessed at rest and during 90 min of recovery following each protocol.

Results After control, delta PWV increased over time (\(P < 0.001\)) and delta RM was unchanged. After higher-intensity interval exercise, delta PWV (\(P < 0.001\)) and delta RM (\(P < 0.001\)) were lower in all fitness groups. After moderate-intensity continuous exercise, delta PWV was not different from control in low-fit adults (\(P = 0.057\)), but was lower in the mid- and higher-fit older adults. Post-exercise AIx75 was higher to control in all fitness groups (\(P < 0.001\)).

Conclusions In older adults, PWV increases during seated rest and this response is attenuated after higher-intensity interval exercise, regardless of fitness level. This attenuation was also observed after moderate-intensity continuous exercise in adults with higher, but not lower fitness levels. Submaximal exercise reveals differences in the arterial stiffness responses between older adults with higher and lower cardiorespiratory fitness.

Keywords Pulse wave velocity · Wave reflection characteristics · Exercise intensity · Ageing

Abbreviations
AIx75 Augmentation index corrected for heart rate
\(\text{cDBP}\) Central diastolic blood pressure
\(\text{cPP}\) Central pulse pressure
\(\text{cSBP}\) Central systolic blood pressure
HR Heart rate
LMM Linear mixed model
MAP Mean arterial pressure
\(\text{Pb}\) Backward pressure wave
\(\text{ Pf}\) Forward pressure wave
PPO Peak power output
PWV Pulse wave velocity
RM Reflection magnitude

Communicated by Keith Phillip George.

1,4 Christopher D. Askew
caskew@nsc.edu.au

1,2 Vasomotor Research Group, School of Health and Sport Sciences, University of the Sunshine Coast, Locked bag 4, Maroochydore DC, Sunshine Coast, QLD, Australia
3 Sunshine Coast University Hospital, Sunshine Coast Hospital and Health Service, Birtinya, Sunshine Coast, Australia
4 Sport and Exercise Science, James Cook University, Townsville, QLD, Australia
5 Queensland Research Centre for Peripheral Vascular Disease, James Cook University, Townsville, QLD, Australia
6 Department of Vascular and Endovascular Surgery, The Townsville Hospital, Townsville, QLD, Australia

Published online: 30 May 2018
**Introduction**

Age-related arterial dysfunction is characterised by thickening of the artery wall (Tsao et al. 2014), a reduction in endothelial and autonomic function (Thijssen et al. 2016), and increased arterial stiffness. Elevated arterial stiffness is one of the earliest detectable manifestations of these adverse structural and functional changes to the vasculature, and contributes to the incidence of hypertension and the development of atherosclerosis (McEniery et al. 2005; Cecelja and Chowienczyk 2012). Carotid to femoral pulse wave velocity (PWV) is the criterion index representing central arterial stiffness (Van Bortel et al. 2012). Stiffening of the peripheral arteries also leads to an early return of the reflected pressure wave contributing to increases in central pulse pressure and arterial wall stress (Nichols et al. 2011). Wave reflection characteristics are represented by the augmentation index (Alx) and reflection magnitude (RM) (Nichols and Singh 2002; Westerhof et al. 2006). Increases in PWV, Alx and RM at rest are independently associated with the future risk of cardiovascular events and mortality (Mitchell et al. 2010; Chirinos et al. 2012); however, arterial stiffness is reduced with regular physical activity (Gando et al. 2016) and aerobic exercise training in older adults (Kim et al. 2016; Fujie et al. 2014, 2015).

The benefits of regular exercise on vascular structure and function are commonly attributed to the repeated, transient, hemodynamic perturbations observed in response to a single bout of exercise (Romero et al. 2017; Green and Smith 2017). Reductions in arterial stiffness are evident after a single bout of sub-maximal exercise in younger adults (Kingwell et al. 1997; Mutter et al. 2017), and are suggested to provide a window of benefit (Luttrell and Halliwill 2015), including a transient reduction in central blood pressure (Millen et al. 2016). On the other hand, pronounced post-exercise increases in central blood pressure and PWV have been reported in younger adults who are obese (Bunsawat et al. 2017) or have untreated hypertension (Gkaliagkousi et al. 2014). These increases in central blood pressure and PWV may be indicative of exercise-related risk in adults with established cardiovascular disease risk factors (Goodman et al. 2016).

While higher levels of cardiorespiratory fitness are associated with lower basal (i.e. resting) arterial stiffness in older adults (Gando et al. 2016), the influence of cardiorespiratory fitness on the post-exercise arterial stiffness response is not well defined. In younger adults, post-exercise PWV is elevated in those with lower as compared to those with higher cardiorespiratory fitness (Moore et al. 2016). With small increases in cardiorespiratory fitness with training, a transient post-exercise decrease in Alx, but not PWV, was reported in middle-aged post-menopausal females (Akazawa et al. 2015). To date, there have been no comparisons of the post-exercise arterial stiffness response between older adults with lower and higher levels of cardiorespiratory fitness.

Higher-intensity interval exercise is increasingly recommended for older adults and clinical populations as it enables individuals to exercise at an intensity that may not be sustained with continuous exercise (Francois and Little 2015). Acute higher-intensity interval exercise is reported to induce a greater reduction in PWV and Alx as compared to a bout of moderate-intensity exercise in younger adults (Tordi et al. 2010; Hanssen et al. 2015); however, the short-term responses of arterial stiffness to a single bout of exercise have not yet been clearly established in older adults. A better understanding of these responses, and how they are influenced by exercise intensity and cardiorespiratory fitness, would provide a greater insight into the potential risks and benefits of exercise among older adults. Therefore, this study aimed to compare the effect of moderate-intensity continuous and higher-intensity interval exercise on indices of post-exercise arterial stiffness, including PWV and wave reflection characteristics (Alx, and RM) between older adults with low, mid and higher levels of cardiorespiratory fitness.

**Methods**

**Experimental overview**

Participants underwent four laboratory visits, each following an overnight fast, refraining from alcohol and exercise for $24$ h, and caffeine for $12$ h, before each visit. Participants were required to consume a standardised meal consisting of oat biscuits ($20$ g carbohydrate, $8$ g fat) $3$ h prior to attending the laboratory. Visit 1 included an incremental maximal cycling test for the determination of cardiorespiratory fitness ($\dot{V}O_2$peak) and peak power output. Three experimental visits were then conducted in a cross-over, counter-balanced randomised order, and consisted of a no-exercise control, a moderate-intensity continuous and a higher-intensity interval exercise protocol. Arterial stiffness was measured at baseline and at multiple time points during $90$ min of supine recovery ($0$, $20$, $40$, $60$ and $90$ min). Lab conditions were standardised for each session in a climate controlled room ($23\pm1^\circ C$). To control for the diurnal variation of blood pressure and arterial stiffness, each individual performed the three visits at the same time of day, separated by $7$ days (Li et al. 2014).
Participants

Fifty-one healthy older males and females aged 71 ± 5 years were recruited through local advertisements. Participants were included if they were aged 60–86 years, able to complete cycling exercise and were non-smokers (> 12 months no smoking history). Participants were excluded if they had a known chronic metabolic or cardiovascular condition, or uncontrolled hypertension (average SBP ≥ 140 mmHg and/or an average DBP ≥ 90 mmHg). Participants were informed of the methods and study design verbally and in writing before providing written informed consent. The study conformed to the Declaration of Helsinki and was approved by the institutional ethics committees.

Maximal incremental cycling test

This test was performed on an electromagnetically braked cycle ergometer (Lode Corival, Groningen, Netherlands). Following a 3-min warm up (0 W), the test began at 20 W and then increased by 10 W each minute until participants reached their maximum load. Prior to the test, participants were fully familiarised with the test procedures and instructed to provide a maximum effort during the test. Participants self-selected a pedal cadence (> 60 rpm) and maintained this throughout the test. Expired gases were collected continuously and data were averaged every 15 s (Parvo Medics, UT, USA) for the determination of oxygen consumption (\(\dot{V}O_2\) \(\text{mL.kg}^{-1}\text{.min}^{-1}\)). Peak cardiopulmonary fitness was determined as the highest 15 s average of \(\dot{V}O_2\) over the last 60 s of maximal exercise (\(\dot{V}O_2\text{peak}\)). Heart rate was measured continuously using a 12-lead ECG (Mortara Inc., WI, USA) and recorded, along with perceived exertion (RPE) using the 0–10 Borg scale (Borg 1998), during the final 10 s of each stage. Peak power output (PPO) at the cessation of exercise was used to establish the exercise intensity in the subsequent experimental visits.

Exercise and control protocols

Following pre-test measurements of blood pressure and arterial stiffness, participants performed 24 min of: (1) moderate-intensity continuous cycling at 40% PPO; or (2) higher-intensity interval cycling consisting of 12 x 60 s bouts at 70% PPO, with each interval separated by 60 s at 10% PPO; or (3) no-exercise control (seated rest). Heart rate and RPE were recorded every 2 min during each protocol. Brachial blood pressure was measured every 6 min using a manual sphygmomanometer. Immediately following each protocol, participants moved back to the supine position for post-protocol measurements of arterial stiffness.

Arterial stiffness measurements

Indices of arterial stiffness (PWV, AIX and RM) were measured in the supine position using the SphygmoCor Xcel device (AtCor Medical, West Ryde, NSW, Australia), at baseline (after 15 min of quiet rest), and 0–90 min post-protocol. Participants were asked to remain quiet and still, before and during each measurement. Wave reflection characteristic measurements preceded PWV measurements, and these procedures are described in detail below.

Wave reflection characteristics: Brachial artery waveforms were recorded from the right upper arm using the SphygmoCor Xcel (AtCor Medical, Sydney, Australia) and following standard guidelines (Hwang et al. 2014). Following triplicate assessment of blood pressure, a corresponding aortic pressure waveform was generated by applying a proprietary digital signal processing and transfer function (Butlin et al. 2012), from which central systolic (cSBP), diastolic (cDBP), central pulse pressure (cPP), mean arterial pressure (MAP), augmentation pressure (AP), and AIXs, were derived. Central pulse pressure is calculated as the difference between cSBP and cDBP. Augmentation pressure is defined as the difference between cSBP and the pressure at the inflection point (the merging of forward and reflected waves). AIX is defined as the augmentation pressure expressed as a percentage of pulse pressure. As AIX is significantly affected by heart rate (Wilkinson et al. 2000), the index was corrected for a heart rate at 75 beats per minute (AIX75). Wave separation analysis was performed using SphygmoCor CVMS software (v.9). This method assumes a triangular-shaped flow wave approximated from the estimated aortic pressure wave (Westerhof et al. 2006). The forward (Pf) and reflected (Pb) pressure waves correspond to the peak and the end of the assumed flow wave, respectively. The reflection magnitude (RM) is calculated as the ratio of the Pb to the Pf (RM = Pb/Pf × 100).

Pulse wave velocity (PWV): To assess carotid-femoral PWV, pulse waveforms were recorded simultaneously from the right carotid artery using a hand-held high fidelity application tonometer, and the right femoral artery using a cuff placed at mid-thigh level. The exact placement of the cuff over the thigh and of the tonometer on the carotid artery was marked with an indelible waterproof skin marker and the distance between the carotid site and the femoral artery was measured and replicated for all repeated sessions. The distance from the in-guinal fold to the femoral cuff was standardised at 200 mm. After a stable carotid pulse signal was detected, the thigh cuff was inflated to 80 mmHg to obtain a concurrent femoral pulse waveform. Measurements were based on 10 s pulse wave traces that were free of artefact and met the quality control threshold of the SphygmoCor Xcel device for pulse-to-pulse variability. PWV was then
determined by calculating the ratio of the distance between the carotid and femoral arteries to the transit time; measured as the time delay between the arrival of the pulse wave at the common carotid artery and the common femoral artery (Wilkinson et al. 2010). The coefficient of variation for resting PWV between visits in this study was 6.9%, which is consistent with previous reports (Millasseau et al. 2005).

**Statistical analyses**

Based on previous literature (Doonan et al. 2013) who reported a post-exercise difference of the change in PWV of 1.2 ± 2.0 m s⁻¹ between males and females, our power calculation revealed that a cohort of 17 participants per group (assuming a between group post-exercise difference in PWV of 1.2 m s⁻¹ with a SD of 2 m s⁻¹ and > 80% power) would be required to reveal a significant difference in the post-exercise PWV response between fitness groups. To differentiate the cohort on the basis of cardiorespiratory fitness, participants were stratified into tertiles based on their VO₂peak. A Linear Mixed Model (LMM) was used to compare anthropometric characteristics and a Pearson’s chi squared test was used to compare categorical data between the three fitness groups. A two-way (group × protocol) LMM was used to compare baseline arterial stiffness indices [PWV (m s⁻¹), AIx 75 (%) and RM (%)] across the study visits. A three-way (group × protocol × time) LMM was used to compare measurements of arterial stiffness indices and central blood pressure among fitness groups (low-, mid- and high-fit groups); across “time” (baseline, 0-, 20-, 40-, 60-, and 90-min post) and between each protocol (control, moderate- and higher-intensity exercise). Data were also analysed as changes from baseline (delta) to account for any baseline variance. Three-way LMM analysis was also used to detect any differences in heart rate, blood pressure and perceived exertion in response to the protocols among the fitness groups, across time (at 2 min intervals for HR and RPE, and at 6 min intervals for BP) during each protocol. Statistically significant interactions were further investigated with multiple comparisons using the least significant difference approach (Rothman 1990). Analyses were conducted using the Statistical Package for Social Sciences (Version 22; IBM SPSS Inc., Chicago, IL). Statistical significance was set at P < 0.05 and exact P values are cited (P values of 0.000 are reported as < 0.001). Data are presented in the text as mean and 95% confidence interval (95% CI) unless otherwise stated.

**Results**

**Participants’ characteristics**

Participants’ characteristics of the complete cohort, and comparisons between the low, mid and high fitness groups, are shown in Table 1. Cardiorespiratory fitness, measured as VO₂peak, was higher in the high-fit group as compared to mid-fit (P < 0.001) and lower-fit groups (P < 0.001) (Table 1).

**Heart rate, mean arterial pressure and perceived exertion during the experimental protocols**

Mean power output (W) during exercise was greater in the higher-fit group [moderate-intensity continuous: mean = 80W, (95% CI 71–91); higher-intensity intervals: mean = 140W (95% CI 133–152)] as compared to the mid [moderate-intensity continuous: mean = 64W, (95% CI 48–74); higher-intensity intervals: mean = 112W (95% CI 91–123), P < 0.001] and low-fit group [moderate-intensity continuous: mean = 52W, (95% CI 45–64); higher-intensity intervals: mean = 91W (95% CI 82–103), P < 0.001]. No differences were observed among the three fitness groups during exercise for mean heart rate (P = 0.12), mean arterial pressure (P = 0.94) and RPE (P = 0.29). Mean heart rate was greater during higher-intensity interval exercise [mean heart rate 109 b·min⁻¹ (95% CI 98–115)] as compared to moderate-intensity continuous exercise [mean heart rate 91 b·min⁻¹ (95% CI 78–100, P < 0.001)], and was higher during both exercise protocols as compared to control (P < 0.05). MAP was greater during higher-intensity interval exercise [mean MAP 108 mmHg (95% CI 98–112)] as compared to moderate-intensity continuous exercise [mean MAP 93 mmHg (95% CI 89–106), P = 0.002] and was also higher during both exercise protocols than during control (P < 0.05). Mean RPE was 4 (95% CI 3–4) during higher-intensity interval exercise as compared to 3 (95% CI 2–3, P < 0.001) during moderate-intensity continuous exercise.

**Arterial stiffness and central blood pressure indices at baseline and in response to exercise**

Arterial stiffness and central blood pressure indices at baseline and during recovery (0–90 min post) after exercise/control protocols for the three fitness groups (low-, mid-, and high-fit) are shown in Tables 2 and 3. The relative change (delta) from baseline in PWV, AIx 75 and RM among the three fitness groups for each protocol are shown in Figs. 1, 2 and 3. Findings are summarised below.

Arterial stiffness indices at baseline were similar across the three separate testing days (pre-exercise/control). AIx 75 at baseline was 9.4% (95% CI 2.3–16, P = 0.10) higher in the lower-fit as compared to the higher-fit group (Table 2). There were no differences in PWV and RM between fitness groups.

We did not observe a three-way (protocol × group × time) interaction for any of the arterial stiffness and central blood pressure indices (Tables 2, 3). In control, PWV increased from baseline at all time-points, whereas after the exercise
Table 1 Participants' characteristics

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Low fit</th>
<th>Mid fit</th>
<th>High fit</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 51)</td>
<td>(n = 17)</td>
<td>(n = 17)</td>
<td>(n = 17)</td>
<td></td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>85</td>
<td>83</td>
<td>85</td>
<td>95</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>72 ± 5</td>
<td>74 ± 5</td>
<td>69 ± 4</td>
<td>70 ± 6</td>
<td>0.801</td>
</tr>
<tr>
<td>Hypertensive (%)</td>
<td>33</td>
<td>30</td>
<td>30</td>
<td>35</td>
<td>0.913</td>
</tr>
<tr>
<td>Anthropometric measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174 ± 8</td>
<td>173 ± 8</td>
<td>172 ± 7</td>
<td>176 ± 9</td>
<td>0.223</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 ± 12</td>
<td>75 ± 12</td>
<td>79 ± 13</td>
<td>76 ± 10</td>
<td>0.545</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>25 ± 3</td>
<td>25 ± 3</td>
<td>26 ± 4</td>
<td>25 ± 3</td>
<td>0.426</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>28 ± 8</td>
<td>27 ± 8</td>
<td>27 ± 5</td>
<td>22 ± 5</td>
<td>0.917</td>
</tr>
<tr>
<td>Waist:Hip ratio</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.738</td>
</tr>
<tr>
<td>Resting heart rate and blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>56 ± 8</td>
<td>59 ± 8</td>
<td>57 ± 6</td>
<td>51 ± 6</td>
<td>0.100</td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>131 ± 18</td>
<td>122 ± 14</td>
<td>124 ± 10</td>
<td>127 ± 10</td>
<td>0.471</td>
</tr>
<tr>
<td>Brachial DBP (mmHg)</td>
<td>76 ± 9</td>
<td>72 ± 9</td>
<td>72 ± 7</td>
<td>73 ± 6</td>
<td>0.682</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>115 ± 6</td>
<td>113 ± 13</td>
<td>114 ± 9</td>
<td>119 ± 7</td>
<td>0.185</td>
</tr>
<tr>
<td>Central DBP (mmHg)</td>
<td>73 ± 7</td>
<td>73 ± 9</td>
<td>73 ± 6</td>
<td>74 ± 6</td>
<td>0.530</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>42 ± 8</td>
<td>40 ± 7</td>
<td>42 ± 7</td>
<td>46 ± 9</td>
<td>0.162</td>
</tr>
<tr>
<td>Medication use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARB/ACE inhibitors (%)</td>
<td>22</td>
<td>12</td>
<td>6</td>
<td>24</td>
<td>0.314</td>
</tr>
<tr>
<td>Antiplaetlets (%)</td>
<td>9</td>
<td>18</td>
<td>6</td>
<td>6</td>
<td>0.412</td>
</tr>
<tr>
<td>Beta-blockers (%)</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0.125</td>
</tr>
<tr>
<td>Calcium channel blockers (%)</td>
<td>10</td>
<td>6</td>
<td>12</td>
<td>6</td>
<td>0.762</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>27</td>
<td>30</td>
<td>35</td>
<td>18</td>
<td>0.662</td>
</tr>
<tr>
<td>Maximal incremental cycling test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂ peak (L min⁻¹)</td>
<td>2.2 ± 0.6</td>
<td>1.7 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>2.8 ± 0.6</td>
<td>0.001</td>
</tr>
<tr>
<td>VO₂ peak (mL kg⁻¹ min⁻¹)</td>
<td>28.9 ± 7</td>
<td>22.3 ± 3</td>
<td>27.5 ± 2</td>
<td>36.3 ± 8</td>
<td>0.001</td>
</tr>
<tr>
<td>Peak heart rate (bpm)</td>
<td>151 ± 15</td>
<td>142 ± 15</td>
<td>157 ± 14</td>
<td>156 ± 10</td>
<td>0.010</td>
</tr>
<tr>
<td>Peak RER (Al)</td>
<td>1.17 ± 0.1</td>
<td>1.23 ± 0.1</td>
<td>1.13 ± 0.1</td>
<td>1.14 ± 0.09</td>
<td>0.020</td>
</tr>
<tr>
<td>Peak power ( Watts)</td>
<td>163 ± 40</td>
<td>130 ± 27</td>
<td>160 ± 27</td>
<td>198 ± 34</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD or frequency (%). P < 0.05 indicates significant difference between fitness groups. Heart rate and blood pressure data are an average of the resting measures collected on the three experimental visits.

Protocols PWV increased from baseline at 90 min only (Table 2). A group x protocol interaction (P < 0.001) revealed that delta PWV was lower after both exercise protocols in the mid- and high-fit groups, as compared with delta PWV in control (Fig. 1). Delta PWV was lower after higher-intensity interval exercise as compared to moderate-intensity continuous exercise in the mid- and low-fit groups (Fig. 1b, c). Conversely, delta PWV was similar after moderate-intensity continuous exercise as compared to control in the low-fit group, but was significantly lower after higher-intensity interval exercise as compared to control (Fig. 1). A1x75 decreased and remained below baseline for 90 min after control; and A1x75 was also significantly lower than baseline 40 to 90 min after both exercise protocols (Table 2). Delta A1x75 was significantly lower after control as compared to both exercise protocols in all fitness groups (Fig. 2). RM was unchanged after the control protocol, and decreased to below baseline levels for 20 min after moderate-intensity continuous exercise and for 90 min after higher-intensity interval exercise in all fitness groups (Table 2). A protocol effect (P < 0.001) revealed that delta RM was significantly lower after both exercise protocols as compared with control, in all the fitness groups (Fig. 3). Further, a group x protocol interaction (P = 0.009) revealed that delta RM was lower after higher-intensity interval exercise as compared to moderate-intensity continuous exercise in the mid- and low-fit groups, but not in the high-fit group (Fig. 3b, c).
<table>
<thead>
<tr>
<th>Fitness groups</th>
<th>Protocol</th>
<th>Time point (min)</th>
<th>P values</th>
<th>Time</th>
<th>Protocol</th>
<th>Group</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0 post</td>
<td>20 post</td>
<td>40 post</td>
<td>60 post</td>
<td>90 post</td>
</tr>
<tr>
<td>PWV (m s$^{-1}$)</td>
<td>Low</td>
<td>CON</td>
<td>$11.1 \pm 2.2$</td>
<td>$12.0 \pm 2.1$</td>
<td>$12.2 \pm 2.5$</td>
<td>$12.2 \pm 2.0$</td>
<td>$12.0 \pm 2.0$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>$11.5 \pm 2.0$</td>
<td>$12.1 \pm 2.2$</td>
<td>$11.9 \pm 2.0$</td>
<td>$12.2 \pm 2.4$</td>
<td>$12.0 \pm 2.0$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>$12.3 \pm 2.3$</td>
<td>$12.1 \pm 2.5$</td>
<td>$11.9 \pm 2.1$</td>
<td>$12.3 \pm 1.9$</td>
<td>$12.5 \pm 1.9$</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>CON</td>
<td>$11.2 \pm 2.0$</td>
<td>$11.9 \pm 2.0$</td>
<td>$12.0 \pm 2.3$</td>
<td>$12.0 \pm 2.4$</td>
<td>$12.5 \pm 2.2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>$11.3 \pm 2.1$</td>
<td>$12.0 \pm 2.0$</td>
<td>$11.7 \pm 2.0$</td>
<td>$11.6 \pm 2.1$</td>
<td>$11.8 \pm 2.1$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>$11.4 \pm 1.9$</td>
<td>$11.9 \pm 1.8$</td>
<td>$11.7 \pm 2.1$</td>
<td>$11.6 \pm 2.1$</td>
<td>$11.6 \pm 1.9$</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>CON</td>
<td>$11.2 \pm 1.4$</td>
<td>$12.0 \pm 1.9$</td>
<td>$11.6 \pm 1.8$</td>
<td>$11.8 \pm 1.8$</td>
<td>$11.9 \pm 1.9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>$11.3 \pm 1.9$</td>
<td>$11.7 \pm 1.8$</td>
<td>$11.3 \pm 1.6$</td>
<td>$11.3 \pm 1.7$</td>
<td>$11.5 \pm 1.9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>$11.3 \pm 2.0$</td>
<td>$11.5 \pm 1.8$</td>
<td>$11.5 \pm 1.9$</td>
<td>$11.5 \pm 1.9$</td>
<td>$11.5 \pm 1.9$</td>
</tr>
<tr>
<td>AIX75 (%)</td>
<td>Low</td>
<td>CON</td>
<td>$24.7 \pm 1.1$</td>
<td>$22.6 \pm 0.9$</td>
<td>$23.6 \pm 1.0$</td>
<td>$21.5 \pm 1.2$</td>
<td>$21.2 \pm 1.3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>$26.5 \pm 1.1$</td>
<td>$22.5 \pm 1.1$</td>
<td>$28.0 \pm 1.1$</td>
<td>$23.0 \pm 1.1$</td>
<td>$22.5 \pm 1.3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>$24.9 \pm 1.1$</td>
<td>$24.9 \pm 1.1$</td>
<td>$24.9 \pm 1.1$</td>
<td>$24.2 \pm 1.2$</td>
<td>$23.0 \pm 1.3$</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>CON</td>
<td>$23.8 \pm 0.9$</td>
<td>$17.0 \pm 0.7$</td>
<td>$18.2 \pm 0.6$</td>
<td>$15.4 \pm 0.8$</td>
<td>$15.8 \pm 0.8$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>$22.7 \pm 0.9$</td>
<td>$21.5 \pm 0.9$</td>
<td>$24.3 \pm 0.9$</td>
<td>$19.0 \pm 0.8$</td>
<td>$18.8 \pm 0.9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>$25.0 \pm 0.9$</td>
<td>$23.2 \pm 0.9$</td>
<td>$25.0 \pm 1.9$</td>
<td>$18.2 \pm 0.9$</td>
<td>$18.8 \pm 0.8$</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>CON</td>
<td>$14.7 \pm 1.0$</td>
<td>$10.7 \pm 0.8$</td>
<td>$13.4 \pm 1.0$</td>
<td>$8.9 \pm 0.9$</td>
<td>$10.0 \pm 0.9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>$15.5 \pm 0.8$</td>
<td>$15.5 \pm 0.8$</td>
<td>$18.3 \pm 0.6$</td>
<td>$13.1 \pm 0.6$</td>
<td>$11.2 \pm 0.9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>$16.3 \pm 0.7$</td>
<td>$17.8 \pm 0.8$</td>
<td>$18.3 \pm 0.8$</td>
<td>$14.7 \pm 0.9$</td>
<td>$12.5 \pm 0.7$</td>
</tr>
<tr>
<td>RM (%)</td>
<td>Low</td>
<td>CON</td>
<td>$73.0 \pm 16$</td>
<td>$77.7 \pm 12$</td>
<td>$79.0 \pm 13$</td>
<td>$78.9 \pm 13$</td>
<td>$82.0 \pm 13$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>$80.4 \pm 13$</td>
<td>$62.0 \pm 13$</td>
<td>$74.9 \pm 10$</td>
<td>$77.6 \pm 13$</td>
<td>$77.9 \pm 14$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>$82.7 \pm 13$</td>
<td>$57.3 \pm 14$</td>
<td>$79.7 \pm 15$</td>
<td>$79.4 \pm 9$</td>
<td>$76.5 \pm 12$</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>CON</td>
<td>$76.2 \pm 11$</td>
<td>$71.7 \pm 10$</td>
<td>$78.7 \pm 13$</td>
<td>$77.2 \pm 12$</td>
<td>$76.8 \pm 14$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>$75.0 \pm 10$</td>
<td>$63.2 \pm 11$</td>
<td>$70.6 \pm 9$</td>
<td>$72.8 \pm 10$</td>
<td>$74.4 \pm 10$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>$78.0 \pm 9$</td>
<td>$63.0 \pm 9$</td>
<td>$69.3 \pm 12$</td>
<td>$69.3 \pm 12$</td>
<td>$70.9 \pm 12$</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>CON</td>
<td>$78.5 \pm 13$</td>
<td>$73.9 \pm 11$</td>
<td>$74.3 \pm 10$</td>
<td>$71.8 \pm 11$</td>
<td>$73.3 \pm 13$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>$77.1 \pm 12$</td>
<td>$70.7 \pm 11$</td>
<td>$75.3 \pm 11$</td>
<td>$78.0 \pm 11$</td>
<td>$73.4 \pm 13$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>$79.8 \pm 10$</td>
<td>$65.0 \pm 12$</td>
<td>$78.9 \pm 12$</td>
<td>$75.1 \pm 10$</td>
<td>$75.6 \pm 10$</td>
</tr>
<tr>
<td>Fitness groups</td>
<td>Protocol</td>
<td>Time point (min)</td>
<td>P values</td>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>----------</td>
<td>-----------------</td>
<td>----------</td>
<td>--------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0 post</td>
<td>20 post</td>
<td>40 post</td>
<td>60 post</td>
<td>90 post</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF (mmHg)</td>
<td>Low</td>
<td>CON</td>
<td>23.4 ± 5.1</td>
<td>24.6 ± 5.8 *</td>
<td>23.6 ± 4.7</td>
<td>22.5 ± 5.0</td>
<td>23.3 ± 4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>23.6 ± 6.2</td>
<td>30.0 ± 7.9 *</td>
<td>24.8 ± 6.3</td>
<td>23.7 ± 6.5</td>
<td>23.9 ± 5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>22.1 ± 4.6</td>
<td>29.9 ± 6.2 *</td>
<td>24.4 ± 5.8</td>
<td>22.8 ± 4.8</td>
<td>23.8 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>CON</td>
<td>25.5 ± 4.8</td>
<td>26.1 ± 6.0 *</td>
<td>23.8 ± 5.9</td>
<td>24.5 ± 6.5</td>
<td>24.7 ± 6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>24.3 ± 4.4</td>
<td>30.6 ± 7.0 *</td>
<td>25.2 ± 5.6</td>
<td>23.9 ± 5.5</td>
<td>24.8 ± 6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>23.9 ± 4.6</td>
<td>30.4 ± 7.0 *</td>
<td>24.9 ± 5.4</td>
<td>24.2 ± 6.0</td>
<td>23.8 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>CON</td>
<td>22.8 ± 5.5</td>
<td>25.1 ± 4.8 *</td>
<td>25.1 ± 4.9</td>
<td>24.5 ± 4.7</td>
<td>24.5 ± 4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>24.3 ± 5.1</td>
<td>28.1 ± 4.4 *</td>
<td>24.5 ± 5.5</td>
<td>22.3 ± 4.9</td>
<td>22.9 ± 4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>23.8 ± 5.0</td>
<td>30.1 ± 6.2 *</td>
<td>22.9 ± 4.6</td>
<td>22.9 ± 5.2</td>
<td>22.2 ± 4.4</td>
</tr>
<tr>
<td>Pb (mmHg)</td>
<td>Low</td>
<td>CON</td>
<td>16.7 ± 3.1</td>
<td>18.9 ± 4.1 *</td>
<td>18.6 ± 4.6</td>
<td>17.6 ± 3.3</td>
<td>19.0 ± 4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>18.6 ± 3.6</td>
<td>18.2 ± 3.2</td>
<td>18.3 ± 3.3</td>
<td>18.1 ± 3.5</td>
<td>18.4 ± 3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>18.2 ± 3.9</td>
<td>16.8 ± 3.0 *</td>
<td>16.9 ± 2.6</td>
<td>17.4 ± 3.3</td>
<td>18.1 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>CON</td>
<td>19.4 ± 3.3</td>
<td>18.5 ± 3.3 *</td>
<td>18.4 ± 3.5</td>
<td>18.1 ± 2.9</td>
<td>18.6 ± 3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>18.0 ± 2.8</td>
<td>19.2 ± 4.2</td>
<td>17.8 ± 3.9</td>
<td>17.2 ± 3.4</td>
<td>18.3 ± 4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>18.5 ± 2.4</td>
<td>18.5 ± 4.6 *</td>
<td>17.1 ± 3.1</td>
<td>16.6 ± 2.8</td>
<td>16.7 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>CON</td>
<td>17.8 ± 4.1</td>
<td>18.7 ± 4.4 *</td>
<td>18.8 ± 4.2</td>
<td>17.7 ± 3.9</td>
<td>18.0 ± 3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>18.7 ± 4.2</td>
<td>19.9 ± 3.9</td>
<td>18.2 ± 3.2 *</td>
<td>17.3 ± 3.2</td>
<td>16.6 ± 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>19.0 ± 3.8</td>
<td>19.4 ± 3.9 *</td>
<td>18.0 ± 3.9</td>
<td>17.1 ± 3.3</td>
<td>16.8 ± 3.4 *</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
CON no-exercise control, MOD moderate-intensity exercise, HIGH higher-intensity exercise, Aih75 augmentation index normalised to a heart rate of 75 bpm, RM reflection magnitude, Pf forward pressure wave, Pb backward pressure wave, PWV pulse wave velocity.

*Significantly different from baseline based on protocol × time post-hoc comparisons.
<table>
<thead>
<tr>
<th>Fitness groups</th>
<th>Protocol</th>
<th>Time point (min)</th>
<th>( P ) value</th>
<th>Time</th>
<th>Protocol</th>
<th>Group</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0 post</td>
<td>20 post</td>
<td>40 post</td>
<td>60 post</td>
<td>90 post</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>Low</td>
<td>CON</td>
<td>60 ±10</td>
<td>56 ±9°</td>
<td>54 ±8°</td>
<td>54 ±8°</td>
<td>56 ±7°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>59 ±7</td>
<td>67 ±9</td>
<td>62 ±8</td>
<td>59 ±7</td>
<td>59 ±7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>58 ±6</td>
<td>71 ±12°</td>
<td>63 ±10°</td>
<td>61 ±9</td>
<td>59 ±8</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>CON</td>
<td>58 ±6</td>
<td>54 ±6°</td>
<td>52 ±4°</td>
<td>51 ±4°</td>
<td>53 ±5°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>56 ±6</td>
<td>67 ±8°</td>
<td>62 ±7°</td>
<td>57 ±6</td>
<td>57 ±5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>56 ±6</td>
<td>67 ±8°</td>
<td>62 ±7°</td>
<td>57 ±6</td>
<td>57 ±5</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>CON</td>
<td>51 ±7</td>
<td>46 ±5°</td>
<td>47 ±6°</td>
<td>46 ±6°</td>
<td>49 ±8°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>52 ±6</td>
<td>59 ±7°</td>
<td>55 ±8°</td>
<td>53 ±6</td>
<td>52 ±5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>51 ±6</td>
<td>64 ±7°</td>
<td>59 ±7°</td>
<td>56 ±7°</td>
<td>53 ±6</td>
</tr>
<tr>
<td>cSBP (mmHg)</td>
<td>Low</td>
<td>CON</td>
<td>111 ±14</td>
<td>118 ±14°</td>
<td>116 ±14°</td>
<td>116 ±13°</td>
<td>117 ±14°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>115 ±15</td>
<td>117 ±13°</td>
<td>112 ±13°</td>
<td>113 ±14°</td>
<td>115 ±12°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>114 ±13</td>
<td>117 ±15°</td>
<td>102 ±12°</td>
<td>110 ±12°</td>
<td>114 ±12°</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>CON</td>
<td>118 ±9</td>
<td>122 ±12°</td>
<td>120 ±12°</td>
<td>121 ±13°</td>
<td>121 ±13°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>114 ±10</td>
<td>121 ±11°</td>
<td>113 ±10°</td>
<td>113 ±10°</td>
<td>117 ±13°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>114 ±9</td>
<td>118 ±12°</td>
<td>113 ±11°</td>
<td>113 ±13°</td>
<td>115 ±12°</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>CON</td>
<td>117 ±11</td>
<td>122 ±11°</td>
<td>122 ±10°</td>
<td>120 ±9°</td>
<td>120 ±11°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>119 ±11</td>
<td>123 ±9°</td>
<td>116 ±8</td>
<td>113 ±10°</td>
<td>114 ±11°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>117 ±9</td>
<td>123 ±7°</td>
<td>113 ±8°</td>
<td>114 ±9°</td>
<td>114 ±10</td>
</tr>
<tr>
<td>cDBP (mmHg)</td>
<td>Low</td>
<td>CON</td>
<td>72 ±8</td>
<td>75 ±8°</td>
<td>73 ±9°</td>
<td>75 ±9°</td>
<td>75 ±8°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>73 ±10</td>
<td>75 ±9°</td>
<td>72 ±10</td>
<td>72 ±10</td>
<td>74 ±12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>73 ±10</td>
<td>77 ±12°</td>
<td>71 ±9</td>
<td>72 ±9</td>
<td>73 ±9</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>CON</td>
<td>74 ±7</td>
<td>77 ±7°</td>
<td>76 ±8°</td>
<td>76 ±8°</td>
<td>78 ±9°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>72 ±6</td>
<td>77 ±8°</td>
<td>73 ±7</td>
<td>74 ±8</td>
<td>75 ±9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>74 ±7</td>
<td>76 ±7°</td>
<td>74 ±8</td>
<td>74 ±9</td>
<td>76 ±10</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>CON</td>
<td>73 ±7</td>
<td>74 ±7°</td>
<td>75 ±7°</td>
<td>74 ±7°</td>
<td>74 ±7°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOD</td>
<td>75 ±6</td>
<td>77 ±7°</td>
<td>74 ±7</td>
<td>73 ±7</td>
<td>73 ±7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIGH</td>
<td>74 ±8</td>
<td>78 ±8°</td>
<td>74 ±7</td>
<td>75 ±9</td>
<td>74 ±8</td>
</tr>
</tbody>
</table>
After control, heart rate decreased below baseline for 90 min in all fitness groups. After both exercise protocols heart rate increased above baseline levels in all fitness groups, before returning to baseline by 60 min post (Table 3). In all fitness groups, cSBP, cDBP and cPP increased above baseline for 90 min after control. After both exercise protocols in all fitness groups, cPP increased immediately after exercise (0 min post), and decreased to below baseline levels for up to 60 min post-exercise (Table 3).

**Discussion**

This study demonstrates that the post-exercise arterial stiffness response is dependent on both the intensity of exercise and the level of cardiorespiratory fitness in healthy older adults. We observed significant increases in PWV following seated rest (control), with negligible changes in RM. Following higher-intensity interval exercise, PWV and RM were lower as compared to control in older adults of all fitness levels. Following moderate-intensity continuous exercise, this relative attenuation in PWV as compared to control was also achieved in the mid- and higher-fit older adults, but not in older adults with low cardiorespiratory fitness.

**Arterial stiffness at rest**

It has previously been suggested that high levels of cardiorespiratory fitness suppress the age-related increases in resting arterial stiffness (Gando et al. 2016). In the current cohort of older adults, we found that resting AIx75 was lowest in those with the highest cardiorespiratory fitness, which is consistent with previous reports (Ramos et al. 2016; Binder et al. 2006; Denham et al. 2016). In contrast, we did not observe differences in the resting measures of PWV between the fitness groups. There are limited studies of the effect of fitness on PWV in older adults, showing that PWV is lower in master athletes (Vaitkevicius et al. 1993) and high-fit post-menopausal females (Gando et al. 2010) when compared to low-fit control groups. In a group of middle-aged adults, with and without the metabolic syndrome, Jae et al. (2010) reported that increases in fitness were associated with reductions in the brachial-to-ankle PWV (Jae et al. 2010). This fitness effect was not seen in a similar cohort when PWV was assessed at the carotid-to-femoral segment (Ramos et al. 2016), which is consistent with our finding in older adults.

The conflicting effects of fitness on resting measures of AIx75 and PWV highlight important differences between the determinants of these indices of arterial stiffness. There is a progressive stiffening of the arterial tree from the ascending aorta and large elastic arteries towards the peripheral muscular conduit arteries (London and Pannier...
Fig. 1. Delta PWV response to control (a), moderate-intensity continuous (b) and higher-intensity interval (c). Delta PWV response between low-fit (circles), mid-fit (squares) and high-fit (triangles). Symbols and error bars represent mean ± SEM; PWV, pulse wave velocity. *Significantly different from control; †higher-intensity protocol significantly different from moderate-intensity protocol.

Carotid-to-femoral PWV quantifies the velocity of the pulse wave through the aorta, which undergoes the greatest stiffening with age (Mitchell et al. 2004). Augmentation index (AIX75) characterizes the magnitude of the reflected pressure wave as it contributes to systolic blood pressure, and has therefore been reported to reflect peripheral arterial stiffness (Nichols 2005). Recent studies have challenged this view, suggesting that augmentation index is largely determined by aortic reservoir pressure (Wang et al. 2003). Aortic reservoir pressure increases with age, probably as a result of impaired aortic compliance and the limited ability to buffer increases in aortic blood volume during cardiac ejection (Davies et al. 2010).

Aortic reservoir pressure has recently been shown to be lower in middle-aged adults with higher cardiorespiratory fitness (Ramos et al. 2016), and therefore may contribute to the lower AIX75 of the higher-fit older adults in the present study. Reflected pressure from the peripheral arteries is reported to be better represented by the reflection magnitude (RM, ratio of forward to backward pressure waves) (Hughes et al. 2013; Millen et al. 2016). Our finding that RM did not differ between the fitness groups further supports the interpretation that differences in resting AIX75 between fitness groups are not likely to be caused by alterations in peripheral reflected wave function.
**Post-exercise arterial stiffness**

During recovery from a bout of aerobic exercise, indices of arterial stiffness have been reported to increase (Gkalingkousi et al. 2014; Hull et al. 2011), decrease (Lane et al. 2013; Heffernan et al. 2007a; Kingwell et al. 1997), or remain unchanged (McClean et al. 2011; Akazawa et al. 2015) as compared with baseline levels. This variance in the post-exercise arterial stiffness response is suggested to be due to the anatomical segment being assessed, the frequency of measures, as well as the age and health status of study participants (Mutter et al. 2017). In the current cohort of healthy older adults, we observed decreases in wave reflection characteristics following exercise, but negligible changes in carotid-femoral PWV. Carotid-femoral PWV has been shown to be less susceptible to acute post-exercise changes when compared with peripheral PWV, i.e. changes in femoral-dorsalis pedis PWV (Heffernan et al. 2007b; Munir et al. 2008). The RM is reported to represent the reflected pressure from the peripheral arteries (Hughes et al. 2013; Millen et al. 2016), which is dependent on changes in downstream arterial function, including any changes as a result of local muscle activity (Hickson et al. 2016). Hence, it is likely that the reductions in wave reflection observed in the present study relate to peripheral vasodilation in the lower limbs during the period immediately following exercise.

We included a no-exercise control condition, which is a strength of the study design and allowed us to control for the influence of time and supine rest on changes in arterial stiffness. Indeed, we observed a progressive rise in PWV, which may reflect an increase in vascular tone (Fok et al. 2012), and a reduction in AIx75, which may be due...
to a redistribution of blood volume and an altered cardiac preload (van de Velde et al. 2017) during the supine control condition. Compared to control, post-exercise PWV and RM were lower following a bout of higher-intensity interval exercise, while post-exercise AIX75 was higher as compared with control. These findings challenge previous studies that did not use a time-adjusted control comparison and reported post-exercise reductions in AIX75 (Hanssen et al. 2015; Lane et al. 2013; Radhakrishnan et al. 2016; Millen et al. 2016). Our finding of a higher AIX75 after exercise as compared to after a period of rest further supports the notion that the augmentation index is largely determined by aortic reservoir pressure, as discussed earlier, which increases with exercise (Climie et al. 2015). McClean et al. (2011) previously observed no differences in post-exercise PWV as compared to control in younger adults; however, the authors only measured PWV immediately following exercise (McClean et al. 2011). Similar to our findings, Wang et al. (2014) reported a lower arterial stiffness (caro-ankle vascular index; CAVI) after both continuous and interval moderate-intensity exercise as compared to control when measured during 40 min of recovery (Wang et al. 2014), although this study also observed a decrease in arterial stiffness from baseline after exercise. This is the first study in healthy older adults to report a lower post-exercise arterial stiffness, where the response is attenuated as compared with control, (PWV and RM) following a bout of higher-intensity exercise.

Compared with higher-intensity interval exercise, the response of post-exercise PWV and RM to moderate-intensity continuous exercise was less pronounced in both the low- and mid-fit groups. This is consistent with the augmented effect of higher-intensity as compared to moderate-intensity exercise on lowering post-exercise arterial stiffness in some (Tordi et al. 2010; Hanssen et al. 2015) but not in

Fig. 3 Delta RM response to control (a), moderate-intensity continuous (b) and higher-intensity interval (c). Delta RM response between low-fit (circles), mid-fit (squares) and high-fit (triangles). Symbols and error bars represent mean ± SEM; RM, reflection magnitude. *Significantly different from control; †higher-intensity protocol significantly different from moderate-intensity protocol.
all studies (Siassos et al. 2016) in younger adults. Intensity-dependent changes in post-exercise arterial stiffness are complex, and may be due to increases in vessel tone and reductions in central pressure following higher intensity exercise (Millen et al. 2016). We observed transient reductions in cPP following exercise in this study, and whilst this has previously been associated with corresponding changes in PWV (Lim et al. 2016), we did not observe an intensity (protocol) effect for cPP and thus it is unlikely to explain our findings. Blood flow and shear stress are augmented with increased exercise intensity (Santana et al. 2013; Bond et al. 2015) and may partly explain the intensity effect observed in post-exercise PWV and RM. Shear-mediated increases in endothelial function (Muir et al. 2008) and the activation of vasodilators e.g. nitric oxide and prostaglandins (Poveda et al. 1998), and inhibition of vasoconstrictors e.g. endothelia-1 expression (Di Francescomarino et al. 2009) have all been associated with a transient lowering of arterial stiffness after exercise. Further, exercise-induced suppression of pro-inflammatory cytokines (Jablonski et al. 2015) and intensity-dependent decreases in post-exercise sympathetic nerve activity (Heffner et al. 2007a) may also contribute to a lower arterial stiffness following higher-intensity exercise.

Post-exercise arterial stiffness has previously been reported to be lower in younger adults with high, compared with low, levels of cardiorespiratory fitness (Moore et al. 2016). A similar trend was observed in the current study following moderate-intensity exercise, where the response of the low-fit group (delta PWV) did not differ to the rises observed during control, whereas participants in the high-fit group demonstrated an attenuation of the PWV response after both exercise intensities as compared to control. This suggests that higher levels of cardiorespiratory fitness may improve the sensitivity or responsiveness of the vasculature to submaximal exercise in older adults, and this may relate to the improved endothelial function and vascular tone seen with training and increased fitness in older adults (DeSouza et al. 2000; Pialoux et al. 2009). It is also possible that the absolute exercise intensity, which was greatest in the high-fit group, may have generated a greater stimulus for post-exercise changes in arterial stiffness and contributed to our observations. However, it is important to note that there were no differences in heart rate, blood pressure or perceived exertion between the groups during exercise.

We found significant differences in post-exercise PWV and RM between the fitness groups, despite there being no difference in the resting measures. These findings support the recent suggestion that subtle underlying differences in arterial stiffness that are undetectable at rest become more pronounced with acute exercise (Keith et al. 2013; Schultz et al. 2017). It has also been reported that the arterial stiffness response to maximal exercise may be a useful tool for detecting small, but clinically relevant changes in vascular health (Bunsawat et al. 2017; Shim et al. 2011) and we also now show that submaximal exercise may reveal important differences in arterial stiffness between adults with higher and lower levels of cardiorespiratory fitness.

As increased PWV and RM during seated rest may reflect increased cardiovascular risk (Mitchell et al. 2010; Chirinos et al. 2012), the attenuation of arterial stiffness with exercise may reflect a transient cardiovascular benefit, or a reduction in risk (Millen et al. 2016). Similar to the relationship between acute and chronic blood pressure responses to exercise, (Kiviniemi et al. 2014), the lowering of arterial stiffness may be additive and contribute to the longer term reductions in resting arterial stiffness after exercise training in older adults (Kim et al. 2016). Whether the acute influence of exercise intensity or cardiorespiratory fitness seen in the present study has any implications for the effect of exercise training on arterial stiffness in older adults remains to be determined.

Limitations

This study included healthy older male and female adults, and we acknowledge that sex-related differences in arterial stiffness (Coutinho et al. 2013) may limit some comparisons. However, it should be noted that the proportion of males and females was similar in each fitness group in this study, and thus is unlikely to have influenced our findings. The inclusion of participants with controlled hypertension (n = 16) may have also influenced our findings, but again the proportion of those with hypertension was similar between groups (30–35%). Anti-hypertensive medication has been reported to lower PWV at rest (Mahmud and Feeley 2008), and may have confounded the differences in arterial stiffness across fitness groups in this study. Finally, we did not include measures of potential mechanisms involved in the changes in arterial stiffness with exercise, and this should be the focus of future research.

Conclusions

In conclusion, the present study suggests that the post-exercise arterial stiffness response is dependent on both the intensity of exercise and the level of cardiorespiratory fitness in healthy older adults. PWV increases during prolonged seated rest in older adults. Moderate-intensity continuous exercise has a positive attenuation on PWV as compared with seated-rest control in those with higher, but not lower, levels of fitness. PWV and RM are lower after higher-intensity interval exercise as compared with control in older adults of all levels of cardiorespiratory fitness. Submaximal exercise reveals differences in arterial stiffness responses
between adults with higher and lower levels of cardiorespiratory fitness.

Acknowledgements This research was funded by grants from the National Health and Medical Research Council (1000967, 1022752, 1079309) and The Townsville Hospital. Professor Jonathan Golledge’s work is supported by fellowships from the NHMRC (117061) and the Queensland Government (Senior Clinical Research Fellowship). Support for this work was also provided through the Inflammation and Healing Research Cluster at the University of the Sunshine Coast.

Author contribution statement All authors read and approved the manuscript. Below is the short description of the manuscript contribution made by each listed author: Conceptualized and designed the experiment: MP, MW, TB, CA. Performed the experiment: MP, MW, TB. Analysed the data: MP, TB, CA. Wrote/rewrote the paper: MP, TB, MW, MN, KG, AL, JC, CA.

Compliance with ethical standards Conflict of interest The authors declare that they have no conflict of interest.

References


 Springer


