Exercise training and Cardioprotection: Interaction with Cardiovascular Risk Factors and Disease.

Hannah McLellan

A thesis submitted in partial fulfilment of requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

February 2024

Abstract

Aims and Methods: Physical activity (PA) and/or exercise training can reduce risk of future cardiovascular events in asymptomatic and individuals with established cardiovascular disease. However, there is large heterogeneity in this response. This thesis aimed to examine some of the factors that may contribute to this. Study 1 was a cohort study including 139,930 individuals looking at the role of moderate-vigorous PA (MVPA) on stroke risk in both medicated and nonmedicated hypertensives vs normotensives (median follow up: 6.75 years). Study 2 was a secondary analysis of markers of cardiovascular function in 338 individuals undergoing supervised exercise training (8-26 weeks) stratified on their baseline endothelial function (FMD%). Study 3 examine the acute effects of moderate-intensity continuous cycling exercise on cerebrovascular function in eight individuals with medicated hypertension (55±6 years) and ten control individuals (54±7 years). **Results**: Study 1 reported lower Hazard Ratios (0.75, *P*=0.02) in those who did more (Quartile 3), MVPA moderate-intensity PA compared to the lowest quartile of MVPA. The shape of the dose-response was not different between hypertensives and normotensives, but this relationship may be altered by hypertensive medication. Study 2 demonstrated that exercise training significantly improved physical fitness, BMI, blood pressure and total cholesterol, irrespective of initial baseline endothelial function. Study 3 showed that post-acute exercise, absolute cerebrovascular reactivity decreased by 0.6 cm/s/mmHg in the control group but increased by 1.03 cm/s/mmHg in the medicated hypertensive group (P=0.02) with no change in peripheral endothelial function (FMD%). **Conclusion**: (i) MVPA reduces stroke risk in the total population, which is not affected by the presence of hypertension. Use of anti-hypertensive medication may interfere with the impact of MVPA on stroke risk. (ii) Individuals with reduced

and preserved a *priori* endothelial function status can obtain benefits from exercise in terms of risk factor modification and fitness change. Therefore, exercise has the potential to be beneficial in all clinical groups. (iii) following acute exercise cerebrovascular function appears to be different in normotensive vs hypertensive individuals (iv) overall, exercise is cardioprotective in healthy individuals and those with cardiovascular risk factors, but further work is needed to determine factors that underpin responsiveness to exercise training including the acute stimulus and role of medication.

Acknowledgements

Firstly, I would like to thank my Director of Studies, Dr Ellen Dawson for all your help, support and guidance throughout my PhD project. We nearly got to the end without any tears! Thank you for always having an open-door policy for me and for spending time trouble shooting problems (especially around statistics). I would also like to thank you for taking over my Director of Studies and giving me the opportunity to finish my PhD. I would also like to thank Dr Niki Hopkins for also being my Director of Studies at the start of my PhD. Thank you for your support and guidance and for constantly making sure that I was not getting too overwhelmed at the start of my PhD. I wouldn't have got to the end of the PhD without you both!

Thank you to my co-supervisor, Professor Dick Thijssen. Thank you for always having answers to my problems and always making me think about the bigger picture. It has been a pleasure to work with you on this PhD project and I hope this can carry on into the future. I would also like to thank Dr Esmée Bakker for all your help on Chapter 3, you are a statistics genius! It has been a pleasure to work so closely with you during my PhD. I am so grateful for all the time that you dedicated to this chapter including helping me understand it all!

A special thanks has to go Team CBF (Gemma, Joe, Katie and Dan). Thank you for all the fun (and stressful!) times in the lab. Gemma, thank you for being the 'Boss' and always wanting to support everyone in anyway that you possibly can. But more importantly, thank you for becoming a friend during this whole crazy PhD journey. Joe, it has been great to have you back as my 'PA'. Thank you for the laughs during those long testing and analysis sessions. I apologise for being your 'full time job' over the past few months, I definitely owe you one! I don't think I would have ever thought about doing a PhD if it wasn't for me helping on your own PhD so I guess I have you to thank for the stress (and good times!) of the past three years?! I have loved being a part of this team and I hope to one day continue working with you all.

It is essential to acknowledge the laboratory technicians' team. None of the research that we do at LJMU would be possible without you all. Thank you for always being there to not only move and fix equipment, but always being a friendly face. I loved gate crashing the occasional quiz and the Monday morning debrief of the football at the weekend. I will miss Dean's snack draw when I needed a treat! Speaking of the lab, I would like to thank every participant who has taken part in the studies within the thesis, this PhD wouldn't have been possible without you all.

To all the postgrads, past and present (too many of you to mention, you know who you are), thanks for everything. Postgrad offices are funny little places whereby people from all backgrounds are thrown together and forced to work closely. Collectively, you guys made the office a fantastic place to work and made the PhD experience so much fun, please still invite me to the after-work pub trips! I can't believe I am saying this, but I will miss office cricket!

To my Dad, Emmsey and Jessica, thank you for all your support during my time at university. Thank you for all being so supportive of me and believing in me when I often don't believe in myself. I can only hope that I have done you all proud!

Luke, thank you for being you. I know you don't fully understand what I have done during this PhD but thank you for just shouting "Cardiovascular Physiology" at everyone when they ask what I do and for always volunteering when I needed someone to practice techniques on. Your pep talks, ability to make me laugh on those stressful days and unwavering support hasn't gone unnoticed. Thank you for being my number one supporter.

Finally, to my mum. I wish you were here to see this achievement, but I know you have been with me every step of the way. Thank you for making me believe that I could always do anything that I put my mind to and being the 'wind beneath my wing'. I did it mum, I hope you are proud up there.

Declaration

I declare that the work contained within this thesis is entirely my own.

Publications directly based on work described in this thesis

McLellan, H.L., Dawson, E.A., Hopkins, N.D., Jones, H., Maiorana, A.J., Hopman, M.T., Haynes, A., Naylor, L.H., Green, D.J. and Thijssen, D.H., 2024. Relation Between Endothelial Dysfunction and Exercise Training-Mediated Adaptation in Cardiovascular Risk Factors, Cardiorespiratory Fitness, and Vascular Health in Humans: A secondary analysis. *Journal of Science in Sport and Exercise*, pp.1-9.

Manuscripts submitted based on work described in this thesis

McLellan, H.L., Dawson, E.A., Thijssen, D.H., Eijsvogels, T.M., Bakker, E.A.. The impact of hypertension on the dose-response association between physical activity and stroke: A cohort study among 139,930 adults from the Netherlands. *Stroke*

Oral communications

McLellan, H.L., Dawson, E.A., Hopkins, N.D., Jones, H., Maiorana, A.J., Hopman, M.T., Haynes, A., Naylor, L.H., Green, D.J. and Thijssen, D.H., 2024. Relation Between Endothelial Dysfunction and Exercise Training-Mediated Adaptation in Cardiovascular Risk Factors,

Cardiorespiratory Fitness, and Vascular Health in Humans: A secondary analysis. Northern Biology Vascular Forum

McLellan, H.L., Dawson, E.A., Thijssen, D.H., Eijsvogels, T.M., Bakker, E.A.. The impact of hypertension on the dose-response association between physical activity and stroke: A cohort study among 139,930 adults from the Netherlands. European College of Sport Science, Paris, France, 2023.

Table of Contents

Chapter 1: General Introduction *14*

1.1 Aims **18**

1.2 Objectives **18**

Chapter 2: Literature Review *20*

2.1 Overview **21**

2.2 Vascular function **23**

2.2.1. Introduction to vascular function 23

2.2.2 The importance of the endothelium for health 24

2.2.3 Measurement of vascular function 25

2.3 Endothelial dysfunction **26**

2.3.1 Pathophysiology of endothelial dysfunction / The role of endothelial dysfunction in atherosclerosis/CVD 26

2.3.2 Exercise training and vascular function. 27

2.3.3 Importance of acute exercise responses 28

2.3.4 Impact of acute exercise on vascular function 29

2.3.5 Effects of exercise training on vascular function (healthy individuals) 30

2.4.6 Effects of exercise training on vascular function in individuals with cardiovascular disease risk factors 31

2.4 Hypertension **32**

2.4.1 Definition of hypertension 32

2.4.2 Pathophysiology of hypertension 34

2.4.3 Hypertension and Stroke 42

2.4.4 Different types of stroke 43

2.4.5 Pathophysiology of hypertension and stroke. 43

2.4.6 Hypertrophy, remodelling and stiffening of cerebral vessels. 43

2.4.7 Treatment for hypertension 44

2.5 Cerebrovasculature and cerebrovascular function **45**

2.5.1 The cerebrovascular system 46

2.5.2 Anatomy of the cerebral vasculature 46

2.5.3 Regulation and control of the cerebral blood flow 47

2.5.4 Cerebrovascular function and the hypertensive brain 56

2.5.5 Cerebrovascular function during exercise 59

2.5.6 Cerebrovascular function following acute exercise 61

2.5.7 Impact of Exercise training in cerebrovascular function in healthy humans 64

2.5.8 Impact of Exercise training in cerebrovascular function in clinical populations 64

2.6 Summary **65**

Chapter 3: The impact of hypertension on the dose-response association between physical activity and stroke: A cohort study among 139,930 adults from the Netherlands. *67*

3.1 Introduction **68**

3.2 Methods **69**

3.2.1 Participants 69

3.2.2 Experimental measures 70

3.2.3 Hypertension 72

3.2.4 Statistic analysis 73

3.3 Results **74**

3.3.1 Participants 74
3.3.2 Physical activity and the risk of stroke 77
3.3.3 Physical activity and stroke risk in hypertensives: impact of medication use 77

3.4 Discussion 80

3.5 Conclusion 83

Chapter 4: Relation between Endothelial Dysfunction and Exercise Training- Mediated Adaptation in Cardiovascular Risk Factors, Cardiorespiratory Fitness, and Vascular Health in Humans: A secondary analysis. 84

4.1. Introduction 85

4.2 Methods 87
4.2.1 Participants 87
4.2.2 Research design 88
4.2.3 Experimental measures 88
4.2.4 Statistical analysis 90

4.3 Results 90
4.3.1 Baseline characteristics 91
4.3.2 Impact of exercise training 91

4.4 Discussion 98

4.5 Conclusion 102

Chapter 5: The effects of acute exercise on (cerebro) vascular function in individuals with normotension and medicated hypertension. 103

5.1 Introduction 104

5.2 Methods 107
5.2.1 Participants 107
5.2.2 Research design 108
5.2.3 Experimental measures 109
5.4.2 Statistical analysis 113

5.3 Results 114
5.3.1 Participants 114
5.3.2 Resting conditions 115

5.3.3 During exercise 120
5.3.4 The effects of acute exercise on cerebral and peripheral vascular function 122

5.4 Discussion 127

5.5 Conclusion 132

Chapter 6: Synthesis of Findings 133

6.1 Aims of Thesis 134

6.2 Summary of Major findings 134

6.3 General discussion of major findings 135
6.3.1 A priori heath status does not alter the cardioprotective effect of increased physical activity. 135
6.3.2 Medication use may alter the cardioprotective effect of acute exercise and exercise training. 136
6.3.3 The role of the endothelium in the response to exercise training. 142
6.3.4 Acute mechanisms that may affect the cardioprotective effect of exercise training. 145

6.4 Methodological considerations and limitations 147

6.5 Summary 149
6.6 Recommendations for clinical practice and future studies 149
Chapter 8: Supplementary Material 152
Chapter 9: References 175

List of Figures

Figure	Heading	Page
2.1	An example graphical representation of dose response relationships between physical activity and cardiovascular mortality that are present within the literature. *Curvilinear association indicates that physically inactive individuals have the highest risk for adverse outcomes, while the most active individuals have the lowest risk. It is important to note that the health benefits of an increase in exercise volume depend on the initial activity status of the individual. U and J-shaped relationships suggest a partial loss of health benefits with higher levels of physical activity.*	22
2.2	Renin-angiotensin system: Classical view. ACE: Angiotensin-converting enzyme. AT1-R: Angiotensin II type 1 receptor. Taken from: Physiology, Renin and Angiotensin system (Fountain et al., 2023).	41
2.3	Acoustic windows used in order to isonate cerebral blood vessels.	48
2.4	A) The classical view of the relationships between mean arterial pressure (MAP) and cerebral blood flow (CBF), i.e. autoregulation (Lassen, 1959). B) an updated view of the relationship indicating a small plateau region (Tan, 2012). This indicates a far more pressure-passive CBF than is conventionally believed, and that more efficacious buffering capacity against increases than decreases in perfusion pressure. Taken from (Willie et al., 2014)	50
2.5	Autoregulation maintains cerebral blood flow relatively constant between 50 and 150 mmHg arterial pressure. The range is right shifted in chronically hypertensive patients. (Ruland and Aiyagari, 2007)	56
3.1	Unadjusted Kaplan–Meier estimates of stroke occurrence for quartiles (Q) of moderate-to-vigorous physical activity during follow-up stratified for total population(A), individuals with normotension (B), and individuals with hypertension (C), individuals with hypertension who are not taking anti-hypertensive medication (D) and individuals with hypertension who are taking antihypertensive medication.	73
3.2	Forrest plot of the quartiles (Q) of MVPA associated with stroke risk for the total population and stratified for blood pressure and medication use.	76
4.1	Cardiovascular risk factor responses of BMI (a), VO2peak (b), Mean arterial pressure (c), Cholesterol (d), Brachial artery (BA) diameter (e) and Flow mediated dilatation (FMD%) (f) in those with endothelial function and endothelial dysfunction group following exercise training.	94

5.1	Figure 1. Schematic of the study design protocol.	105
5.2	Middle cerebral artery velocity (MCAv) (a), end tidal volume of CO_2 (b), mean arterial pressure (c), heart rate (d), RPE (e) in response to moderate intensity cycling in individuals with normotension and medicated hypertension.	117
5.3	individual data points (black lines) and mean (red line) for absolute CVR pre and post exercise for normotensive (A) and medicated hypertensives (B).	120

List of Tables

Table	Heading	Page
2.1	Blood pressure guidelines for normal, evaluated and hypertension from the American Heart Association (Whelton et al., 2018)	32
2.2	Identification of cerebral arteries using transcranial Doppler Ultrasound.	48
3.1	Baseline characteristics for the total population (n=139,949) and individuals with normotension (N=78,309) and hypertension (n=61,621).	72
3.2	Hazard ratios (HR) with 95% confidence intervals (95% CI) for the association between moderate-to-vigorous physical activity and stroke.	75
4.1	Study characteristics of included studies. NAFLD: Non-alcoholic fatty liver disease, PCOS polycystic ovary syndrome T2D type 2 diabetes	90
4.2	Subject characteristics of the study population stratified by endothelial function into preserved endothelial function (P-EF, n=123) and reduced endothelial function (R-EF, n=215).	91
4.3	Characteristics of the groups divided by endothelial function into the preserved endothelial function (P-EF) group and subjects with reduced endothelial function (R-EF) before and after exercise interventions	92
5.1	Baseline characteristics for the normotension and medicated hypertension group.	111
5.2	Baseline haemodynamics (taken during measurements for static autoregulation) for the normotension and medicated hypertension group	112
5.3	Power spectral and transfer function analysis of cerebral autoregulation during spontaneous changes in BP and CBFv.	113
5.4	Power spectrum densities of forced oscillations in mean arterial pressure and cerebral blood flow velocity during squat-stand manoeuvres (0.10Hz, low frequency).	115
5.5	Cerebrovascular reactivity to 5% carbon dioxide.	119
5.6	Vascular function for participants with normotension and medicated hypertension	122

Chapter 1: General Introduction

Cardiovascular disease (including cerebrovascular disease) is the leading cause of death worldwide (World Health Organisation, 2021). Physical activity (PA) and/or exercise training is important in both preventing and reducing reduce risk of future cardiovascular events in asymptomatic and individuals with established cardiovascular disease (Mora et al., 2007, Lee et al., 2012, Lee et al., 2003b, Eijsvogels et al., 2016). However, there is heterogeneity in individuals' responses to training which may relate to a number of factors, some of which will be explored in this thesis (Mora et al., 2007, Green et al., 2008, Joyner and Green, 2009, Tinken et al., 2010, Ashor et al., 2015, Green et al., 2017, Pedralli et al., 2020).

There is currently a debate as to whether health status affects the dose response association between PA and event rate (Eijsvogels et al., 2016, Moore et al., 2012, Bakker et al., 2021). An epidemiological study including 142,493 adults from the Netherlands reinforced the benefits of moderate-to-vigorous (MVPA) on reducing major adverse cardiovascular events (MACE) and all-cause mortality but found that a curvilinear association was found in healthy individuals and individuals with cardiovascular risk factors (CVRF), whilst individuals with CVD demonstrated a linear association, suggesting a constant reduction of risk with higher volumes of MVPA (Bakker et al., 2021). Therefore, more research is warranted to better understand the interactions between PA and/or exercise training, cardiovascular risk factors and disease for effective preventative strategies.

To date, no research has looked at the influence of individual cardiovascular risk factors on the association between MVPA and event rate. Hypertension is the leading modifiable risk factor of cardiovascular and cerebrovascular disease (including stroke) (Mills et al., 2020). Both pharmacological and lifestyle interventions are needed for the prevention and

management of hypertension (Valenzuela Ruiz et al., 2020). Regular PA has been shown to reduce stroke risk (Lee et al., 2003a, Kiely et al., 1994, Jeong et al., 2017) which is at least partly explained by the reduction in blood pressure (Pinckard K, 2019). Antihypertensive medication is also often prescribed alongside lifestyle interventions, and independent of these lifestyle interventions antihypertensive medication effectively reduces risk. Interestingly, both anti-hypertensive medication and lifestyle interventions may also have interacting effects. Previous work investigating the effect of antihypertensive medications on exercise training suggest that medication may interfere with exercise induced adaptations, with some studies suggesting antihypertensive drugs attenuate and others indicate these drugs promote exercise-induced effects (Dimeo et al., 2012, Sumukadas et al., 2014, Baptista et al., 2018, Sjúrðarson et al., 2022). Therefore, it is vitally important that the influence of antihypertensive medication is considered alongside changes in PA.

Whilst changes in traditional cardiovascular disease risk factors explain some of the cardioprotective effects of training (Mora et al., 2007), it is possible there are also direct effects of exercise on vascular health, including improvements in endothelial function (Green et al., 2008, Joyner and Green, 2009, Tinken et al., 2010, Ashor et al., 2015, Green et al., 2017, Pedralli et al., 2020). Endothelial dysfunction is a well-established response to exposure of cardiovascular disease risk factors. Endothelial function, measured as flow mediated dilation (FMD%), is largely nitric oxide (NO)-mediated (Green et al., 2014a) and predicts future CV events (Green et al., 2011, Inaba et al., 2010, Ras et al., 2013). It is therefore considered an early marker of future atherosclerotic vascular risk and a non-invasive window into vascular health status. It has been suggested that those with *a priori* endothelial dysfunction (low FMD), improve endothelial to a greater degree than those with a healthy, functioning

endothelium (normal FMD) following exercise training (Maiorana *et al.*, 2003; Green *et al.*, 2014).

Long term benefits of exercise training are likely related to the repeated acute responses to acute bouts of exercise (Dawson et al., 2018) therefore, understanding the acute effects of exercise is essential (Thijssen et al., 2018) as it will help to understand the complex interplay between exercise, cardiovascular risk and cardioprotective effects. Understanding the acute effects of exercise is important for optimising exercise prescription for individuals with various cardiovascular diseases (Luan et al., 2019) that will target key mechanistic pathways linked to improved vascular and brain function (Lucas et al., 2015, Mulser and Moreau, 2023). Impairment in FMD following acute exercise, provides a barometer of cardiovascular risk (Vita and Keaney Jr, 2002) and may reflect a transient period of increased risk for exercise related cardiac events immediately following physical exertion (Mittleman et al., 1993, Siscovick et al., 1984, Whang et al., 2006, Albert et al., 2000, Willich et al., 1993, Smyth et al., 2016). Consequently, assessing immediate cardiovascular and cerebrovascular responses could elucidate whether there is a transient period of risk immediately following exercise or provides insights on the stimulus for long-term adaptation. This can contribute to effectiveness of cardioprotective strategies and prevent adverse events during and after exercise (Vita and Keaney Jr, 2002, Mittleman et al., 1993, Siscovick et al., 1984, Whang et al., 2006, Albert et al., 2000, Willich et al., 1993, Smyth et al., 2016). However, there is limited research on the acute effects of exercise on cerebrovascular function which is an important barometer of stroke, dementia, and cognitive decline (Chrissobolis et al., 2011, Miller et al., 2010). This is vitally important in individuals who are diagnosed with hypertension as they are at high risk of cerebrovascular disease (Jones et al., 2003, Meissner, 2016).

1.1 Aims

The overarching aim of this thesis was to investigate the relationship between cardiovascular risk factors and exercise training cardiovascular responses.

The specific aims of this thesis were to:

1. Examine influence of a singular cardiovascular risk factor with regular physical activity on event outcomes.
2. Investigate whether *a priori* endothelial dysfunction alters the cardioprotective effects of exercise training,
3. Investigate the potential mechanisms of acute exercise on (cerebro)vascular function following moderate intensity aerobic exercise.
4. Examine the influence of medication use on the cardioprotective effects of exercise.

1.2 Objectives

The aims outlined above will be achieved through the following objectives:

In line with Aim 1:

1. Perform analysis of a cohort study to understand the impact of hypertension on the dose response relationship between physical activity and stroke.

In line with Aim 2:

2. This will use data from previous exercise training interventions in healthy individuals, individuals with cardiovascular risk factors and those with cardiovascular disease where FMD data were collected pre and post exercise. Individuals will be categorised as having either preserved endothelial function or reduced endothelial function based on FMD reference intervals (Holder et al., 2021) to determine if this impacts on exercise training induced changes in vascular function.

In line with Aim 3:

3. Investigate the effect of an acute bout of moderate intensity continuous cycling on (cerebro) vascular function in individuals with normotension and medicated hypertension.

In line with Aim 4:

4. In the cohort study (i.e., Aim 1), examine whether the use of antihypertensive medication alters the dose response relationship between physical activity and stroke.
5. In the acute exercise study (i.e., Aim 3), examine whether antihypertensive medication alters the acute effects of exercise on (cerebro) vascular function.

Chapter 2: Literature Review

2.1 Overview

Cardiovascular disease (CVD) including cerebrovascular disease is the leading cause of death worldwide (World Health Organisation, 2021). CVDs are a group of diseases that affect the heart and blood vessels. Specifically, cerebrovascular diseases refer to a group of conditions that impair cerebral blood flow to the brain and primarily involves blood vessels in the brain (Capildeo et al., 1978). Stroke is the most common manifestation of cerebrovascular disease and can have long-term consequences for neurological, neuropsychiatric, and vascular outcomes (Singh et al., 2018) as well as mortality. Physical inactivity, poor nutrition, dyslipidemia, hyperglycemia, high blood pressure and obesity are traditional risk factors for CVD (Bays et al., 2021) and stroke (Boehme et al., 2017). Endothelial dysfunction is a well-established consequence of CVD risk factors as it has previously been found that the magnitude of endothelial dysfunction increases in proportion with the accumulation of CV risk factors (Hashimoto et al., 2000). High blood pressure is an important modifiable risk factor of CVD/stroke that causes damage the endothelium which results in thickening of the artery walls and formation of atherosclerotic plaques (Gonzalez-Guerra et al., 2021, Poznyak et al., 2022). Endothelial dysfunction within coronaries/cerebral circulation is a major contributing factor CVD events (Hadi et al., 2005, Boulanger, 2016) and cerebrovascular disease (Ashby and Mack, 2021). It is important to note that while there are numerous risk factors that can lead to endothelial damage and CVD, exercise training is a known therapeutic intervention that can help mitigate vascular damage in response to CVD risk factors. However, it is possible that *a priori* cardiovascular health status may alter the cardioprotective effects of exercise compared to healthy individuals (Eijsvogels et al., 2016, Moore et al., 2012, Bakker et al., 2021). Some studies have found a linear association between PA and mortality

reductions (Moholdt et al., 2008, Stewart et al., 2017, Jeong et al., 2019), whereas others have reported a reverse J-shaped curve or a U-shaped relationship (Mons et al., 2014, Wannamethee et al., 2000, Williams and Thompson, 2014, Keteyian et al., 2012) (Visual representation of the relations Figure 2.1). Recently, a curvilinear association was found in healthy and cardiovascular disease risk factor individuals whilst CVD patients demonstrated a linear association, suggesting a constant reduction of risk with higher volumes of MVPA (Bakker et al., 2021). Therefore, it is important to further investigate what factors of CVD and CVD risk factors that may contribute to this altered cardioprotective effects of exercise.

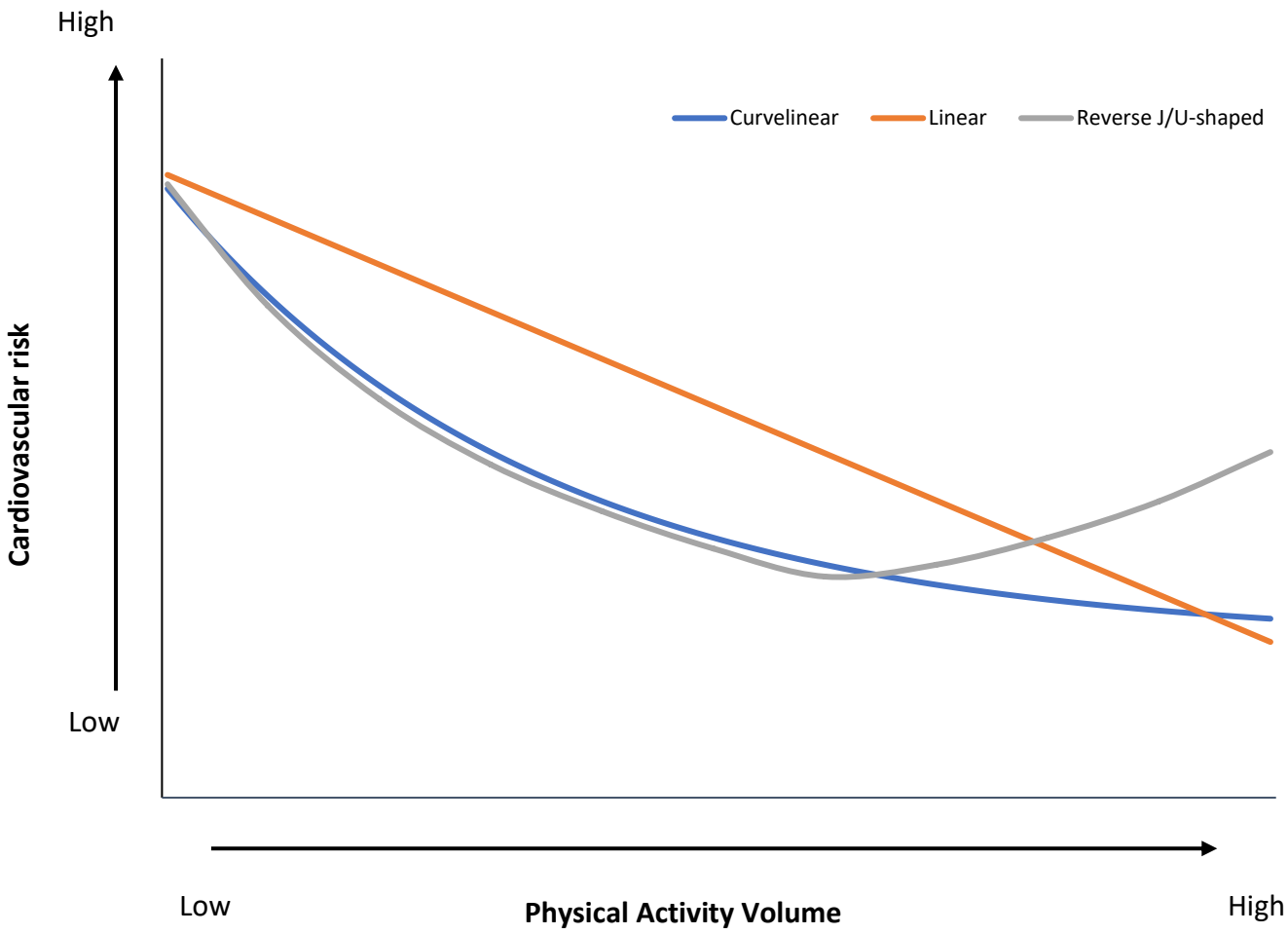


Figure 2.1 An example graphical representation of dose-response relationships between physical activity and cardiovascular mortality that are present within the literature.

Curvilinear association indicates that physically inactive individuals have the highest risk for adverse outcomes, while the most active individuals have the lowest risk. It is important to note that the health benefits of an increase in exercise volume depend on the initial activity status of the individual and can depend on health status of the individual (Bakker et al., 2021). U and J-shaped relationships suggest a partial loss of health benefits with higher levels of physical activity (Mons et al., 2014, Wannamethee et al., 2000, Williams and Thompson, 2014, Keteyian et al., 2012)*. Linear relationships suggest that as physical activity volume increases, cardiovascular risk continues to decrease* (Moholdt et al., 2008, Stewart et al., 2017, Jeong et al., 2019),

This review will summarise:

1. The importance of vascular function and health
2. Exercise-induced adaptations of vascular health
3. Hypertension as a risk factor for stroke
4. Hypertension and its effect on cerebrovascular function
5. Exercise-induced adaptations of cerebrovascular function

2.2 Vascular function

2.2.1. Introduction to vascular function

The vascular endothelium is a monolayer of endothelial cells (EC) on the intima lining the blood luminal surface of the whole cardiovascular tree. However, EC display different structures and phenotypes in the arteries, veins and capillaries (Ghitescu and Robert, 2002). Initially, the endothelium was regarded as a relatively inert cell layer acting as a semi-permeable barrier between the blood and interstitium. However, key discoveries since the

1970s have highlighted the vital importance of the endothelium and have shown that it is an active metabolic and endocrine organ (Garland et al., 2011), with a strategic placement between blood flow and the artery wall providing a multitude of regulatory roles to maintain homeostasis of many (patho)physiological processes (Petty and Pearson, 1989, Durand and Gutterman, 2013).

2.2.2 The importance of the endothelium for health

The endothelium plays a vital role in maintaining cardiovascular health, with pathophysiological alterations in endothelial structure and function underpinning most major cardiovascular diseases. The endothelium regulates vascular tone by releasing vasoconstrictors (e.g., thromboxane, endothelin-1 and angiotensin) or vasodilators (e.g., nitric oxide (NO), prostacyclin (PGI_2), bradykinin, endothelium derived hyperpolarising factors (EDHF)) in response to physical and chemical stimuli (Davignon and Ganz, 2004). The maintenance of a correct balance between these vasoactive molecules is crucial; imbalance leads to an impairment of endothelium-dependent vasodilation, which is the functional characteristic of endothelial dysfunction.

NO is of particular interest as it is a potent vasodilator in the vascular system, which is also known to control vascular wall inflammation, inhibit platelet aggregation and modulate vascular smooth muscle cell proliferation thus protecting the vessel wall against the development of atherosclerosis and thrombosis (Moncada and Higgs, 2006). NO is a gaseous free radical signalling molecule which is short lived that diffuses to the adjacent muscle layer causing relaxation. First identified in the 1980s (Furchgott and Zawadzki, 1980), NO is

synthesised from L-arginine in endothelial cells by the action of endothelial NO synthases (eNOS) (Förstermann, 2006) in a reaction that requires oxygen and the reduced cofactors tetrahydrobiopterin (BH_4) and nicotinamide adenine dinucleotide phosphate (NADPH) (Alkaitis and Crabtree, 2012). NO then diffuses to vascular smooth muscle cells where it binds to guanylate cyclase and forms cyclic guanosine monophosphate. This leads to smooth muscle relaxation and subsequent vasodilation (Green et al., 2004). The activity of eNOS and the production of NO can be stimulated by increases in intimal shear stress (Pohl et al., 1986). Elevation of shear stress, primarily into the antegrade direction, upregulates NO synthesis by activating calcium and potassium channels on the endothelial cell surface resulting in potassium efflux and calcium influx which increases intracellular calcium. This in turns results in eNOS detaching from caveolin which upregulates the production of NO (Förstermann and Sessa, 2012).

2.2.3 Measurement of vascular function

In humans, vascular function can be measured using the flow mediated dilation (FMD) technique which is a non-invasive assessment that is able to predict future cardiovascular events (Inaba et al., 2010, Ras et al., 2013, Vita and Keaney Jr, 2002, Thijssen et al., 2011) and correlates with coronary artery endothelial function (Celermajer et al., 1992, Takase et al., 1998). FMD is endothelium-dependent and partly nitric oxide (NO)-mediated (Thijssen et al., 2019). FMD measures changes in brachial artery diameter in response to a period of ischemia (typically 5 minutes) by inflating a cuff distal from the imaged artery around the forearm to supra-systolic level (Thijssen et al., 2019). Cuff deflation can lead to increases in shear-stress-mediated augmented NO production in endothelial cells resulting in vasodilation of the artery

following an increase in luminal blood flow and internal wall shear stress (Thijssen et al., 2011). Several meta-analyses have explored the prognostic value of FMD and revealed that a 1% increase in brachial artery FMD is associated with an 8-13% reduction in CVD risk in heterogeneous populations (Ras et al., 2013, Inaba et al., 2010, Matsuzawa et al., 2015).

2.3 Endothelial dysfunction

2.3.1 Pathophysiology of endothelial dysfunction / The role of endothelial dysfunction in atherosclerosis/CVD

Atherosclerosis is an inflammatory process which underlies most CVD conditions, resulting in the thickening of the artery wall, plaque development and sometimes plaque rupture resulting in cardiovascular complications (Ross, 1999). Endothelial dysfunction is the initial step in the pathogenesis of arteriosclerosis and consequently, is widely accepted as an early event in the pathogenesis of cardiovascular diseases (Boulanger, 2016). Reduced NO bioavailability results in the initiation, progression and complications of atherosclerosis through numerous means including lipid damage, inflammatory response, vasoconstriction, platelet aggregation, smooth muscle cell proliferation and migration, leukocyte adhesion as well as oxidative stress (Chen et al., 2018). A healthy endothelium provides antioxidant, ant-inflammatory, and antithrombotic functions and contributes to the maintenance of vascular tone, serving as a gatekeeper for organ/tissue homeostasis and blood pressure control (Eelen et al., 2015, Ross, 1999).

Development of endothelial dysfunction is a well-established response to exposure to cardiovascular risk factors. Both traditional and novel CVD risk factors initiate a chronic inflammatory process alongside a reduction in vasodilators and anti-thrombotic factors,

whilst levels of vasoconstrictors and pro-thrombotic factors increase. Endothelial dysfunction occurs in association with CVD risk factors, contributes to inflammation in the vascular wall, as well as to increased lipoprotein oxidation, smooth muscle cell proliferation, extracellular matrix deposition, cell adhesion, and thrombus formation in conducting arteries (Durier et al., 2003, Ross, 1999). Studies have found that the magnitude of endothelial dysfunction increases in proportion with the accumulation of CV risk factors (Hashimoto et al., 2000).

Endothelial dysfunction is characterised by an imbalance between vasodilation and vasoconstriction, elevated reactive oxygen species (ROS), and proinflammatory factors, as well as a deficiency of nitric oxide (NO) bioavailability. Reduced NO bioavailability may result from reduced activity of eNOS, uncoupling of eNOS, scavenging of NO by superoxide anions and related reactive oxygen species (Endemann and Schiffrin, 2004, Beckman and Koppenol, 1996, Kissner et al., 1997). ROS may uncouple the eNOS-catalysed reduction of molecular oxygen from the oxidation of L-arginine resulting in the production of ROS superoxide anion instead of reducing NO (Münzel et al., 2005). An imbalance of NO and ROS (i.e. elevated oxidative stress), may promote endothelial dysfunction. ROS production also occurs in the media and adventitia, which also impairs NO signalling within vascular tissues (Sorescu et al., 2002, Pagano et al., 1997).

2.3.2 Exercise training and vascular function.

It is well established that exercise provides cardioprotective effects and is effective in the primary and secondary prevention of CVD (Eijsvogels et al., 2016, Kodama et al., 2009, Alves et al., 2016). Remarkably, the exact underlying mechanisms of the cardioprotective effect of

exercise are not fully understood (Mora et al., 2007). Part of the magnitude of the CVD risk reduction (≤ 59%) can be explained by the beneficial effect of exercise on several CVD risk factors (Mora et al., 2007, Taylor et al., 2006, Hamer et al., 2012). These findings imply that the cardioprotective effect of exercise is larger than expected based on the improvement in these risk factors. The unexplained part of the cardioprotective effect of exercise training is often referred to as the risk factor gap (Green et al., 2008). The risk factor gap may be explained by changes in vascular function. It is well established that exercise directly impacts vascular function as a result of hemodynamic effects (Green et al., 2017). Exercise has mechanical effects on arteries due to repeated exposure to increases in blood pressure, blood flow and arterial shear stress that occurs during each exercise bout (Green et al., 2017). Improved vascular health occurs in response to exercise training and improves vascular function through increases in bioavailability of endothelium derived substances such as nitric oxide, and modifies arterial structure including outward remodelling and modifying wall-to-lumen ratios (Green and Smith, 2018). Therefore, direct effects of exercise on the vasculature provides a plausible contribution to the reduction in cardiac events associated with exercise training (Green et al., 2008).

2.3.3 Importance of acute exercise responses

To understand the stimulus contributing to vascular adaptation following exercise training, studies have examined the acute impact of exercise on vascular function. An acute bout of exercise exerts direct effects on the vasculature via the impact of hemodynamic stimuli such as increases in shear stress and transmural pressure (Green et al., 2017) and is likely the stimulus for exercise training induced benefits. Research has shown that acute post-exercise changes in vascular function can predict exercise training responses (Dawson et al., 2018)

therefore, understanding the acute effects of exercise is important as a single bout of acute exercise may relate to future clinically relevant cardioprotection (Thijssen et al., 2018).

2.3.4 Impact of acute exercise on vascular function

There is variability among the literature examining the effects of acute exercise on vascular function. Measurements taken 30-60 minutes post exercise have reported conflicting findings with some studies reporting an increase (Goel et al., 2007, Harris et al., 2008, Harvey et al., 2005, Johnson et al., 2012, Hanson and Casey, 2023), decrease (Harris et al., 2008, Jurva et al., 2006, Rognmo et al., 2008, Caldwell et al., 2023) or no change (Harvey et al., 2005, Rognmo et al., 2008, Johnson et al., 2012) in FMD following exercise. Studies that have taken multiple FMD measures after exercise training have shown that the timing of the postexercise FMD assessment dictate the directionality of the change in FMD following exercise (Dawson et al., 2013). Post exercise FMD is also altered by exercise intensity, with higher exercise intensities (>80% $\dot{V}O_{2max}$) typically resulting in a larger decrease in FMD immediately postexercise (Birk et al., 2012, Johnson et al., 2012), whereas most (Cosio-Lima et al., 2006, Padilla et al., 2006, Zhu et al., 2010) but not all (Tyldum et al., 2009, Thijssen et al., 2006) studies of low moderate intensity exercise (50–80% $\dot{V}o_{2max}$) have reported an increase in FMD after exercise. Type of exercise also alters post exercise FMD response. A study performing 30 minute of handgrip dynamic contractions with high contractility demonstrated an association between high mean blood pressure during exercise and decreased local vascular function following exercise (Gonzales et al., 2011). Furthermore, acute aerobic lower limb exercise has been shown to increase (Farsidfar et al., 2008, Harris et al., 2008), decrease (McGowan et al., 2006, Harris et al., 2008) or show no change (Silvestro et al., 2002) in FMD in individuals with endothelial dysfunction. It has been suggested that postexercise FMD

follows a biphasic response with a decrease in FMD immediately after exercise followed by a normal to supranormal FMD and ultimate normalization within 24–48 h after exercise (Gonzales et al., 2011). The varied responses amongst the literature are likely to be related to the study population, the exercise duration, mode and intensity (Dawson et al., 2013) and individuals' cardiorespiratory fitness levels (Landers-Ramos et al., 2023). Various mechanisms have been used to explain the changes in FMD such as shear stress, alterations in oxidative stress, blood pressure and SNS activity (Dawson et al., 2013) however this still remains unclear. Overall, interpretation of post exercise FMD is needs to be taken with caution as the FMD response following acute exercise is dependent upon timing of post exercise measure, exercise intensity, type of exercise and individual's cardiovascular health status.

2.3.5 Effects of exercise training on vascular function (healthy individuals)

Reviews investigating exercise interventions in general healthy adult populations found aerobic (Ashor et al., 2015, Early et al., 2017, Campbell et al., 2019), resistance (Early et al., 2017, Ashton et al., 2020, Zhang et al., 2021, Silva et al., 2021) and combined (Ashor et al., 2015, Early et al., 2017) exercise training all positively impacted FMD. However, others have reported no improvement in vascular function following exercise training in healthy individuals (Maiorana et al., 2001, Thijssen et al., 2007, Østergård et al., 2006). The differences between studies may be explained by the timing of assessments. It has been suggested that the there is an initial improvement in artery function (e.g. FMD) in the initial weeks of training, however, as exercise training continues, the structural adaptation (an outward remodelling of the artery) normalises shear rate levels which may result in NO-mediated endothelial function (FMD) returning to initial levels (Tinken et al., 2008).

Consequently, FMD may appear unchanged pre to post training, due to a short-term initial increase which is no longer apparent longer term due to an increase in artery size.

2.4.6 Effects of exercise training on vascular function in individuals with cardiovascular disease risk factors

As highlighted previously, endothelial dysfunction is present in individuals with cardiovascular disease risk factors and established cardiovascular disease. Therefore, exercise interventions that improve endothelial function in this population may be vitally important. Exercise training has been shown to improve cardiovascular disease risk factors which can improve endothelial function. However, exercise training-induced change in vascular function do not correlate with changes in traditional cardiovascular risk factors, indicating that cardioprotective effects of exercise training through improvement in endothelial function are independent of improvement in risk factors (Green et al., 2014b).

Exercise training following 8 and 12 weeks of aerobic exercise in individuals with Type 2 Diabetes Mellitus, vascular function (FMD) is improved (Schreuder et al., 2015, Naylor et al., 2016). A meta-analysis in overweight and obese older adults who have a high risk for developing cardiovascular disease found that regular aerobic exercise for more than 24 weeks improved FMD and exercise for more than three times per week improved FMD (Li et al., 2023). A meta-analysis showed that aerobic, resistance and combined exercise training improved endothelium dependent vasodilation in heart failure patients (Vuckovic et al., 2013) whilst 12 weeks cardiac rehabilitation has been shown to improve endothelial function in men with coronary artery disease (Edwards et al., 2004). FMD increased following 12 weeks water-based exercise training, but not gym-based circuit exercise training in individuals with

coronary heart disease (Scheer et al., 2023). In individuals with mild hypertension, 12 weeks of brisk walking 5 to 7 times per week and 12 weeks of cycling have shown to improve blood pressure endothelial function in individual with hypertension (Higashi et al., 1999, Moriguchi et al., 2005).

2.4 Hypertension

2.4.1 Definition of hypertension

Blood pressure (BP) is the pressure or force that is exerted on the artery walls, when blood is passing or circulating within blood vessels. BP is the product of cardiac output and systemic vascular resistance. Cardiac output is determined by stroke volume and heart rate; where stroke volume is related to preload, afterload, and myocardial contractility. Peripheral resistance is determined by functional and anatomic changes in small arteries and arterioles. Blood pressure can be measured in millimetres of mercury (mmHg) and is typically reported as systolic pressure over the diastolic pressure (e.g., 120/80 mmHg). Systolic blood pressure (SBP) represents the maximum pressure that the blood exerts on a vessel wall during the contraction phase of the cardiac cycle. Diastolic blood pressure (DBP) refers to the pressure that the blood exerts during relaxation phase of the cardiac cycle. Hypertension refers to persistent elevation of blood pressure. High blood pressure has traditionally been defined as 140/90 mmHg or greater. However, the American Heart Association has recently revised its definition of Stage 1 hypertension to include individuals with SBP between 130 and 139 mmHg or DBP between 80 and 89 mmHg (Table 2.1). This change was prompted by studies demonstrating beneficial effects of lowering BP below the 120/80 mmHg threshold

(Lewington et al., 2003, Guo et al., 2013, Whelton et al., 2018) and effectively increased the number of Americans categorised as hypertensive from 32% to 46% (Whelton et al., 2018).

Table 2.1 Blood pressure guidelines for normal, evaluated and hypertension from the American Heart Association (Whelton et al., 2018).

BP Classification	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)
Normal	<120	<80
Elevated	120-129	<80
Stage 1 hypertension	130-139	80-89
Stage 2 hypertension	≥140	≥90
Hypertension crisis	≥180	≥120

Hypertension is generally classified into two main types: primary (essential) and secondary hypertension. Primary hypertension is high blood pressure that is not related to another medical condition and consequently does not have a specific cause. It is often influenced by genetic factors, lifestyle choices (e.g., diet, physical activity, and stress) and aging. Conversely, secondary hypertension is a result of another medical condition typically related with the kidneys, arteries, heart, or endocrine system.

The incidence and prevalence of hypertension is continuing to increase around the world. According to the World Health Organisation (WHO), in 2023 an estimated 1.23 billion adults aged 30-70 years have hypertension. One of the major global targets for noncommunicable diseases is to reduce the prevalence of hypertension by 33% between 2010 and 2030 (World Health Organisation, 2023). However, the number of people aged 30-70 with hypertension

doubled from 1990 to 2019, from 331 million women and 317 million men in 1990 to 626 million women and 652 million men in 2019 (Zhou et al., 2021). Hypertension is the most common preventable risk factor for cardiovascular disease, including coronary artery disease, heart failure, stroke, myocardial infarction, atrial fibrillation, peripheral artery disease as well as chronic kidney disease, and cognitive impairment. Consequently, it is the leading single contributor to all cause death and disability worldwide (Forouzanfar et al., 2016). Successful prevention and treatment of hypertension is key to reducing disease burden, improving the lives of the population, and reducing economical cost. In individuals of 40-69 years of age, a 20 mmHg rise of SBP or a 10 mmHg rise of DBP regardless of baseline values is associated with more than a doubling of the risk for stroke or ischaemic heart disease mortality (Lewington et al., 2002). Therefore, it is imperative to find interventions that effectively reduce blood pressure.

2.4.2 Pathophysiology of hypertension

The pathophysiology of hypertension is not fully understood as it involves multiple different pathways (Figure 2.1). In the 1940s, Dr Irvine Page proposed the Mosaic theory of hypertension which suggested that hypertension could be mediated by the central nervous system, cardiovascular factors, endocrine factors, and perturbations of renal function (Page, 1949). Page further revised this, stating that that hypertension is caused by multiple factors, including neural and chemical perturbations, alterations of vascular calibre and elastance, cardiovascular reactivity, blood volume, and viscosity (Page, 1963) (Figure 2.1). Over the years, Dr Page modified his paradigm, and new concepts regarding oxidative stress, inflammation, genetics, sodium homeostasis, and the microbiome have arisen that allow further refinements of the Mosaic Theory further highlighting there are many factors that

contribute to hypertension. (Harrison et al., 2021). However, it is clear that the pathophysiology of hypertension involves a complex of multiple vascular effectors including activation of the sympathetic nervous system, the renin angiotensin-aldosterone system and the inflammatory mediators.

Hypertension can lead to cardiovascular disease through several different pathways. Some of these have been discussed in more detail, including endothelial dysfunction (See section 2.4.2.1), oxidative stress (See section 2.4.2.2), increased peripheral resistance and cardiac output (See section 2.4.2.3), upregulation of the renin-angiotensin system (See section 2.4.2.4), and impairment in the sympathetic autonomic nervous system (See section 2.4.2.3).

2.4.2.1 Endothelial dysfunction and Hypertension

Although it is well established that endothelial dysfunction is a predictor of atherosclerosis and future cardiovascular events, its role for hypertension is less well understood. Endothelial dysfunction may precede the development of hypertension (Savoia et al., 2011). Endothelial dysfunction may contribute to the increased peripheral resistance by several mechanisms that lead to the enhanced constriction and vascular remodelling (i.e., structural, mechanical, and functional alterations) of resistance arteries, which is associated to the development and complications of hypertension (Savoia et al., 2011, Murray et al., 2021) (See section 2.4.2.3).

In hypertensive individuals, it is possible that in the presence of reduced NO availability, a compensatory pathway is activated involving production of a hyperpolarizing factor, to sustain endothelium-dependent vasodilation. This compensatory pathway can be further depressed by the simultaneous presence of essential hypertension and

hyperhomocysteinaemia, another cardiovascular risk factor (Taddei et al., 2001). Finally, reduced NO availability can increase the biological activity of endothelin-1 (ET-1), a potent vasoconstrictor and mitogenic substance. Thus, while in healthy conditions the vasoconstrictor effect of ET-1 is partially blunted by endothelial ET_B-receptor mediated NO production, in essential hypertensive patients this protective mechanism is lacking due to impaired NO availability. As NO not only causes vasodilatation but is also a potent inhibitor of platelet aggregation, smooth muscle cell proliferation, monocyte adhesion and adhesion molecule expression, a dysfunctional endothelium loses its capacity to protect the vessel wall against the development of atherosclerosis and becomes a promoter of atherosclerotic vascular damage associated with hypertensive disease (Taddei et al., 2001).

2.4.2.2 Increased peripheral resistance and cardiac output

Peripheral vascular resistance is characteristically elevated in hypertension because of alterations in structure, mechanical properties, and function of small arteries. Remodelling of these vessels contributes to high blood pressure and its associated target organ damage (Folkow, 1982, Mulvany and Aalkjaer, 1990). The narrowing of blood vessels (vasoconstriction) leads to an increased resistance to blood flow in the peripheral (systemic) circulation. This increased resistance requires the heart to pump blood with more force to overcome the resistance and maintain adequate blood flow to organs and tissues. If there is prolonged constriction of smooth muscle within the arterioles, this will lead to hypertrophy and thickening of the vessel. Endothelial dysfunction may participate to the increased tone of resistance arteries through the activation of the renin-angiotensin system (RAS), ET-1, catecholamines and growth factor production leading to vasoconstriction, vascular

remodelling and then to increased resistance to blood flow and ultimately to increased resistance to blood flow ultimately to increased peripheral blood pressure (Gallo et al., 2022). Blood pressure mediation is achieved by a balance of cardiac output and peripheral vascular resistance. The heart responds to increased peripheral resistance by a cascade of increasing stroke volume, increasing cardiac output, placing strain on the heart muscle and contributing to the development of hypertension.

2.4.2.3 Oxidative stress

There are a number of processes that can influence vascular tone or lead to upregulation of pro-atherosclerotic processes and pathways that lead to increased inflammation, and promotion of growth factors or damage. ROS regulates prostaglandin production and signalling, which is important in regulating vascular function. The ROS hydrogen peroxide (H_2O_2) upregulates the production of other vasoconstrictors such as thromboxane, prostacyclin and prostaglandin E2 (Hernanz et al., 2014). Oxidative stress prostanoids by constitutive (COX-1) and inducible (COX-2) cyclooxygenases which increases vasoconstriction and reduces vasodilation (Wong et al., 2013, Hernanz et al., 2014). COX can also produce ROS by oxidising NADPH (Hernanz et al., 2014, Martínez-Revelles et al., 2013). NADPH is also increased as a consequence of inflammation. Production of ROS increases the inflammatory response (Zhang et al., 2017); inflammation induced by angiotensin II increases vascular ROS (NOX and NADPH oxidases) formation (Takac et al., 2012). In hypertension, oxidative stress impairs cell signalling and post translation modification (oxidation and phosphorylation) of proteins which can cause cell and tissue damage (Touyz et al., 2020). In particular, protein phosphatases such as tyrosine phosphatases and protein serine/threonine phosphatases are

inactive in the oxidised state, resulting in increased phosphorylation and activation of downstream protein targets involved in cell growth and vascular inflammation which may contribute to vascular remodelling and hypertension development (Touyz et al., 2020, Tabet et al., 2008).

ROS regulate G protein-coupled receptors (GPCR) (Spiegelberg, 2013) and within vascular smooth muscle cells, activation of ROS-mediated GPCR influences cell contraction, growth, migration, collagen deposition, and matrix metalloprotease activation, cellular processes important in regulating vascular tone and structure. GPCR-mediated signalling also stimulates activation of transcription factors and proinflammatory genes, chemokine and cytokine production and recruitment and activation of inflammatory cells that cause vascular inflammation (Ushio-Fukai, 2009, Mochin et al., 2015) which can contribute to hypertension.

2.4.2.4 Sympathetic Autonomic nervous system

The sympathetic (SNS) and parasympathetic (PNS) nervous systems are the two major components of the autonomic nervous system which are fundamental in maintenance the physiological homeostasis of the cardiovascular system. Impaired sympathetic function is firmly established in the development, maintenance and the pathophysiology of many cardiovascular diseases including hypertension (Esler et al., 2006, Grassi et al., 2015) through stimulation of the heart, peripheral vasculature, and kidneys, causing increased cardiac output, increased vascular resistance, and fluid retention (Mark, 1996). The specific cause of the increased sympathetic activity in hypertension is only partially known; genetic influences,

behavioural (salty food preference), psychosocial (mental stress) and lifestyle (physical inactivity) factors all appear to be involved (Dibona and Esler, 2010, Osborn et al., 2007).

Increases SNS activity also causes the release of noradrenaline. In the heart, noradrenaline binds to adrenergic receptors, leading to an increase in heart rate (positive chronotropic effect) and the force of cardiac contractions (positive inotropic effect). This results in an increase in cardiac output, and therefore BP. Noradrenaline also causes increased peripheral resistance due to enhance vasoconstriction induced by noradrenaline binding to the alpha-one receptors (Beevers et al., 2001, Bolívar, 2013). This contributes to increases in total peripheral resistance, which again increases BP. Noradrenaline also causes an increase in renin which increases blood pressure as described above (See section 2.4.2.5). Furthermore, there is also enhanced renal resorption of salt and water, which results in an increased BP.

The mechanisms of increased sympathetic nervous system activity in hypertension also involve alterations in baroreflex and chemoreflex pathways at both peripheral and central levels. The SNS is involved in short-term regulation of blood pressure in response to transient changes in arterial blood pressure via baroreflex mechanisms (Cowley Jr et al., 1973). One hypothesis for the development of hypertension relates to the functional impairment of reflexogenic areas, arterial baroreceptors and cardiopulmonary volume receptors in the heart and chemoreceptors in the carotid arteries, that physiologically exert inhibitory effects on central sympathetic drive (Grassi et al., 2010). Consequently, increased SNS leads to increased BP through numerous pathways and is therefore an important mechanism for hypertension.

2.4.2.5 Renin and angiotensin system

The renin-angiotensin-aldosterone system (RAAS) is a hormonal system that helps regulate blood pressure and fluid balance. It is stimulated by fluid volume depletion and inhibited in fluid overload (Fyhrquist and Saijonmaa, 2008). Renin, angiotensin and aldosterone have central roles in regulating sodium, affecting the pressure-natriuresis relationship and therefore blood pressure. Increases in sympathetic nervous system activity can increase the activation of the RAAS. Whilst the RAAS is present throughout all tissues of the body, its role in systemic blood pressure control lies predominantly in the kidney.

Consequently, the kidney plays a vital role in hypertension. Renin is the rate limiting step for the activation of the circulating renin-angiotensin system (RAS) and its synthesis and secretion by the kidney is tightly regulated (Guessoum et al., 2021). Renin is a circulating enzyme secreted from the granular cells (juxtaglomerular cells which synthesise, store, and secrete renin) in the kidney under the stimulation of a decrease in arterial blood pressure, increased sympathetic activity in response to a fall in arterial blood pressure or a reduced sodium chloride level. Renin is important in the pathophysiology of hypertension as it enables the conversion of Angiotensinogen, produced by the liver, to Angiotensin I. Angiotensin I is relatively inactive and is converted by Angiotensin Converting Enzyme (ACE) to form Angiotensin II in the lungs. In hypertension there may be an overactivation of the RAAS, leading to the release of angiotensin II which increases blood pressure by various mechanisms including constricting resistance vessels, stimulating aldosterone synthesis and release, and renal tubular sodium reabsorption, stimulating thirst and release of antidiuretic hormone and enhancing sympathetic outflow from the brain (Oparil et al., 2003).

Angiotensin II is also a potent vasoconstrictor that increases peripheral vascular resistance, leading to hyperplasia and hypertrophy of vascular smooth muscle cells in resistance arteries, ultimately elevating arterial pressure. Angiotensin II also stimulates the release of aldosterone from the kidneys which leads to sodium and fluid retention (White, 1994), further increasing blood pressure. Angiotensin II also induces the expression of growth factors, cytokines, chemokines, and adhesion molecules. All of these are involved in cell growth/apoptosis, fibrosis and inflammation (Mezzano et al., 2001, Sadoshima, 2000, Ruiz-Ortega et al., 2001, Higuchi et al., 2007, Savoia et al., 2011) which contributes to vascular remodelling and atherosclerosis. Aldosterone induces vascular dysfunction and remodelling, increases generation of reactive oxygen species (ROS) and inflammation (Sun et al., 2002, Schiffrin, 2006, Briet and Schiffrin, 2013) which contribute to high blood pressure.

In summary, the RAAS plays a pivotal role in hypertension by contributing to elevation in blood pressure (Figure 2.2). The release of renin upregulates the formation of angiotensin II, promoting vasoconstriction, increasing peripheral resistance, and stimulates the release of aldosterone and antidiuretic hormone, causing the retention of sodium and water. Consequently, these processes contribute to an expansion of blood volume and an overall rise in blood pressure. In hypertensive individuals, the dysregulation of the RAAS can lead to sustained vasoconstriction and fluid retention, accentuating the elevation in blood pressure. Medications targeting this system, such as ACE inhibitors and ARBs, are commonly employed to mitigate these effects and manage hypertension effectively.

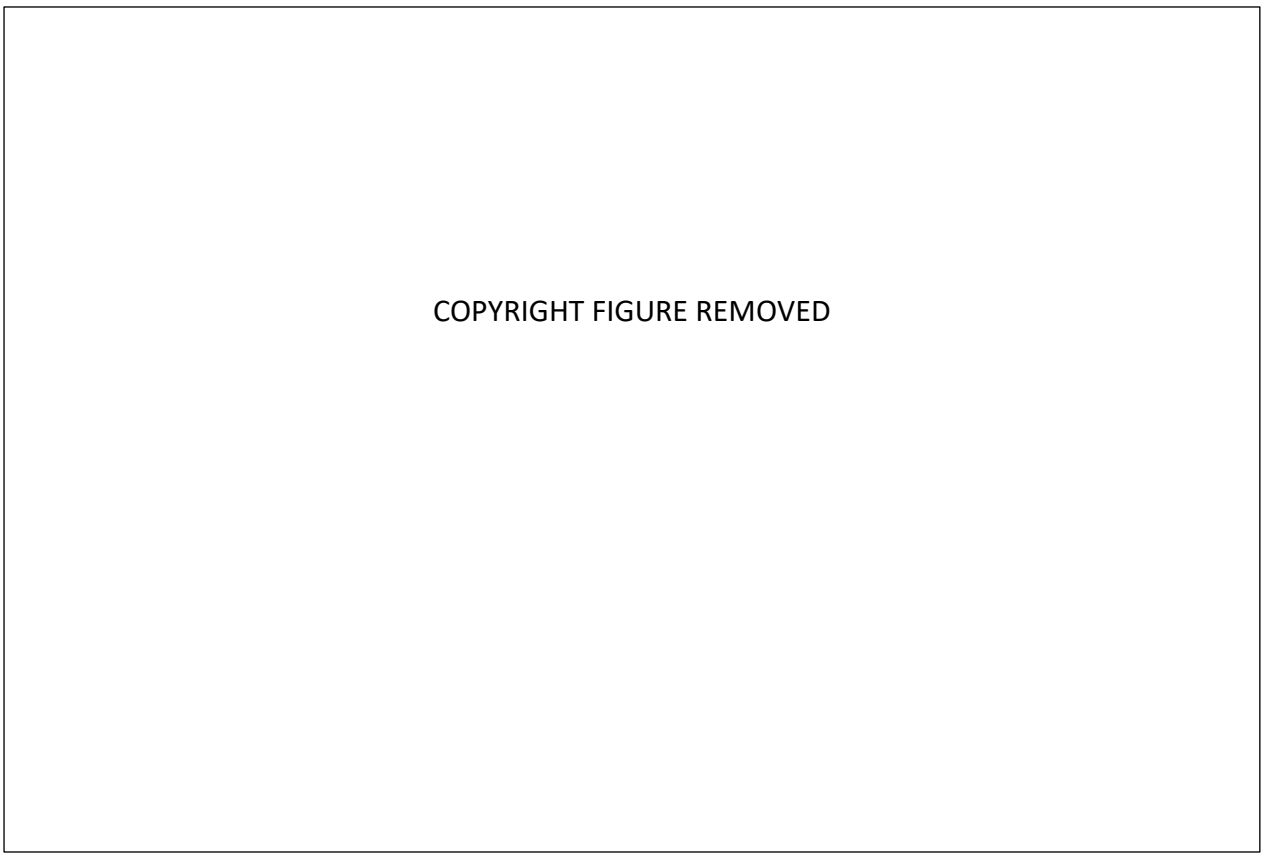


Figure 2.2 Renin-angiotensin system: Classical view. ACE: Angiotensin-converting enzyme. AT1-R: Angiotensin II type 1 receptor. Taken from: Physiology, Renin and Angiotensin system (Fountain et al., 2023).

2.4.3 Hypertension and Stroke

Stroke is one of the main causes of death and disability in many countries (Venketasubramanian et al., 2017). Hypertension is the single most important modifiable risk factor for stroke; with more than half of all strokes worldwide attributed to hypertension (Lawes *et al.*, 2008; O'Donnell *et al.*, 2016). Elevated systolic blood pressure, with or without an accompanying increase in diastolic blood pressure, has been shown to increase the risk of stroke (Kannel et al., 1981). A meta-analysis of clinical trials showed that with 10 mmHg reduction in systolic blood pressure, stroke risk reduced by 41% (Law et al., 2009).

2.4.4 Different types of stroke

There are two main types of stroke: haemorrhagic and ischemic. Haemorrhagic stroke involves bleeding within the brain and results from a variety of conditions including uncontrolled hypertension and aneurysms. Two types of haemorrhagic strokes exist: intracerebral and subarachnoid, which are associated with brain tissue damage emerging from the rupture of a cerebral artery within or on the surface of the brain, respectively. There are 2 types of ischaemic stroke: thrombotic and embolic. Thrombotic strokes are caused by a blood clot forming in an artery inside or leading to the brain, as a result of atherosclerosis. Thrombotic strokes are further stratified into lacunar and nonlacunar strokes. Lacunar infarcts are small infarcts in the deep cerebral white matter, basal ganglia, or pons which are thought to stem from the occlusion of a small perforating artery feeding the subcortical areas of the brain (Bamford et al., 1991). Embolic strokes, occur when an embolus (a piece of intravascular itinerant object) breaks loose and travels through the bloodstream to a part of the brain and occluding a small cerebral artery reducing cerebral blood flow. Most emboli are caused by atrial fibrillation, endocarditis, mitral stenosis, or atherosclerosis (Hisham and Bayraktutan, 2013). Hypertension is associated with all ischemic stroke subtypes.

2.4.5 Pathophysiology of hypertension and stroke.

The role of hypertension in stroke is complex and multifaceted. Hypertension has been associated with causing deleterious effects to the structural integrity of the brain and is the number one risk factor for stroke and age-related cognitive decline (Dahlöf, 2007).

2.4.6 Hypertrophy, remodelling and stiffening of cerebral vessels.

Increased shear stress on the blood brain barrier (BBB) is likely to contribute to the pathophysiology of stroke (Johansson, 1999). Hypertrophy and remodelling are adaptive responses aimed at reducing stress on the vascular wall and protecting downstream microvessels from the effect of increased pressure (Baumbach and Heistad, 1988, Laurent et al., 2005). Hypertension causes chronic increases in shear stress on the endothelium, altering endothelial structure and function, which promotes the development of atherosclerotic plaques in the cerebral arteries which may lead to arterial occlusions and ischemic injury (Dahlöf, 2007, Alistair, 2002). Hypertension alters the structure of cerebral blood vessels by producing vascular hypertrophy and remodelling and by promoting atherosclerosis in large cerebral arteries and lipohyalinosis in penetrating arterioles (Dickinson, 2001, Faraci and Heistad, 1990). Furthermore, smooth muscle cells undergo hypertrophy and hyperplasia and grow inward encroaching into the lumen of the artery which increases wall thickness and reduces the lumen of the artery (Baumbach and Heistad, 1988). The increase in shear stress also promotes the release of several vasoactive substances, such as endothelin, nitric oxide, cytokines, and reactive oxygen species which eventually play a key role in hypertrophy, remodelling, and stiffening (Yu et al., 2011). The increase in pulse pressure as a result of vascular stiffening is a good predictor of stroke (Baumbach and Heistad, 1988). Hypertension impairs the function of cerebral blood vessels which increases the risk of stroke (Faraci and Heistad, 1990).

2.4.7 Treatment for hypertension

Given the importance of hypertension in the pathophysiology of stroke, there is compelling evidence that suggest that controlling blood pressure contributes to stroke prevention as well

as prevention of other cardiovascular diseases. Lifestyles changes and pharmaceutical interventions are used to reduce risk factors such as hypertension and therefore reduce the risk of stroke. Lifestyle modifications including diet modifications, weight management and increases in physical activity, should be used as a first line treatment (Pescatello et al., 2015).

Clinical trials for individuals on antihypertensive medication have shown that there are 38% fewer strokes in individuals achieving mean blood pressure reductions of 10 to 12 mmHg systolic BP and 5 to 6 mmHg DBP (MacMahon, 1996). At present, several antihypertensive agents, including diuretics, beta-adrenergic blocking agents (β-blockers), angiotensin-converting enzyme inhibitors (ACEIs), ARBs, and calcium channel blockers (CCBs) are available to effectively lower BP (Hisham and Bayraktutan, 2013). In a meta-analysis of 23 randomised trials with stroke outcomes, antihypertensive drug treatment reduced risk of stroke by 32% in comparison to no drug treatment (Psaty et al., 2003). Another meta-analysis evaluated different classes of drugs used in subjects with a baseline blood pressure >140/90 mmHg to prevent stroke. This analysis found thiazide diuretics to have a relative risk (RR) of 0.63, beta-blockers to have a RR of 0.83, ACE inhibitors to have a RR of 0.65, and CCBs to have a RR of 0.58. Each drug type reduced the risk of stroke compared to placebo (Wright et al., 2018). The studies presented highlight the importance of antihypertensive medication in control blood pressure and reducing stroke risk.

2.5 Cerebrovasculature and cerebrovascular function

Stroke is a heterogeneous syndrome caused by multiple disease mechanisms, but all result in a disruption of cerebral blood flow with subsequent tissue damage. Transcranial Doppler

ultrasound has been used for the evaluation of cerebrovascular disease as it provides insight into a wide range of intracranial and extracranial vascular pathological conditions and their effects on cerebral haemodynamics (Sarkar et al., 2007).

2.5.1 The cerebrovascular system

Despite only occupying 2-3% of total human body mass, the brain requires 15% of cardiac output and ~20% of available oxygen for normal function due to its high metabolic function (Willie and Smith, 2011, Claassen et al., 2021). Control of sufficient cerebral blood flow (CBF) is critical to maintain normal cognitive function, as both hypoperfusion (insufficient CBF) and hyperperfusion (excessive CBF) can cause damage to the brain. Hypoperfusion can cause damage through ischemic injury whilst hyperperfusion can result in the breakdown of the blood-brain barrier which can cause seizures, headaches, encephalopathy and stroke (ischemic and haemorrhagic) (Tzeng and Ainslie, 2014, Fantini et al., 2016). The brain controls its own blood flow responds to changes in carbon dioxide (CO_2), neural activity and blood pressure (Payne, 2016). Differentially, the peripheral vessels are tolerant to changes in flow and pressure, responding with vasodilation or vasoconstriction, dependent on the endothelial response to a given stimulus (Vlachopoulos et al., 2011).

2.5.2 Anatomy of the cerebral vasculature

The cerebral vasculature is made up of highly specialised structures that assure constant brain perfusion. Regulation of blood flow to the brain is an extremely complex process as the brain has a limited capacity to store energy (Brown and Ransom, 2007). In a normal physiological state, total blood flow to the brain is remarkably constant due to part to the prominent

contribution of large arteries to vascular resistance (Faraci and Heistad, 1990). Blood flow to the brain is supplied by two internal carotid arteries (ICAs) and two vertebral arteries (VA). The ICA bifurcate into the middle cerebral artery and anterior cerebral arteries with the ICAs carrying ~80% of the total blood flow to the brain. The two VA join distally to form the basilar artery (BA) which bifurcates into two posterior cerebral arteries. Proximally, the anterior (ICA and its branches) and the posterior circulation (vertebral arteries and its branches) arteries come together at the base of the skull to form the circle of Willis (Agarwal and Carare, 2021). The circle of Willis gives rise to three pairs of main arteries: Anterior (ACA), middle (MCA) and posterior cerebral arteries (PCA) (Figure 2.3).

2.5.3 Regulation and control of the cerebral blood flow

CBF is defined as the rate of blood delivered by the arteries to the capillary bed in brain tissue and is calculated as the volume of blood in millilitres per 100 g of the cerebral tissue per minute (Bertsch et al., 2009). CBF is regulated by metabolic demand, myogenic and neurogenic factors (Ainslie and Duffin, 2009). The myogenic response refers to the active behaviour of the vasculature to respond to changes in BP. CBF is dependent on cerebral perfusion pressure (CPP) and inversely proportional to cerebral vascular resistance (Ainslie and Duffin, 2009, Edvinsson and Krause, 1993). Cerebrovascular resistance refers to the resistant forces acting on blood flow through the brain with resistance to flow occurring mostly in the cerebral arteries and capillary beds, with increasing vascular tone in turn increasing resistance (Ainslie and Duffin, 2009).

Transcranial Doppler (TCD) ultrasound provides rapid, noninvasive, real-time measures of cerebral blood velocities (CBv) and can be used to assess the physiological health of the cerebral blood vessels. TCD ultrasonography is a transmitter that emits pulsed ultrasound waves from a probe that can be focused on the vessels of interest. The Doppler shift creates a reflection of ultrasound waves from the moving red blood cells within the isolated blood vessel that are returned to the receiver unit within the Doppler probe. The Doppler shift is the difference between the transmitted and received ultrasound signals (Aaslid, 1986). Cerebral vessels can be isonated from four different acoustic windows; Transorbital, Transtemproal, Submedibular and Tranforaminal (Figure 2.3). Vessel identification is achieved based on knowledge of the circle of Willis (Willie and Smith, 2011). Understanding of isonation depths and flow direction of the vessel of interest is vital in order to acquire reliable and valid signals (Table 2.2).

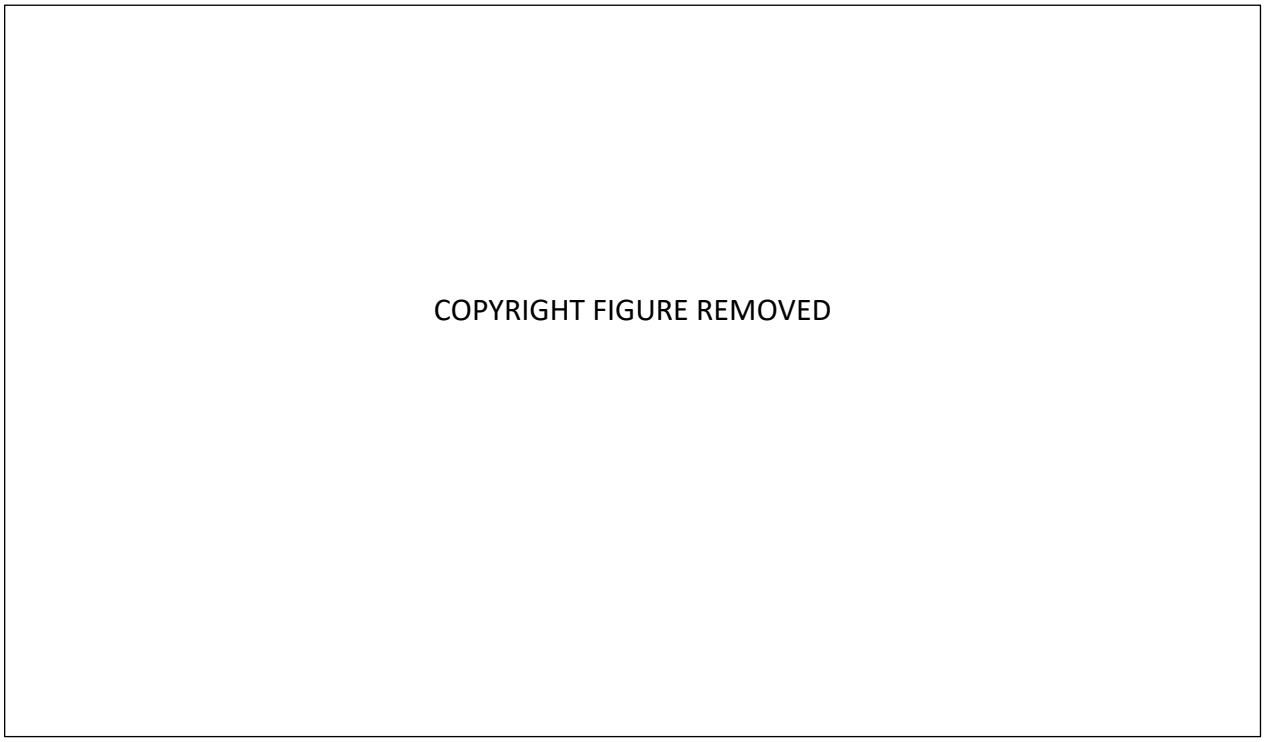


Figure 2.3. Acoustic windows used in order to isonate cerebral blood vessels.

Table 2.2 Identification of cerebral arteries using transcranial Doppler Ultrasound.

Vessel	Probe Direction	Depth	Mean velocity	Flow Direction
ACA	Anterior	60-80	50 ±11	Away
MCA	Perpendicular	30-60	55 ± 12	Toward
PCA	Posterior	60-70	39 ± 12	Toward

Regulation of blood flow to the brain is an extremely complex process. CBF is largely controlled by changes in vascular resistance in parenchymal arterioles. The large pial arteries on the surface of the cortex contain multiple layers of vascular smooth muscle cells (Wei et al., 1980). These pial vessels branch into penetrating arterioles containing a single layer of

vascular smooth muscle cells (Nishimura et al., 2007). The vascular smooth muscle cells will then relax or contract depending upon the stimulus. The regulation of CBF involves numerous mechanisms that are driven primarily through the following key factors: blood pressure (cerebral autoregulation), cerebral metabolism (neurovascular coupling), chemical control (cerebrovascular reactivity) and the autonomic nervous system. These mechanisms and factors will be discussed below.

2.5.3.1 Blood pressure (Cerebral autoregulation).

CA refers to the brains intrinsic ability to maintain a relatively constant blood flow, despite changes in arterial BP (or more accurately, cerebral perfusion pressure, CPP) (Paulson, 1990). CBF is maintained despite changing BP due to changes in cerebrovascular resistance (Kety et al., 1948, Willie et al., 2014). Arterioles constrict (vasoconstrict) when the pressure increases and relax (vasodilate) when pressure decreases. The primary driver of CA is CPP. As the brain depends on a continuous supply of oxygenated blood to prevent cerebral injury, an intact cerebral autoregulation is imperative to product against hypo- and hyperperfusion. Impaired CA has been observed in patients with traumatic brain injury (Czosnyka et al., 1996, Lang et al., 2003, Liu et al., 2015), ischemic and haemorrhagic strokes (Oeinck et al., 2013) and cerebrovascular stenosis (Reinhard et al., 2003). Impaired autoregulation in different pathological conditions can result in cognitive dysfunction, neurological damage, worse outcome, and increased mortality, even in non-brain injured patients (Longhitano et al., 2021).

The first indication of CA was noted in 1896, when it was stated that '*In all physiological conditions, a rise in arterial blood pressure accelerated the flow of blood through the brain and a fall slackens it*' (Bayliss et al., 1895). Lassen in 1959 constructed a plot of average BP and total brain blood flow from seven studies involving 11 different patient groups having a range of drug- and/or pathology-induced BP levels. This plot suggested that a plateau region is present in which CBF appears to be stable between ~60-150 mmHg (Lassen, 1959). However, advancements in research have suggested that it should no longer be referred to as a plateau phase as more recent studies have shown that the relationship between this BP and CBF within this autoregulatory range can vary between an upward or even downward slope (Claassen, 2016, Liu et al., 2016, Numan et al., 2014, Lucas et al., 2010) (Figure 2.4).

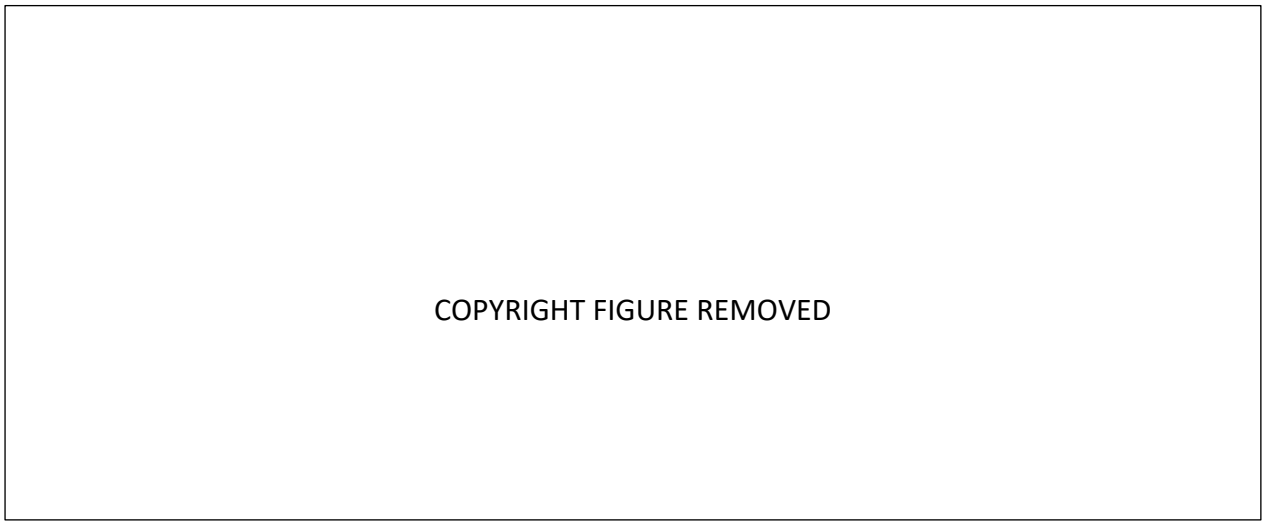


Figure 2.4 A) The classical view of the relationships between mean arterial pressure (MAP) and cerebral blood flow (CBF), i.e. autoregulation (Lassen, 1959). B) an updated view of the relationship indicating a small plateau region (Tan, 2012). This indicates a far more pressure-passive CBF than is conventionally believed, and that more efficacious buffering capacity against increases than decreases in perfusion pressure. Taken from (Willie et al., 2014).

CA can be classified as static (sCA) or dynamic (dCA). sCA refers to the relationship between BP and CBF when both variables have reached a steady state (Panerai, 1998, Panerai et al., 1998). sCA evaluates the overall efficiency of the autoregulatory action (i.e. the change in cerebrovascular resistance in response to manipulation of ambulatory blood pressure but

does not address the time in which this change in cerebrovascular resistance is achieved, this is where dCA is vitally important as allows for measurements of changes in BP and CBF occurring over seconds (Aaslid et al., 1989, Tiecks et al., 1995). dCA looks at the cerebral pressure–flow relationship during transient changes in BP, such as during changes in posture (Willie et al., 2014). Originally, the foundation of dynamic CA came from a study investigating the temporal relationships between middle cerebral artery velocity (MCAv) and mean arterial pressure (MAP) during the release of inflated thigh occlusion cuffs that induce rapid transient hypotension (Aaslid et al., 1989). However, this was not well tolerated by individuals and is subject to variability in BP responses (Willie et al., 2014). Nowadays, dCA is typically assessed using the combination of TCD and finger photoplethysmography to examine the dynamic response between CBv and BP when transient changes in BP are induced. Simple squat-stand or sit-to-stand protocol is now commonly used as it produces transient changes in BP with less discomfit than thigh occlusion cuffs (Lipsitz et al., 2000).

2.5.3.2 Cerebral metabolism (neurovascular coupling)

CBF is closely linked to metabolic activity, as activation of regions of the brain results in changes in local CBF (Willie et al., 2014). Neurovascular coupling (NVC) describes a close temporal and regional linkage between neural activity and CBF responses. The brain is well equipped to provide suitable blood flow for a given metabolic demand due to two components: extremely high vascularisation, as well as redundant and sophisticated regulation of blood flow (Attwell et al., 2010). The neurovascular unit is made up of 3 key components: the vascular smooth muscle, the neuron, and the astrocyte gial cell. Changes in neural activity causes changes in local blood flow mediated by transmissions through the

astrocyte gial cell. The present understanding of the mechanisms underpinning neurovascular coupling are not fully understood (Phillips et al., 2016). Activation of the neurovascular unit causes the release of glutamate. Both neurons and astrocytes respond to increased extracellular glutamate to transmit direct and indirect vasoactive signals for the appropriate delivery and distribution of CBF (Attwell et al., 2010). The activation of both neuron and astrocytes causes the release of vasoactive substances (NO, adenosine or arachidonic acid) causing relaxation of the smooth muscle. Pericytes (small cells expressing contractile tissue in the capillaries) may also play a role in the regulation of blood flow during neurovascular coupling. Neurons are more often closer to pericytes than arterioles, creating the plausible scenario whereby neuronal activation first alters resistance through modulations in pericyte tone on capillaries, and signals are then transmitted 'up-stream' to arterioles. Pericytes and vascular smooth muscle cells hyperpolarise which initiates either vasodilation (via release of NO, adenosine or arachidonic acid) or vasoconstriction (release of endothelin or thromboxane) of the local cells (Muoio et al., 2014), increasing or decreasing CBF respectively.

NVC is impaired in many clinical conditions including stroke, spinal cord injury, Alzheimer's, diabetes and hypertension (Phillips et al., 2016). A possible explanation for impaired NVC in individuals with hypertension is due to increased activation of angiotensin I receptors on cerebral blood vessels and increased oxidative stress which inhibits neuronal and astrocytic vascular dilators (De Silva and Faraci, 2013, Takeda et al., 2009).

2.5.3.3 Chemical control (cerebrovascular reactivity)

Measuring the cerebrovascular response to changes in CO_2 concentration reflects an index of the ability of the cerebrovascular beds to dilate or constrict Is termed cerebrovascular reactivity (Ainslie and Duffin, 2009). The brain is extremely susceptible to changes in partial pressure of arterial carbon dioxide ($PaCO_2$) and hypoxia (Kety and Schmidt, 1948, Ainslie and Duffin, 2009). An increase in $PaCO_2$ (hypercapnia) results in cerebral arteriolar vasodilation, whilst a decrease in $PaCO_2$ (hypocapnia) will result in vasoconstriction leading to a reduction in CBF (Ainslie and Duffin, 2009). Cerebrovascular reactivity (CVR) provides an index of reactivity of the intracranial vessels in response to changes in blood flow or other physiological factors i.e. changes in CBv in response to a vasoactive stimulus (Fierstra et al., 2013). This physiological response can be viewed as a defence mechanism, whereby elevation in $PaCO_2$ lead to an increase in CBF in an attempt to "wash out" CO_2 from brain tissue (Ainslie and Duffin, 2009). Increased CO_2 alters plasma pH which activate K^+ channels in the vascular smooth muscle which results in the relaxation of cerebral vessels (Jackson, 2005). The increase in CBF is limited by the vasodilator reserve of the cerebral vessels i.e. the ceiling for this is not much greater than the limits of subjective tolerance to hypercapnia in most participants and seems to be in the region of ~15-20 mmHg above an individual's normocapnic baseline (Willie et al., 2012) or 10-15% inhaled CO_2 (Sicard and Duong, 2005). The typical increase in CBF is approximately 3-6% and the decrease is 1-3% in flow per mmHg change in CO_2 from rest (Willie et al., 2014).

In clinical and research settings, CVR is often reported as an index of cerebrovascular health, as it represents integrative functionality of the cerebrovascular mechanisms of tone regulation and cellular pH maintenance (Gupta et al., 2012). The mechanisms responsible for the role of CO_2 in regulating vascular tone and pH are not fully identified, it could relate to pH

levels. More specifically, alterations in CO_2 levels cause changes in plasma pH levels. Increases in plasma CO_2 levels (causes acidosis) activates potassium (K^+) channels in the vascular smooth muscle which causes dilation (relaxation) of the cerebral blood vessels (Jackson, 2005, Ainslie and Duffin, 2009). Studies have also highlighted that shear stress is increased following hypercapnia (Ainslie and Duffin, 2009) which can mediate NO vasodilation of the cerebral vessels, suggesting that this measure can be used to quantify cerebrovascular endothelial function (Carter et al., 2016).

CVR changes in response to CO_2 has been used extensively to assess cerebrovascular regulation in healthy and clinical populations. The Rotterdam study in 1695 individuals investigated the association between cerebrovascular reactivity and cardiovascular mortality (independent of stroke) and found that lower cerebrovascular reactivity to a 5% CO_2 stimulus was associated with increased risk of all cause death (Portegies et al., 2014). Reduced CBF and lower responses to CO_2 stimulus have also been observed in individuals with clinical diseases such as atrial fibrillation (Junejo et al., 2019), stroke (Markus and Cullinane, 2001) and dementia (SS Meel-van den Abeelen et al., 2014). Thus, CVR has become a common functional test to assess brain vascular health (Burley et al., 2020).

2.5.4 The Selfish Brain Hypothesis

The Selfish Brain Hypothesis proposes that the brain plays a central role in regulating blood pressure to ensure adequate cerebral perfusion for metabolic needs which may result in increased blood pressure (Hart, 2016). This theory suggests that hypertension is a protective mechanism which protects the brain from ischemic damage. The first study to measure CBF and cerebrovascular resistance in individuals with hypertension reported that CBF was similar

to values reported in young normotensive men however, cerebrovascular resistance was elevated in the hypertensive group (Kety et al., 1948). This research group originally suggested that the elevation in cerebral vascular resistance was either causal in hypertension or a consequence of hypertension. Previous evidence has demonstrated that human vessels show hypertrophy and reduced lumen size in individuals with hypertension compared to control individuals (Dickinson and Thomson, 1959, Rizzoni et al., 2009). These alterations are viewed to be caused by high blood pressure as a mechanism to protect smaller downstream arterioles, capillaries, and venules from high pressure. The Selfish Brain Hypothesis suggests that cerebral vascular changes (vascular damage, vessel hypertrophy and elevated cerebral vascular resistance) could occur before the onset of hypertension (Hart, 2016). The hypothesis does not dismiss that vascular hypertension leads to remodelling of the cerebral vessels but suggests that other factors could lead to cerebrovascular damage and increased cerebrovascular resistance before the onset of hypertension. This hypothesis suggests that congenital cerebral anatomic abnormalities, vascular damage from inflammation and increased levels of ROS and/or alterations in the collagen type and elastic content of cerebral vessels, may lead to increased cerebral vascular resistance before the onset of hypertension with high blood pressure causing further cerebrovascular remodelling as the disease progresses (Hart, 2016). However, the majority of evidence supporting the Selfish Brain Hypothesis has been in animals, with only some preliminary data from this research group suggesting that this may also occur in humans.

2.5.5 Cerebrovascular function and the hypertensive brain

As highlighted in section 2.4.5, hypertension has been associated with alterations in vascular structure and function due to hypertrophy, remodelling and stiffening of cerebral vessels which can alter cerebrovascular function. As cerebral blood flow is determined by responses of the cerebral arteries, it is important to understand the importance of hypertension on cerebrovascular function measures. The focus of this section is to explore how cerebrovascular function measures interact with the unique challenges posed by hypertension.

2.5.5.1 Hypertension and Cerebral autoregulation

Cerebral autoregulation is often impaired in hypertensive individuals. Whilst the cerebral circulation retains its ability to autoregulate, it shifts the autoregulation curve to the right so that higher levels of blood pressure are needed to maintain CBF in the autoregulated range (Figure 2.5) (Paulson et al., 1990). These alterations were postulated to be due to altered reactivity which may expose individuals with hypertension to periods of decreased CBF (Paulson, 1990). The shift in autoregulation to the increase in myogenic tone is induced by an increase in Ca^{2+} sensitivity of myocytes (Chrissobolis and Sobey, 2006). These changes may relate to alterations in the structure of the artery wall, reduced vascular lumen and increased stiffness of the cerebral resistance vessels (Asmar et al., 1997, Baumbach and Heistad, 1988).

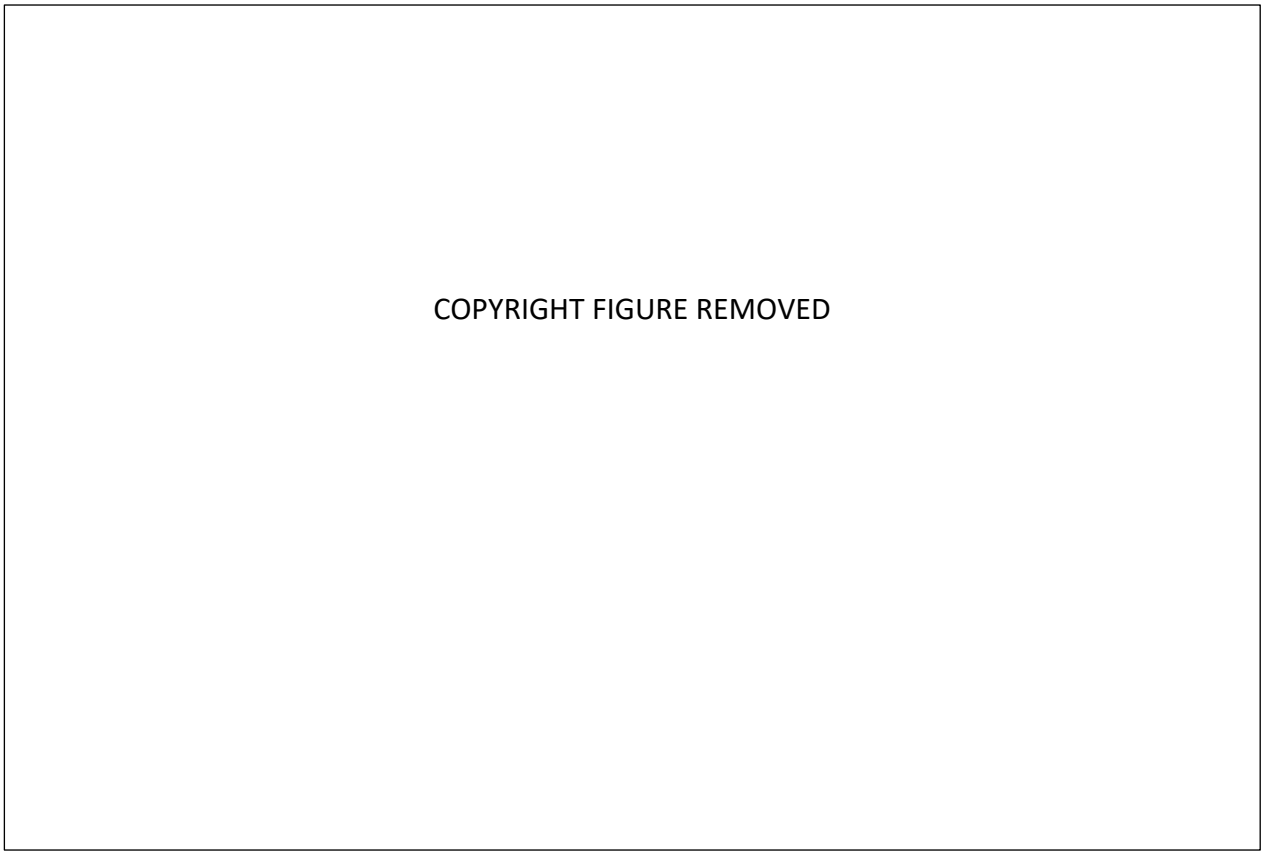


Figure 2.5. The range is right shifted in chronically hypertensive patients (Ruland and Aiyagari, 2007).

2.5.5.2 Hypertension and cerebrovascular reactivity

In hypertensive patients, TCD measurements after vasodilatory stimuli (such as intravenously administered acetazolamide, breath holding, and hyperventilation) have indicated decreased reactivity of the cerebral micro-vasculature, (Ficzere et al., 1997, Maeda et al., 1994, Magyar et al., 2001, Fujishima et al., 2001, Pieniażek et al., 2001) which can be improved by the administration of antihypertensive treatment (Pieniażek et al., 2001).

2.5.5.3 Hypertension and neurovascular coupling

Limited research in humans has investigated the effects of hypertension on neurovascular coupling. The increase in CBF in posterior parietal and thalamic areas produced by cognitive

tasks is reduced in patients with chronic untreated hypertension relative to normotensive individuals (Jennings et al., 2005). The attenuated CBF response was associated with a lower cognitive performance (Jennings et al., 2005). Overall, there is evidence that hypertension may influence CBF, which could impair the structural and functional integrity of the brain.

2.5.6 Cerebrovascular function during exercise

It is important to consider what happens to CBF during acute exercise as the response of cerebral vasculature is different to other peripheral vasculature due to the smaller vascular bed and it being strongly regulated by cerebral autoregulation and $PaCO_2$ (Ogoh and Ainslie, 2009). Despite an increase of cardiac output of around 300-600% (Jarvis et al., 2007) and skeletal muscle blood of 800-100%, (Jorfeldt et al., 1978), CBF only increases around 10-30% to maintain adequate substrate delivery (Smith and Ainslie, 2017). Studies using TCD have shown that the onset of exercise there is an increase in CBv (Ogoh et al., 2005a, Ogoh et al., 2007a, Ogoh et al., 2005b, Ogoh et al., 2008). Research suggests that during an acute bout of aerobic exercise, blood pressure progressively increases up to maximal intensities by ~20-30%, whilst CBv increases linearly with exercise intensity up to approximately 60-70% maximal oxygen uptake (VO_{2max}) (Smith and Ainslie, 2017). However, during resistance exercise rapid fluctuations in blood pressure occur which results in insufficient cerebral autoregulation therefore MCAv varies with MAP (Edwards et al., 2002, Romero and Cooke, 2007). Studies on the MCAv during dynamic resistance exercise are ambiguous with some reporting increases (Koch et al., 2005, Moralez et al., 2012, Romero and Cooke, 2007, Hartwich et al., 2010, Kim et al., 2007), no change (Edwards et al., 2002) or decreases (Dickerman et al., 2000). In order to ensure that an adequate oxygen delivery to working

skeletal muscles during exercise, systemic blood pressure and cardiac output increase which challenges the cerebrovasculature (Marsden et al., 2012) highlighting the importance of an intact cerebral autoregulation. It is believed that is more proficient in counteracting brief hypertension compared to hypotension, possibly due to the dangers associated with increased hypertension (Marsden et al., 2012). CA is maintained during mild to moderate exercise in young and older adults overall, data suggests that increases in resting CBv is likely to contribute to positive cerebrovascular adaptations (Smith and Ainslie, 2017)

CBF at rest is influenced by many factors, however P_aCO_2 is the primary factor and mediator of CBF in response to exercise. P_aCO_2 increases with exercise until exercise intensity reaches about a threshold of ~70-80% VO_{2max}. Individuals will hyperventilate during high intensity exercise which induces hypocapnia, causing a reduction in P_aCO_2 which causes CBFv to plateau or decrease towards baseline (Smith et al., 2014). This response to CBF occurs despite continued elevations of arterial blood pressure, cardiac output and increasing oxygen consumption demands of the brain (Moraine et al., 1993). CVR is increased during aerobic exercise, in order to maintain CO_2 homeostasis in the brain (Ogoh et al., 2008).

Given that the individual cardiorespiratory responses during incremental exercise (i.e. arterial blood pressure, P_aCO_2 and cardiac output) occur concurrently but independently of one another, it is difficult to differentiate the independent influences of PaCO2 and arterial blood pressure on CBF regulation during exercise (Smith and Ainslie, 2017). More research is warranted to understand the precise mechanisms that affect CBF during exercise in both healthy individuals and clinical populations who often have impaired cerebrovascular

function especially as exercise interventions are often implemented to improve cerebrovascular function (Smith and Ainslie, 2017).

In non-stroke populations, older adults demonstrate an increase in MCAv between 10% and 30% during low- to moderate-intensity dynamic exercise (Braz and Fisher, 2016). A systematic review and meta-analysis reported that individuals after stroke may have attenuated cerebrovascular haemodynamics; as measured by the MCAv during acute moderate-intensity exercise which show ∼8–12% increase in MCAv during moderate-intensity exercise (Moncion et al., 2022). Aerobic exercise training is beneficial for improving cardiovascular health and function after stroke. However, high quality research is needed to examine the potential role of exercise training on improving cerebrovascular haemodynamics after stroke (Moncion et al., 2022).

Physiological response during resistance exercise (RE) differ compared to aerobic exercise. resistance training provides substantial challenges to blood flow regulation due to changes in blood pressure. The exact blood pressure response is dependent upon contraction type and intensity which are mediated by increases in both systolic and diastolic pressures

During dynamic RE, rapid fluctuations in blood pressure occur which results in insufficient cerebral autoregulation therefore MCAv varies with MAP (Edwards et al., 2002, Romero and Cooke, 2007)

2.5.7 Cerebrovascular function following acute exercise

Relatively little is known about the immediate effects of exercise on cerebrovascular blood flow and function. A single bout of cardiovascular exercise affects CBF in a number of ways:

An increased heart rate results in a higher blood flow that allows for enhanced oxygen, excitatory neurotransmitter and nutrient supply to the brain (Olivo et al., 2021). CBF may also increase as exercise may induce an increase in cerebral perfusion due to the mental effort required and the associated increase in cortical activation (Billinger et al., 2021, Hiura et al., 2010).

As with peripheral vascular function (FMD), the acute effect of aerobic exercise on CBv are inconsistent among the literature with studies reporting increases, decreases or no change in CBF following exercise (Robertson et al., 2015, Hellstrom et al., 1996, MacIntosh et al., 2014, Williamson et al., 2009, Williamson et al., 2004, Willie et al., 2013, Ainslie et al., 2007). Inconsistent results across studies may be due to small sample sizes, differences in measurement of different cerebral vessels or regions, variable duration and intensity of the exercise stimulus, and differences in timings of post exercise measurements as outlined below.

Cerebral Reactivity: Exercise intensity is important to consider when looking at cerebrovascular reactivity. Research has shown that in healthy adults, one bout of high intensity interval training (25 minutes completed at 85–90% heart rate reserve) significantly lowered CVR (7% CO_2 closed circuit rebreathing) to hypercapnia immediately and 1 h post exercise but was restored to baseline levels 2 h following exercise but remained unchanged following moderate intensity exercise (45 minutes completed at 50-60% heart rate reserve (Burma et al., 2020). However, another study found that exercise intensity had no effect on CVR to both hypercapnia and hypocapnia 1 and 3 hours post exercise cessation (6% CO_2 4-minute open circuit), following moderate and high intensity exercise (Weston et al., 2022). It

is also important to highlight the difference measurement techniques of CVR used within the studies which makes it difficult to truly understand the impact of acute exercise on cerebrovascular reactivity.

Cerebral autoregulation: Current understanding of CA immediately following exercise training is limited. During the post exercise reduction in BP and heart rate, there is conflicting evidence for the stability of CA. The type of exercise may influence CA responses following acute exercise. One study found BP was lower in the hour following a 10-min and to a greater extent, a 30-min bout of aerobic exercise as compared to sitting whilst MCAv remained unchanged suggesting that CA is not impaired following aerobic exercise (Perdomo et al., 2019). CA was also maintained following mountain marathons (Murrell et al., 2007) and 4 hours of continuous running (Murrell et al., 2009). However, during the recovery phase following dynamic resistance exercise, cerebral autoregulation was temporarily disturbed (Koch et al., 2005, Ogoh et al., 2005b). Results are conflicting on the effect of exercise intensity of CA. Studies have reported impaired CA following maximal exercise, as assessed by the rate of regulation during suprasystolic thigh-cuff release (Bailey et al., 2011). However, following moderate intensity exercise, CA has been unaltered (Ogoh et al., 2007b, Perdomo et al., 2019). Furthermore, CA at the mean phase of MCAv velocity profile was found to be stable during the first 10 minutes following the end of mild, moderate and heavy cycling (Ogoh et al., 2005b). However, when looking at 3 different time points following acute aerobic exercise bout, MCAv was maintained despite the presence of post exercise hypotension and exercise induced hypocapnia (Willie et al., 2013). Differences in findings could be due to different methods of CA assessment and the types of exercise performed.

2.5.8 Impact of exercise training in cerebrovascular function in healthy humans

Exercise training has been shown to have beneficial effects on cerebrovascular health (Bliss et al., 2021) including improved endothelial function and cerebral angiogenesis. Even though cerebral blood flow declines with age, maintaining a high cardiorespiratory fitness can protect individuals against some of the decline in cerebrovascular function (Ainslie et al., 2008). This protection is, in part, suggested to be due to established benefits of exercise training on cardiorespiratory fitness (Weston et al., 2014). There are conflicting findings when examining the effects of exercise training interventions in order to improve CBF and cerebrovascular function to counteract this age-related decline. A recent review has suggested that short-term exercise training has little impact on resting MCAv (Smith et al., 2021). Eight weeks of moderate intensity cycling increase CBv in postmenopausal women (Akazawa et al., 2012), young health females (Bailey et al., 2016) and sedentary older men (Kleinloog et al., 2019). Others have found that 12 weeks of aerobic exercise does not improve CBv in healthy young and older individuals (Murrell et al., 2013). Further research has shown that the environment that we train in may alter the response of exercise training. Eight weeks of exercise training in a thermoneutral environment or a cold environment both showed no change in CBv or CVR, however exercise in the cold was shown to enhance dCA. Interestingly, the changes in dCA were not directly explained by greater CBv or haemodynamics during acute exercise in young healthy males (Miller et al., 2022). In summary, short-term exercise training may improve cerebrovascular blood flow and function; however, more research is needed to elucidate the type and intensity of exercise needed to elicit these changes.

2.5.9 Impact of Exercise training in cerebrovascular function in clinical populations

Clinical populations often have impaired CBF and cerebrovascular function therefore exercise interventions that have the potential to improve cerebrovascular function have real potential clinical benefit. Eight weeks of aerobic exercise in individuals with chronic obstructive pulmonary disease resulted in no change in dCA, CVR or CBv (Lewis et al., 2019). A review in stroke survivors exercise training does not appear to significantly change resting hemodynamic variables, including MCAv and CBF, although this finding must be interpreted with caution because of small sample sizes (Moncion et al., 2022). However, a study showed that following 6 months of aerobic treadmill exercise in stroke survivors improved CVR (Ivey et al., 2011) yet no such changes were evident following 18 weeks of training in congestive heart failure patients (Tanne et al., 2005). Research is limited within clinical populations. More research is warranted as responses to exercise training may differ between clinical conditions, different exercise modalities and different exercise training durations.

2.6 Summary

In summary, exercise is important for improving risk of cardiovascular and cerebrovascular events and mortality. However, individuals with cardiovascular disease risk may show altered responses to physical activity as they may not demonstrate the same cardiovascular risk reduction compared to healthy individuals (Moholdt et al., 2008, Stewart et al., 2017, Jeong et al., 2019, Mons et al., 2014). This finding requires more in-depth analysis in order to understand why individuals with cardiovascular disease risk factors may show the impaired response, either acutely or chronically, but also in relation to the study population that is examined. This information is important to optimise, but also to personalise, the benefits of exercise training in those need exercise the most. Hypertension is a key modifiable risk factor

for cardiovascular disease and stroke (Mills et al., 2020) and can impact cerebral blood flow however there is limited evidence on the acute responses of exercise on cerebrovascular function. Understanding this can help further understand the chronic adaptations of regular exercise training and can contribute to effectiveness of cardioprotective strategies and prevent adverse events during and after exercise (Vita and Keaney Jr, 2002, Mittleman et al., 1993, Siscovick et al., 1984, Whang et al., 2006, Albert et al., 2000, Willich et al., 1993, Smyth et al., 2016).

Chapter 3: The impact of hypertension on the dose-response association between physical activity and stroke: A cohort study among 139,930 adults from the Netherlands.

3.1 Introduction

Stroke remains a significant health problem, with a predicted increased incidence of 34% between 2015 and 2035 (Stevens et al., 2017). This highlights the need for preventive strategies to lower the risk for stroke, and there by reducing the health and socio-economic effects pertaining to stroke.

Several studies found potential health benefits of physical activity (PA) in the prevention of stroke (Tajiri et al., 2019, Jeong et al., 2017, Kono et al., 2015). For example, ≥30 minutes of moderate-to-vigorous (MV) PA during 1 to 2 times per week resulted in a 16% risk reduction for the risk of stroke compared to inactive individuals (Jeong et al., 2017). Similarly, 3 to 4 times/week and ≥5 times/week of MVPA sessions were also associated with 21 and 22% a lower risk of stroke compared to inactivity, respectively (Jeong et al., 2017). In addition to the risk of stroke, MVPA is also associated with a lower risk of developing hypertension, an important modifiable risk factors for stroke (Pinckard K, 2019). Some studies have suggested that the positive responses to PA may become stronger with the prevalence of risk factors, such as hypertension (Wannamethee and Shaper, 1992, Moholdt et al., 2008, Mons et al., 2014, Stewart et al., 2017, Jeong et al., 2019). Supporting this, a recent study in 142,493 adults found that individuals with cardiovascular risk factors may have larger benefits of MVPA (Bakker et al., 2021), as the dose-response association between MVPA and major cardiovascular events and mortality became stronger and contained larger risk reductions in individuals with risk factors compared to individuals without risk factors or with established CVD. The dose-response relation between physical activity and the risk for stroke, therefore,

may be affected by presence of hypertension, one of the key risk factors. Furthermore, anti-hypertensive pharmacological treatments may also interfere with the benefits of exercise training on the risk for clinical events.

Therefore, the aim of our study was to examine the dose-response association between MVPA and stroke and to determine whether this association differed between those with *a priori* hypertension and normotension. Secondly, we aimed to evaluate whether the use of anti-hypertensive medication alters this dose-response association. In line with recent work, we expect that those with hypertension will demonstrate a stronger inverse association between MVPA and the risk of stroke. In addition, we hypothesize that anti-hypertensive medication may attenuate the benefits of MVPA on stroke, as recent exercise training studies evaluating the impact of drugs on exercise mediated cardiovascular adaptations was blunted with simvastatin (Mikus et al., 2013) and angiotensin converting enzyme inhibitors whilst angiotensin-converting (Sjúrðarson et al., 2022).

3.2 Methods

3.2.1 Participants

This study used data from the Lifeline Cohort Study. Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviours of 167,729 persons living in the North of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-

morbidity and complex genetics (Stolk et al., 2008, Scholtens et al., 2015). All individuals who lived in the northern Netherlands were eligible to take part, other than those with 1) severe psychiatric or physical illness (e.g. individuals with cancer and associated reduced life expectancy), 2) life expectancy <5 years; and 3) lack of fluency in Dutch. Participants who were ≥18 years old were include (N=152,737). Participants were excluded (N=12,807) due to 1) missing data for PA, blood pressure and CVD health status; 2) limited ability to be physically active; 3) inability to merge individual data with registry data; and 4) the presence of cardiovascular disease (Supplemental Figure 3.1). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline was used to report our findings (Supplemental Table 3.1). All participants provided written informed consent. The Lifelines Cohort study was conducted according to the principles of the Declaration of Helsinki and approved by the University Medical Centre Groningen Medical Ethical Committee.

3.2.2 Experimental measures

Physical examination and questionnaire. Participants visited one of the Lifeline research sites for a physical examination and completed a baseline questionnaire between 2006 and 2013. Baseline data were collected for 167,729 participants, aged from 18 to 93 years. Every 1.5 years a follow up questionnaire was administered to assess the occurrence of a stroke.

The physical examination included anthropometric assessment, blood pressure (BP) measurements and blood draw. Anthropometrics included height (Stadiometer) and weight (Standard weighing scale), which were used to calculate body mass index (BMI; kg/m^2). Ten blood pressure measurements were taken during a 10-minute period using an automated

sphygmomanometer (Dynamap, PRO 100 or PRO 100V2) placed around the upper right arm (or the left arm if contraindications were present) with participants in a seated position, the two last successive blood pressures were averaged. Blood samples were taken after >8 hour fasting for measurements of low-density lipoprotein (LDL), glucose and serum creatinine. Renal function (estimated glomerular filtration rate, eGFR) was estimated (Foundatio, 2002).

Baseline questionnaires included questions on demographics, health status and lifestyle. Demographics included age, sex, postal code, income, and education level. Income was estimated using the postal codes and data of Statistics Netherlands (Statistics Netherlands, 2020) when not reported. Education level was categorised in low, moderate, and high. Medical history included self-reported information on medication use, presence of CVD, comorbidities and other illnesses including cancer, arthritis, multiple sclerosis, and Parkinson disease. Lifestyle factors included smoking status, alcohol consumption, and hours of sleep per night. Smoking status was categorised as currently, previously, and never. High alcohol consumption was defined as >14 drinks/week or >4 drinks/day for men and >7 drink/week or > 3 drink/day for women (Fleming, 2004).

Habitual Physical Activity Volumes. Baseline physical activity was assessed using the Short Questionnaire to Assess Health-enhancing Physical Activity (SQUASH). SQUASH divides habitual physical activity into transportation, occupation, household and leisure domains, and asks for the duration and intensity of an individual's typical weekly activities over the past 3 months (Wendel-Vos et al., 2003). Weekly physical activities were converted to the average amount of metabolic equivalent of task (MET) minutes per week based (Ainsworth et al., 2011). MET minutes were calculated by multiplying the MET values of each activity by the

duration (i.e., minutes/week). Only activities with ≥3 MET value were included, since these activities relate to moderate to vigorous activities as specified in the World Health Organisation PA guidelines (Bull et al., 2020). Individuals were categorised into 4 quartiles of least (Q1) to most (Q4) physically active based on self-reported MVPA volumes. Q1 included the least active individuals who spent <1830 MET min/week, Q2 included individuals with 1830-3617 MET min/week, Q3 included individuals with 3618-7175 MET min/week and Q4 included individuals with >7175 MET min/week.

Clinical outcomes. The primary endpoint was the occurrence of a stroke. Hospital registry data of statistics Netherlands were used to determine the primary outcome. When hospital registry data were not available, self-reported stroke during follow-up were used instead. Self-reported stroke was measures after a median follow-up of 1.1, 2.1, and 3.8 years. The date at which the questionnaire was completed, was used for the event date of self-reported stroke. Participants were followed until the first stroke event. Participants who did not reach the endpoint were censored at the end of the last assessment or at the date they died, whichever occurred first.

3.2.3 Hypertension

Hypertension. Participants were divided as hypertensive or normotensive based on the BP evaluation performed at baseline, whilst using the recent classifications of hypertension following the updated National Institute for Health Care Excellence (NICE) guidelines (i.e., ≥130/80 mmHg). In addition, those who reported being diagnosed by a physician with hypertension and used blood pressure lowering medication were included in the

hypertensive group. Those who did not report being diagnosed with hypertension and had a baseline blood pressure of ≤129/79 mmHg were categorised into the normotensive group.

Medicated. For our secondary analysis, we have divided the hypertensive group into a medicated and non-medicated group. Individuals were categorised into the medicated group if they were prescribed Angiotensin Converting Enzyme (ACE) inhibitors, angiotensin blockers, beta blockers, diuretics, or calcium antagonists.

3.2.4 Statistic analysis

Baseline characteristics were described for the normo- and hypertensive group. Normally distributed data were presented with mean (± standard deviation; SD) and non-normally distributed data were presented with the median (interquartile range; Q_{25} to Q_{75}).

Stratified Kaplan–Meier curves and log-rank tests were performed to assess differences in the outcome between the quartiles of physical activity. Multivariable Cox regression model was conducted to estimate the association between physical activity and stroke using hazard ratios (HRs) with 95% confidence intervals (CIs). First, an unadjusted model was performed (model 1). Model 2 was adjusted for age (years), sex (male/female), body mass index (BMI; (kg/m^2)), income (per 1,000 euros), education level (low/moderate/high), smoking (pack years), kidney function (glomerular filtration rate, $mL/min/1.73m^2$), glucose (mmol/L) and low-density lipo-protein (LDL; mmol/L). Model 3 included the variables of model 2 and was additionally adjusted for systolic blood pressure (mmHg), diastolic blood pressure (mmHg), use of acetylsalicyclic acid, anti-platelets and anti-hypertensive medication. The analyses were performed for the total population and were stratified for hypertensive and normotensive individuals. We also tested interaction in terms of blood pressure

(normotensive versus hypertensive) and the 4 quartiles of MVPA. In addition, to examine whether medication use altered the dose-response association of MVPA in hypertensive individuals, we performed stratified analyses and tested the interaction term.

Since the number of missing values was relatively small (<14%), the complete case was used for the analyses. All statistical analyses were performed in R version 3.5.2. P values < 0.05 were considered statistically significant.

3.3 Results

3.3.1 Participants

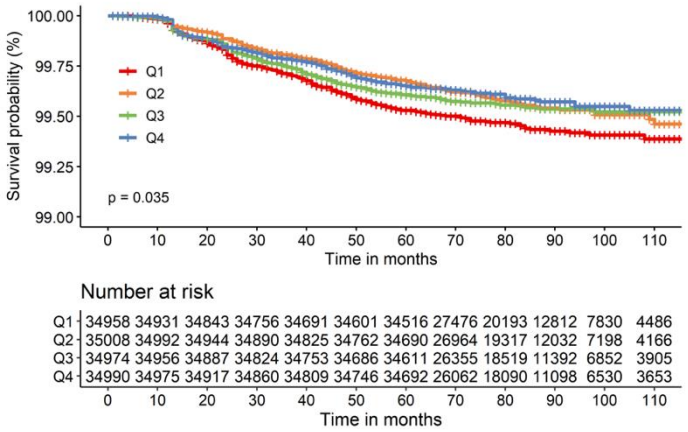
In total, 139,930 participants were included in our analysis. 41% of the population was male with an overall mean age of 41 (13) years (Table 3.1). The median follow up was 6.75 years [Q_{25} 5.83; Q_{75} 7.92] with a total number of 640 strokes (0.46%; Figure 3.1). Individuals with hypertension were more often male, older, had a lower education level and higher BMI, used more often medication, and had more comorbidities. Within the hypertensive group, medicated individuals were more often female, older, had a lower education level, and had more comorbidities compared to non-medicated (Supplemental table 3.3).

Table 3.1. Baseline characteristics for the total population (n=139,949) and individuals with normotension (N=78,309) and hypertension (n=61,621). Data are presented as mean (SD), median [Q25,Q75], and *n* (%).

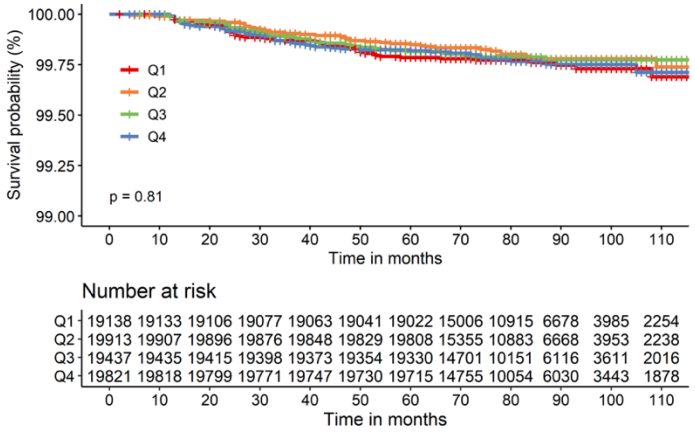
	Total population N=139,930	**Normotensive N= 78,309**	**Hypertensive N= 61,621**	**P-value normotensives vs hypertensives**
General characteristics				
Sex (male)	57,457 (41%)	24,479 (31%)	32,978 (54%)	<0.001
Age	44.12 (12.61)	40.67 (11.69)	48.51 (12.37)	<0.001
Income x 1000/year	27 [25, 30]	27.10 [25.00, 30.30]	27.00 [25.00, 29.90]	<0.001
Education level				<0.001
low	39,828 (29%)	18,085 (24%)	21,743 (36%)	
moderate	55,134 (40%)	32,465 (42%)	22,669 (38%)	
high	41,845 (30%)	26,207 (34%)	15,638 (26%)	
BMI (median [IQR])	25 [23, 28]	24.30 [22.30, 26.90]	26.70 [24.40, 29.50]	<0.001
Lifestyle characteristics				
Smoking status				<0.001
Never	64,616 (47%)	38,288 (50%)	26,328 (43%)	
Previous	44,804 (32%)	22,187 (29%)	22,617 (37%)	
Current	28,977 (21%)	16,872 (22%)	12,105 (20%)	
Alcohol consumption (high)	32,162 (24.3)	17,616 (24%)	14,546 (25%)	<0.001
Medication use				
Antiplatelet	176 (0.1%)	73 (0.1%)	103 (0.2%)	<0.001
Anti-hypertensive	7,568 (5%)	197 (0.3%)	7,371 (12%)	<0.001
Anti-coagulant	759 (0.5%)	256 (0.3%)	503 (0.8%)	<0.001
Acetylsalicylic acid	2,386 (2%)	677 (0.9%)	1,709 (3%)	<0.001
Beta-blocker	6,330 (5%)	952 (1%)	5,378 (9%)	<0.001
Calcium antagonists	2,244 (2%)	261 (0.3%)	1,983 (3%)	<0.001
Diuretics	4,859 (4%)	170 (0.2%)	4,689 (8%)	<0.001
Statins	6,322 (5%)	1622 (2%)	4,700 (8%)	<0.001
Alternative cholesterol lowering medication	311 (0.2%)	97 (0.1%)	214 (0.3%)	<0.001
Anti-diabetics	2,113 (2%)	520 (0.7%)	1,596 (2.6%)	<0.001
Health characteristics				
Diagnosed hypertension	12,291 (9%)	0 (0%)	12,291 (20%)	<0.001
Diagnosed hypercholesterolemia	17,983 (13%)	6,352 (8%)	11,631 (19%)	<0.001
Diagnosed diabetes	3,405 (2%)	835 (1%)	2,570 (4%)	<0.001
Systolic blood pressure	124 [115, 134]	116 [110, 122]	136 [130, 144]	<0.001
Diastolic blood pressure	73 [67, 79]	68 [65, 73]	81 [74, 85]	<0.001
Total cholesterol	5.00 [4.40, 5.70]	4.80 [4.20, 5.50]	5.20 [4.60, 5.90]	<0.001
LDL cholesterol	3.23 (0.91)	3.07 (0.88)	3.42 (0.92)	<0.001
HDL cholesterol	1.40 [1.20, 1.70]	1.50 [1.30, 1.80]	1.40 [1.20, 1.60]	<0.001
Triglycerides	0.97 [0.71, 1.39]	0.87 [0.65, 1.19]	1.14 [0.82, 1.62]	<0.001
Renal function	98 [87, 100]	100 [90, 100]	95 [84, 100]	<0.001

Data is presented as mean (SD), median [Q25, Q75] and n (%).

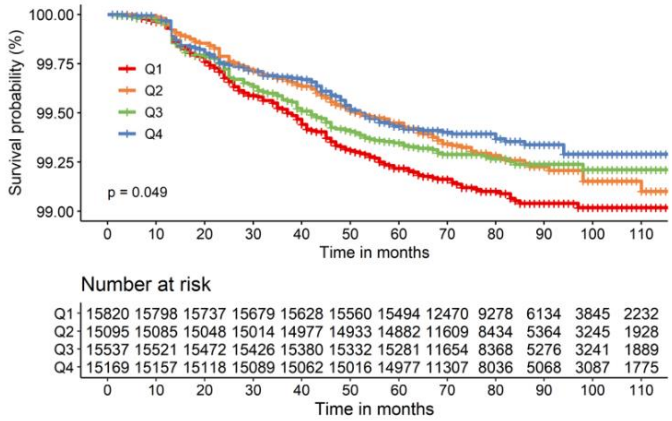
(A) Total Population



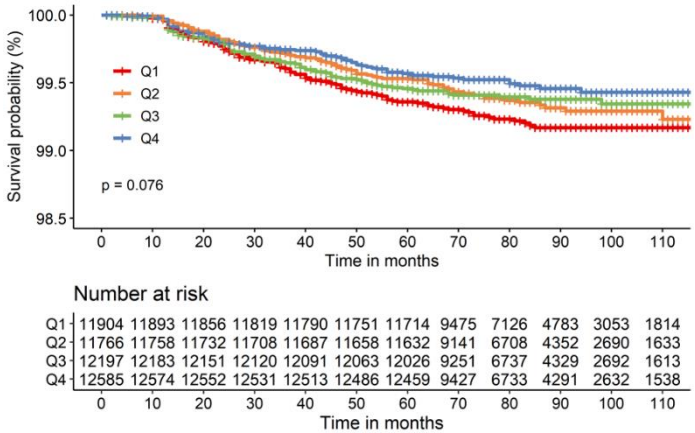
(B) Normotension



(C) Hypertension



(D) Hypertension without antihypertensive medication



(E) Hypertension with antihypertensive medication

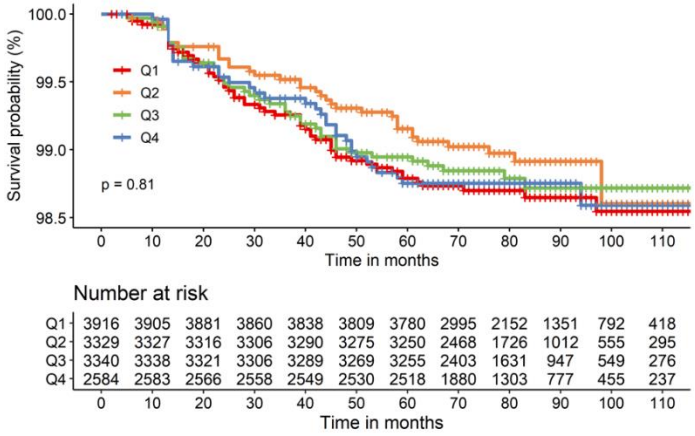


Figure 3.1 Unadjusted Kaplan–Meier estimates of stroke occurrence for quartiles (Q) of moderate-to-vigorous physical activity during follow-up stratified for total population(A), individuals with normotension (B), and individuals with hypertension (C), individuals with hypertension who are not taking anti-hypertensive medication (D) and individuals with hypertension who are taking antihypertensive medication.

3.3.2 Physical activity and the risk of stroke

There was a significant negative and linear trend between MVPA and stroke risk for the total population and in the hypertensive subgroup, which disappeared partly after adjusting for confounding factors (Model 2 and 3; Table 3.2). After adjustments for confounders (model 3) and using MVPA categories, a significantly lower HR was found in Q3 compared to the least active individuals (Q1) within the total population and in individuals with hypertension (Table 3.2 and Figure 3.2). No significant association was found in Q2 and Q4 compared to Q1. Although we found no significant association between MVPA and stroke risk in normotensive individuals, estimated HRs were largely comparable to the hypertensive participants. In addition, there was no significant interaction between each of the MVPA quartiles for stroke risk and normotension versus hypertension ($P>0.05$), suggesting that dose-response curves between MVPA and stroke are not different between normotensive and hypertensive individuals.

3.3.3 Physical activity and stroke risk in hypertensives: impact of medication use

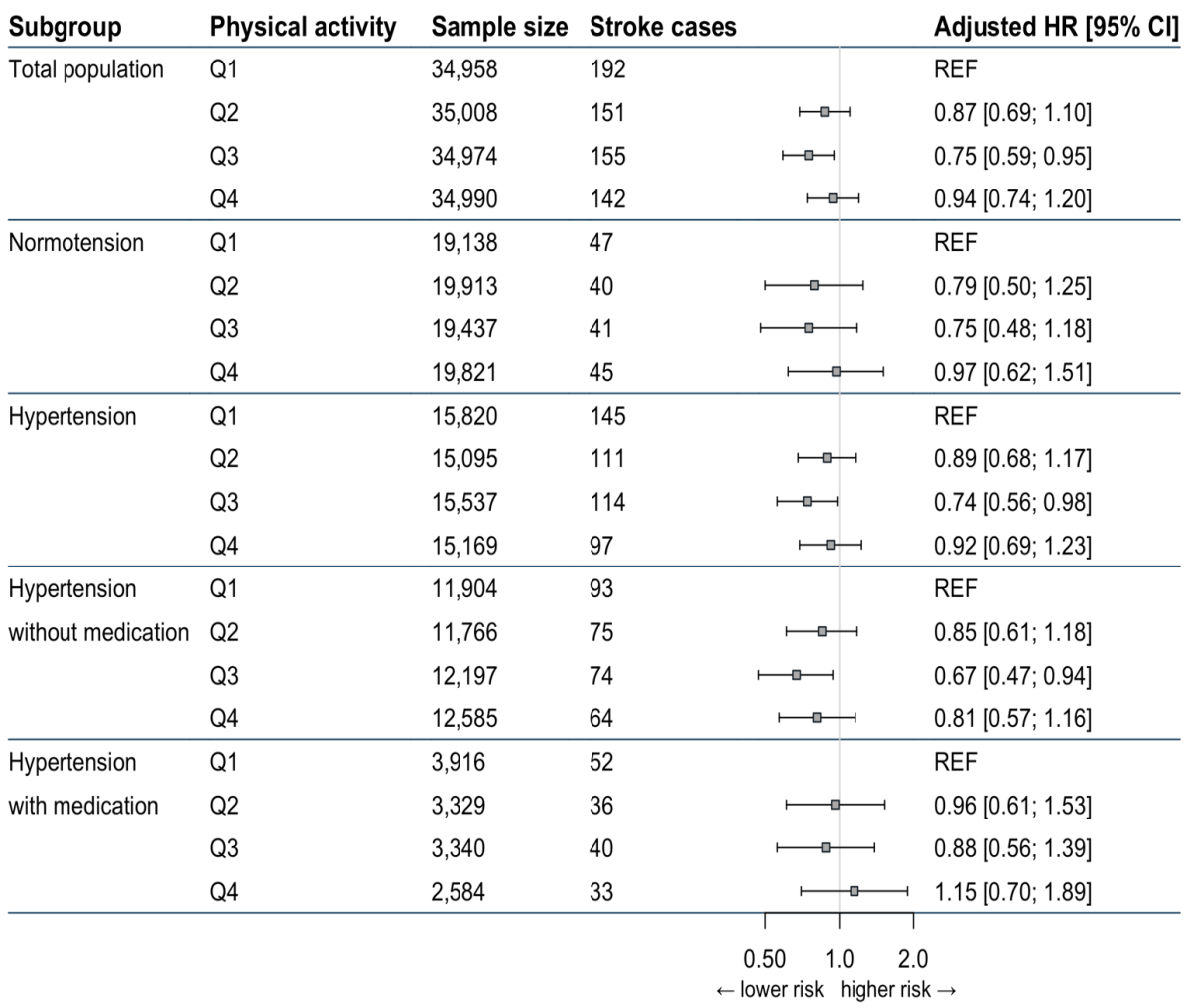
Within hypertensive individuals, after adjustment for confounders, there was a significant reduction in Q3 compared to Q1 for MVPA and stroke risk in those without medication (Supplemental 3.2 and Figure 3.2). In medicated hypertensive individuals, no significant association was found between PA and stroke risk, with estimated HRs being slightly higher compared to the non-medicated hypertensive population (Supplemental 3.2 and Figure 3.2). However, the p-value for interaction between PA and medication use was non-significant ($P>0.05$).

Table 3.2. Hazard ratios (HR) with 95% confidence intervals (95% CI) for the association between moderate-to-vigorous physical activity and stroke.

Total PA (MET min/week)	N sample	N outcomes	Model 1 (unadjusted) HR with 95% CI, P value	Model 2* HR with 95% CI, P value	Model 3# HR with 95% CI, P value
Total population					
Continuous	139,930	640	1.00 [1.00-1.00]	1.00 [1.00-1.00]	1.00 [0.999-1.00]
***P* for linear trend**			0.002	0.28	0.35
Quartiles					
Inactive Q1<1830	34,958	192	REF	REF	REF
Q2 1830-3617	35,008	151	0.79 [0.64-0.98], *P* = 0.03	0.87 [0.69-1.09], *P* = 0 .22	0.87 [0.69-1.10], *P* = 0.23
Q3 3618-7175	34,974	155	0.81 [0.66-1.01], *P* = 0.06	0.74 [0.58-0.94], *P* = 0.01	0.75 [0.59-0.95], P = 0.02
Q4 >7175	34,990	142	0.75 [0.60-0.93], *P* = 0.009	0.93 [0.73-1.18], *P* = 0.53	0.94 [0.74-1.20], *P* = 0.64
Normotension					
Continuous	78,309	173	1.00 [1.00-1.00]	1.00 [1.00-1.00]	1.00 [1.00-1.00]
***P* for linear trend**			0.80	0.82	0.83
Quartiles					
Inactive Q1<1830	19,138	47	REF	REF	REF
Q2 1830-3617	19,913	40	0.82 [0.54-1.25], *P* = 0.36	0.80 [0.51-1.26], *P* = 0.34	0.79 [0.50-1.25], *P* = 0.32
Q3 3618-7175	19,437	41	0.87 [0.57-1.32], *P* = 0.51	0.76 [0.48-1.19], *P* = 0.23	0.75 [0.48-1.18], *P* = 0.22
Q4 >7175	19,821	45	0.94 [0.62-1.41], *P* = 0.76	0.97 [0.63-1.51], *P* = 0.91	0.97 [0.62-1.51], *P* = 0.90
Hypertension					
Continuous	61,621	467	1.00 [0.999-1.00]	1.00 [1.00-1.00]	1.00 [1.00-1.00]
***P* for linear trend**			>0.001	0.26	0.29
Quartiles					
Inactive Q1<1830	15,820	145	REF	REF	REF
Q2 1830-3617	15,095	111	0.80 [0.63-1.03], *P* = 0.08	0.89 [0.68-1.16], *P* = 0.39	0.89 [0.68-1.17], *P* = 0.41
Q3 3618-7175	15,537	114	0.81 [0.63-1.03], *P* = 0.09	0.73 [0.56-0.97], *P* = 0.03	0.74 [0.56-0.98], *P* = 0.03
Q4 >7175	15,169	97	0.70 [0.54-0.92], *P* = 0.008	0.91 [0.68-1.21], *P* = 0.51	0.92 [0.69-1.23], P = 0.56

*Model 2 is adjusted for age, sex, BMI, income, educational level, alcohol consumption, smoking, kidney function, serum glucose, low-density lipoprotein cholesterol. # Model 3 is adjusted for age, sex, BMI, income, education level, alcohol, smoking (pack years), kidney function, BMI, serum glucose, systolic blood pressure, diastolic blood pressure, low-density lipoprotein cholesterol., use of acetylsalicylic acid, anti-platelets, antihypertensive medication.

Figure 3.2. Forrest plot of the quartiles (Q) of MVPA associated with stroke risk for the total population and stratified for blood pressure and medication use.



HRs were adjusted for age, sex, BMI, income, education level, alcohol, smoking (pack years), kidney function, BMI, serum glucose, systolic blood pressure, diastolic blood pressure, low-density lipoprotein cholesterol., use of acetylsalicylic acid, anti-platelets, antihypertensive medication (model 3). 95% CI, 95% confidence interval; HR, hazard ratio.

3.4 Discussion

Previous research has demonstrated the importance of PA on stroke risk reduction, however emerging research has suggested that the dose-dependent association of regular PA may be altered in the presence of cardiovascular risk factors and/or medication use. This study presents the following findings. First, we reinforce the benefits of regular PA, as engagement in MVPA is associated with a lower risk for stroke, which remained significant upon adjustment for potential confounders. Second, the shape of the dose-response association does not importantly differ between hypertensive (n=61,621) and normotensive (n=78,309) individuals. However, the association remained only significant in the hypertensives after stratification. Thirdly, the benefits of regular MVPA seem to be only stronger in non-medicated individuals with hypertension, but further studies are necessary to confirm this preliminary result. Our findings highlight that a physically active lifestyle is beneficial for reducing stroke risk.

Our study reinforces findings from previous studies highlighting that more PA is associated with a reduction in the risk for stroke in the general population. For example, a study in the USA, with a median follow up of 18.8 years and 648 ischemic stroke events, showed a significant reduction in ischemic stroke (and other subtypes) with increasing PA levels among middle-aged men and women. Another prospective cohort study in South Koreans with 13 years follow-up showed that exercising between 3–4 or 5–6 times/week showed the lowest stroke risk compared to physically inactive individuals (Kim et al., 2019). In contrast to others, our study further examined whether the association between MVPA and stroke depends on BP levels. We found that the shape of the dose-response association between MVPA and

stroke risk was not significantly different between individuals with normotension or hypertension. Nevertheless, the association was not significant in the normotensive group. A possible explanation for this observation is due to the low event rate present in the normotensive group (i.e., >60% less events in the normotensives *versus* the hypertensives). In addition, compared to other studies, our follow-up time was shorter (i.e., median 6.8 years) and we included a relatively young population, which suggests that a longer follow-up in the normotensives who have a lower *a priori* risk for stroke may be required. Furthermore, HRs in Q4 in the present study are higher that Q3, however this may be explained by possible measurement error in MVPA classification, Specifically, Q4 may contain individuals who exaggerated their PA levels but due to the nature of questionnaires and the absence of an objective PA measurement we can only speculate. Importantly, our findings show that the presence of hypertension does not attenuate the dose-response relationship between MVPA and stroke. This finding supports previous studies examining the effect of PA and cardiovascular disease and mortality (Bakker et al., 2021, Joseph et al., 2019). Our study provides an important public health message; that benefits from a physically active lifestyle in relation to stroke remain equally present in those who have already developed hypertension.

For our secondary aim, we compared the effects of PA on stroke risk between medicated and unmedicated hypertensive individuals. Whilst reinforcing our initial results, in that PA is associated with lower risks for stroke in unmedicated hypertensive individuals, such effects were not observed in medicated individuals with hypertension. One potential explanation of the latter observation is that the sample size and event rate of the medicated hypertensives was relatively low. Although the medicated hypertensive individuals might be underpowered,

the analysis showed higher HRs in this subgroup, which might suggest an attenuated and smaller effect of MVPA on the risk of stroke in the medicated group of individuals with hypertension. One potential explanation could be that those within the medicated group had higher BP levels and/or disease severity. Previous research has suggested that those with established CVD may not benefit to the same extent as healthy individuals (Bakker et al., 2021, Stewart et al., 2017, Jeong et al., 2019). In addition, the attenuated effect may relate to the use of medication itself. However, previous work is conflicting whether antihypertensive medication may interfere or potentiate the benefits of PA or exercise training (Dimeo et al., 2012, Sumukadas et al., 2014, Baptista et al., 2018, Sjúrðarson et al., 2022). Apart from the antihypertensive medication, other drugs that are commonly prescribed to hypertensive individuals such as statins (21% in our medicated hypertensives) may also have an effect on the benefits of PA (Mikus et al., 2013). Therefore, further research is warranted to understand whether antihypertensive medication affects the benefits of PA on stroke risk. Nonetheless, we believe it is important to emphasise that, even in the presence of hypertension and medication use, other research has suggested that when combined, exercise and medication strengthen the BP effects of medication alone and should be recommended and implemented as indicated according to existent professional treatment algorithms (Whelton et al., 2018, Pescatello et al., 2021).

A particular strength of this study is that it includes a large population (n=139,930) with outcome data based on national hospital registry data. One potential limitation of our study is that MVPA was self-reported, which is susceptible for overestimating true PA volumes (Celis-Morales et al., 2012). Nonetheless, categorising individuals across quartiles allowed comparison between the least active individuals and those engaged in larger volumes of

MVPA, and ultimately self-reported MVPA data is likely to underestimate the true effect of MVPA on health benefits (Celis-Morales et al., 2012). Another limitation relates to the relatively low event rate of stroke in the normotensive population, and relatively small sample size of medicated hypertensive individuals. Nonetheless, HRs in both populations, albeit with larger confidence intervals, provide insight into the effects of PA.

3.5 Conclusion

Our study reinforces the benefits of regular MVPA on stroke risk reduction, especially in hypertensive individuals. However, we found that the shape of dose-dependent association between MVPA and risk of stroke was not different between individuals with hypertension and normotension. Furthermore, our data provide preliminary support that in individuals with hypertension, the use of antihypertensive medication may be associated with a smaller benefit of MVPA on the risk of stroke, albeit effects of PA in this subgroup have unlikely disappeared. Future studies are warranted to better understand the effects of regular MVPA on the risk of stroke in medicated hypertensive individuals and the potential underlying mechanisms, which can aid in optimising the benefits of MVPA in the prevention of stroke.

Chapter 4: Relation between Endothelial Dysfunction and Exercise Training- Mediated Adaptation in Cardiovascular Risk Factors, Cardiorespiratory Fitness, and Vascular Health in Humans: A secondary analysis.

4.1. Introduction

Cardiovascular disease (CVD) is the leading causes of death worldwide and is one of the most serious health problems throughout the world (Mensah et al., 2019, Roth et al., 2020). Regular exercise training and/or physical activity is a conventional and non-pharmological strategy that effectively reduces the risk for development and progression of cardiovascular disease (Fiuza-Luces et al., 2018). Benefits of exercise are only partly explained through improvements in traditional cardiovascular disease risk factors, e.g., blood pressure (Huang et al., 2013), body weight (Leung et al., 2008), glucose homeostasis (MacLeod et al., 2013), cholesterol (Dunn et al., 1997), and cardiorespiratory fitness (Blair et al., 1995). Benefits of exercise training also relate to improvements in vascular health, including endothelial function (Green et al., 2008, Green et al., 2017, Tinken et al., 2010, Ashor et al., 2015).

Endothelial function, measured as flow mediated dilation (FMD%), is largely nitric oxide (NO)-mediated (Green et al., 2014a) and predicts future CV events (Green et al., 2011). It is therefore considered an early marker of future atherosclerotic vascular risk and a non-invasive window into vascular health status. Recently, age and sex-specific flow mediated dilation (FMD) reference intervals for healthy individuals have been published (Holder et al., 2021). It has been consistently reported that age and sex-specific differences in FMD are present (Celermajer et al., 1994, Taddei et al., 1995, Seals et al., 2011, Yao et al., 2014, Hopkins et al., 2015) with sex altering the age-related decline in FMD (Holder et al., 2021). FMD references intervals that account for these differences allow for a clinical interpretation of FMD.

Some epidemiological evidence suggests that individuals with cardiovascular disease (CVD) gain less benefit from regular physical activity, in terms of relative risk reduction for all-cause mortality and morbidity, than apparently healthy individuals (Moholdt et al., 2008, Mons et al., 2014, Jeong et al., 2019). In addition, a recent study demonstrated that cardiovascular (CV) health status alters the dose-response between moderate to vigorous physical activity (MVPA) and incident morbidity and mortality among 143,070 adults (Bakker et al., 2021). Healthy individuals and those with CVD risk factors presented with a curvilinear relation, whilst those with established CVD presented a more gradual, linear relation, suggesting a smaller risk reduction from regular physical activity in those with CVD (Maiorana et al., 2011, Green et al., 2014a). This could have implications for the benefits of exercise training and interventions in these individuals.

Whilst *a priori* cardiovascular health status may impact reductions in risk factors and CV mortality and morbidity, currently, it is not known whether *a priori* endothelial dysfunction impacts upon exercise-training adaptation in CVD risk factors and fitness. Therefore, the aim of this study was to investigate whether *a priori* endothelial dysfunction is associated with distinct training-induced improvement in traditional CVD risk factors, cardiopulmonary fitness, or vascular function by performing a secondary analysis in a large cohort of 338 individuals who performed supervised exercise training. Supported by previous work on endothelial (dys)function (Maiorana et al., 2011, Green et al., 2014b), it was hypothesised that those with *a priori* endothelial dysfunction would show smaller improvements in CV risk factors and cardiopulmonary fitness compared to those with *a priori* preserved endothelial function.

4.2 Methods

4.2.1 Participants

Endurance exercise training studies performed in our laboratories (Liverpool, Perth, Nijmegen) which met the following criteria were included in this analysis : 1) completion of moderate-intensity supervised exercise ≥ 8 weeks; 2) the exercise sessions consisted of endurance (aerobic) exercise involving large muscle groups or combined aerobic and resistance exercise (CARE) sessions; 3) exercise training was performed ≥ 2 times per week with a duration of ≥30minutes; 4) measurements of baseline FMD and age were available to categorize *a priori* endothelial function based on Holder *et al.*, 2021 equations*;* 5) endothelial function (FMD) measurements were performed strictly adhering to expert consensus guidelines (Thijssen et al., 2019, Thijssen et al., 2011); 6) the exercise study was approved by the local ethics committees and conformed to the standards of the Declaration Helsinki. Participants were asked not to modify their diet and lifestyle factors during the programme, no studies controlled the participants' diet throughout their study. Smokers were excluded as well as studies which included children, adolescents, or pregnant women. Additionally, any changes in brachial artery diameter or FMD that was >3 times the overall standard deviation were removed. This led to the inclusion of 19 studies (Table 1) with 338 participants for this secondary analysis, with a wide range of different health statuses included: healthy young, older sedentary, individuals with non-alcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), type 2 diabetes, elite rowers, and individuals with CVD risk. Please see Table 1 for further details.

We performed a power calculation, to achieve a power of 80% with level of significance of 5%

(two side) based on the ability to detect a 1% difference in the change in FMD over time between the two groups, with a standard deviation of the difference of 2.5%, we need to include at least 99 participants in each group.

4.2.2 Research design

Participant characteristics and traditional CV risk factors were recorded pre and post exercise training. All physiological measures pre and post the exercise intervention were performed following an overnight fast and participants were asked to abstain from strenuous exercise for >24 hours and caffeine and alcohol for >12 hours before testing. Post measurements were completed 1-4 days following the last exercise session. Presence of pre-training endothelial dysfunction was determined using published FMD reference values (Holder et al., 2021). Specifically, individuals were categorised as possessing "reduced endothelial function, R-EF" when pre-training FMD was lower than the age- and sex specific FMD reference values using the 50th percentile as the cut-off value (Holder et al., 2021) and preserved endothelial function (P-EF) when at or above the 50th percentile for age- and sex specific FMD reference values.

4.2.3 Experimental measures

Participant characteristics Participant characteristics were measured pre and post the exercise training intervention. Height, weight, body mass index (BMI), waist circumference and waist to hip ratio were measured using standard methods. Body fat percentage was measured either via dual X-Ray Absorptiometry (DXA) or skin fold measurements using standard techniques. Measurements of blood pressure were conducted after ≥5-minute rest

in a seated or supine position using a manual or automated sphygmomanometer and were repeated at least twice and were averaged (Supplemental table 4.1).

Cardiopulmonary fitness Peak oxygen consumption (VO_{2peak}) was measured during a maximal graded exercise test on a treadmill, a cycle ergometer or a rower was used. VO_{2peak} values are presented relative to body weight (ml/kg/min) (Supplementary Table 4.1).

Endothelial function FMD assessments were performed in a quiet, temperature-controlled laboratory at the same time of day to avoid diurnal effects. Participants were asked to avoid alcohol, caffeine consumption and vigorous exercise for ≥12 hours before testing. Participants rested for ≥15 minutes in the supine position. The participants' arm was extended and positioned at an angle ~80 degree from the torso. A rapid inflation and deflation pneumatic cuff was positioned on the forearm and inflated to suprasystolic pressure to induce ischemia. B-mode images were obtained with a ≥7.5 MHz multifrequency linear array probe attached to a high-resolution ultrasound machine was used to image the brachial artery in the distal one-third of the upper arm. One study used a Megas ultrasound device (Esaote, Firenze, Italy), whilst all other studies used either the Aspen Acuson (Mountain view, CA, USA) or a Terason, t3000, (Aloka, Burlington, MA, USA) (Supplemental table 4.1). Baseline diameter, flow and shear stress measurements were recorded for ≥1 minute before the forearm cuff was inflated for 5 minutes. Diameter and flow recordings resumed 30 seconds prior to cuff deflation and continued for 3 minutes post deflation. FMD was calculated as peak artery diameter following hyperaemia, expressed as % increase. All analysis was performed using custom designed edge detection and wall tracking software which is largely independent of investigator bias (Woodman *et al.*, 2001).

Cardiovascular blood parameters Venous blood samples were taken to assess fasted glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol as well as triglycerides. The blood samples were analysed in accredited laboratory facilities.

4.2.4 Statistical analysis

The effect of *a priori* endothelial function on improvements in endothelial function and CVD risk factors in response to exercise training were determined using a two-way mixed design general linear model, with a within subject factor of time (pre- versus post-training) and a between subject factor of group (endothelial function vs endothelial dysfunction). Data analysis was performed using SPSS (Version 26; SPSS Inc., Chicago, IL). Statistical significance was delimited at $P < 0.05$ and exact P values are cited (P values of '0.000' provided by the statistics package are reported as <0.001).

4.3 Results

A total of 338 participants were included in the study. Following the age- and sex-based reference values, we classified 123 (56 female, 67 male) participants as possessing preserved endothelial function (P-EF) and 215 (69 female, 146 male) as having *a priori* reduced endothelial function (R-EF). The duration of exercise training varied, with n=127 having undertaken an 8-week exercise intervention (P-EF: n=40, R-EF: n=87), n=164 completed a 12-week intervention (P-EF: n=58, R-ED: n=106), n=15 completed a 16-week intervention (P-EF: n=5, R-EF: n=10), and n=32 completed a ≥24-week intervention (P-EF: n=20, R-EF: n=12).

Details of the exercise training interventions are summarised in Table 4.1. Exercise intensity was prescribed as a percentage of VO_{2max} or heart rate reserve. Where the exercise intervention was progressive, an average intensity was calculated, data is presented as decimals.

4.3.1 Baseline characteristics

Prior to training, no differences between P-EF versus R-ED in general characteristics or in cardio-respiratory fitness were found (Table 4.2). Regarding traditional CVD risk factors, we found significantly higher baseline systolic blood pressure, triglycerides and fasted glucose levels in R-EF, whilst no differences were found for diastolic or mean blood pressure, cholesterol, LDL and HDL (Table 4.2). As a consequence of the group allocation, the R-EF-group demonstrated a significantly lower FMD%, compared to those with P-EF.

4.3.2 Impact of exercise training

Cardiovascular risk factors. Exercise training caused modest but significant reductions in body weight, BMI, percentage body fat, waist circumference, blood pressure (diastolic, systolic and mean) and total cholesterol (all $P<0.05$). The magnitude of these changes were not different between preserved P-EF and R-ED (Table 4.3). Exercise training did not significantly alter serum levels of glucose, triglycerides, HDL and LDL (Table 4.3).

Cardiorespiratory fitness. Exercise training improved cardiorespiratory fitness in those with preserved endothelial function and with reduced endothelial function ($F_{1,291}$ = 136.199,

P<0.001). This effect of training on VO_{2peak} was not significantly different between groups with both groups showing a significant improvement (interaction, $F_{1,291}$ = 1.402, *P*=0.237) (Table 4.3).

Brachial artery diameter and endothelial function Brachial artery FMD increased over time in response to exercise training ($F_{1,335}$=10.092, *P*=0.002), which differed between groups (interaction effect; $F_{1,335}$=42.942, *P*<0.001) (Table 2). Post-hoc tests indicated little change in FMD% in preserved EF, whilst an increase was found in R-EF (P<0.001). Exercise training significantly increased resting brachial artery diameter ($F_{1,322}$ = 9.334, P=0.002). The magnitude of this increase in resting diameter following exercise training was not different between groups ($F_{1,322}$=0.056, P=0.813).

Table 4.1 Study characteristics of included studies. NAFLD: Non-alcoholic fatty liver disease, PCOS polycystic ovary syndrome T2D type 2 diabetes.*Unpublished

Author (year)	Group size (P-EF/R-EF)	Study Population	Type of exercise	Training weeks	Frequency (days/week)	Duration (minutes)	Intensity
Tinken 2008	12 (4/8)	Healthy young males	Aerobic	8	3	30	0.8
Black 2009	11(4/7)	Older sedentary males and females	Aerobic	12	4	30	0.3
Birk 2012	9 (3/6)	Healthy men, recreationally active	Aerobic	8	3	30	0.8
Pugh 2016	9 (3/6)	NAFLD males and females	Aerobic	16	3.5	37.5	0.45
Sprung 2013	6 (2/4)	PCOS	Aerobic	16	3.5	37.5	0.45
Buckley 2018	13 (4/9)	Increased CVD risk	CARE	12	1-3		0.5-0.75
Buckley 2020	33 (8/25)	Increased CVD risk	CARE	12	1-3		0.5-0.75
Miller 2022	17 (7/10)	Healthy	Aerobic	8	2.5	55	0.7
Maxwell 2021	10 (4/6)	CVD Risk	Aerobic	8	3	50	0.7
Thijssen 2007	8 (5/3)	Older men	Aerobic	8	3	30	0.7
Schreuder 2014a	13 (3/10)	Older men	CARE	8	3	60	0.725
Schreuder 2014b	23 (6/17)	Healthy and T2D	CARE	8	3	60	0.725
Poelkens*	13 (12/1)	Healthy and CVD risk	Aerobic	26	3	45	0.725
Scholten 2012	37 (19/18)	Pre-eclamptic women, control	Aerobic	12	3/2.5	60/55	0.725
Brenda 2015	14 (7/7)	Manifest CVD	Aerobic	12	2	45	0.83
Green 2003	35 (8/27)	CVD risk and manifest CVD	CARE	8	3	55	0.8
Naylor 2006	25 (8/17)	Elite Rowers	CARE	12	13	135	
Haynes 2021	19 (8/11)	Older subjects	Aerobic	24	3	32.5	0.512
McKeown*	31 (8/23)	Healthy	CARE	12	2.5	60	0.75

Table 4.2 Participant characteristics of the study population stratified by endothelial function into preserved endothelial function (P-EF, n=123) and reduced endothelial function (R-EF, n=215).

The preserved endothelial function group consisted of 58 healthy participants, 46 cardiovascular disease risk, 19 manifest cardiovascular disease. The reduced endothelial function group consisted of 88 healthy participants, 74 cardiovascular disease risk, 19 manifest cardiovascular disease. When n is different to P-EF, n=123 and R-EF, n=215 this has been stated as n=P-EF, R-EF in the table

Participant characteristics	**P-EF** (n=123, *58 healthy, 46 CVD risk, 19 CVD)*	**R-EF** (n=215, *88 healthy, 74 CVD risk, 19 CVD)*	**P values**
Age (years)	46 ± 17	48 ± 17	0.297
Sex (% male)	54	68	**0.016**
Height (m)	1.73 ± 0.08	1.73 ± 0.10	0.815
Weight (kg, n=115-202)	84.6 ± 19.4	88.0 ± 87.4	0.487
Body mass index (kg/m^2,n=115-202)	28.2 ± 5.9	29.2 ± 5.4	0.500
Waist:Hip (n=107-186)	0.88 ± 0.07	0.93 ± 0.09	0.162
Waist circumference (cm, n=45-61)	97.37 ± 15.21	102.72 ± 15.68	0.289
VO_{2peak} (mL/kg/min, n=107-186)	30.36 ± 11.31	29.98 ± 12.41	0.751
Vascular function			
Flow-mediated dilation (%)	8.80 ± 2.58	3.69 ± 1.58	**<0.001**
Baseline diameter brachial artery (mm, n=110-214)	3.72 ± 0.82	4.16 ± 0.80	**<0.001**
Health Characteristics			
Systolic blood pressure (mmHg, n=96-166)	123 ± 14	131 ± 16	**<0.001**
Diastolic blood pressure (mmHg, n=96-166)	74 ± 9	77 ± 11	0.118
Mean Arterial Pressure (mmHg, n=113,202)	91 ± 10	94 ± 11	0.108
Total Cholesterol (mmol/L, n=69-119)	4.84 ± 1.09	4.99 ± 1.18	0.192
LDL Cholesterol (mmol/L, n=59-83)	2.97 ± 0.96	2.98 ± 0.86	0.319
HDL Cholesterol (mmol/L, n=66-107)	1.26 ± 0.37	1.20 ± 0.35	0.310
Triglycerides (mmol/L, n=59-86)	1.20 ± 0.68	1.69 ± 1.12	**0.009**
Glucose (mmol/L,56-69)	5.32 ± 1.43	6.18 ± 2.65	**0.002**

Table 4.3 Characteristics of the groups divided by endothelial function into the preserved endothelial function (P-EF) group and participants with reduced endothelial function (R-EF) before and after exercise interventions. P-values were determined using a two-way mixed design ANOVA. Data is presented as mean ± SD (95% Confidence interval).

	P-EF		**R-EF**		**P-value**		
	Pre	**Post**	**Pre**	**Post**	**Group**	**Time**	**Group*Time**
Weight (kg)	85.3 ± 19.6 (81.8-88.7)	84.5 ± 19.2 (81.1-87.9)	87.6 ± 18.6 (85.0-90.3)	87.12 ± 18.4 (84.5-89.7)	0.257	**<0.001**	0.446
Body mass index (kg/m^2)	28.5 ± 5.9 (27.5-29.6)	28.3 ± 5.8 (27.3-29.3)	29.1 ± 5.5 (28.3-29.9)	28.9 ± 5.3 (28.2-29.7)	0.334	**<0.001**	0.367
Body Fat (%)	35 ± 8 (33-37)	34 ± 8 (32-36)	34 ± 9 (32-36)	33 ±9 (31-35)	0.708	**<0.001**	0.278
Waist:Hip	0.88 ± 0.07 (0.86-0.91)	0.87 ± 0.09 (0.85-0.90)	0.95 ± 0.09 (0.93-0.97)	0.92 ± 0.09 (0.90-0.95)	**0.001**	**0.001**	0.132
Waist Circumference (cm)	98.6 ± 15.4 (91.0 – 103.0)	96.77 ± 16.6 (88.0-101.0)	103.9 ± 14.3 (94.8-104.3)	101 ± 173 (93.1-103.3)	0.440	**<0.001**	0.210
Systolic blood pressure (mmHg)	124 ± 14 (120-127)	119 ± 14 (116-121)	130 ± 16 (128-133)	126 ± 14 (124-128)	**<0.001**	**<0.001**	0.737
Diastolic blood pressure (mmHg)	74 ± 9 (72-77)	71 ± 9 (69-73)	76 ± 11 (75-78)	74 ± 10 (73-76)	**0.037**	**<0.001**	0.233
Mean arterial pressure (mmHg)	91 ± 10 (89-93)	87 ± 10 (85-89)	93 ± 11 (92-95)	90 ± 10 (89-92)	**0.013**	**<0.001**	0.309
VO_{2peak} (mL/kg/min)	30.01 ± 11.18 (27.71- 32.30)	32.84 ± 11.10 (30.52-35.16)	30.18 ± 12.52 (28.44-31.92)	32.49 ± 12.80 (30.73-34.25)	0.954	**<0.001**	0.237
Glucose (mmol/L)	5.32 ± 1.40 (4.79-5.85)	5.30 ± 1.25 (4.87-5.73)	6.26 ± 2.53 (5.93-6.79)	6.01 ± 2.02 (5.73-6.42)	**0.005**	0.132	0.193
Triglycerides (mmol/L)	1.19 ± 0.68 (0.96-1.42)	1.18 ± 0.68 (0.98-1.34)	1.60 ± 1.0 (1.41-1.79)	1.53 ± 0.85 (1.37-1.71)	**0.006**	0.433	0.567
Total cholesterol (mmol/L)	4.80 ± 1.05 (4.57-5.05)	4.72 ± 1.1 (4.49-4.96)	4.96 ± 0.99 (4.77-5.14)	4.80 ± 0.94 (4.62-5.98)	0.460	**0.004**	0.413
High-density lipoprotein (mmol/L)	1.26 ± 0.37 (1.18-1.34)	1.27 ± 0.36 (1.19-1.35)	1.17 ± 0.31 (1.11-1.24)	1.18 ± 0.30 (1.12-1.24)	0.076	0.456	0.896
Low-density lipoprotein (mmol/L)	2.91 ± 0.94 (2.67-3.14)	2.89 ± 0.99 (2.66-3.12)	3.07 ± 0.85 (2.88-3.26)	2.95 ± 0.82 (2.75-3.14)	0.476	0.071	0.197
Flow-mediated dilation (%)	8.63± 2.78 (8.29-9.02)	8.21 ± 3.62 (7.71-8.72)	3.70 ± 1.51 (3.42-3.98)	4.99 ± 2.24 (4.61-5.36)	**<0.001**	**0.002**	**<0.001**

Diameter (mm)	3.73 ± 0.82 (3.58-3.89)	3.81 ± 0.79 (3.66-3.97)	4.16 ± 0.80 (4.05-4.27)	4.23 ± 0.85 (4.17-4.34)	**<0.001**	**0.002**	0.813

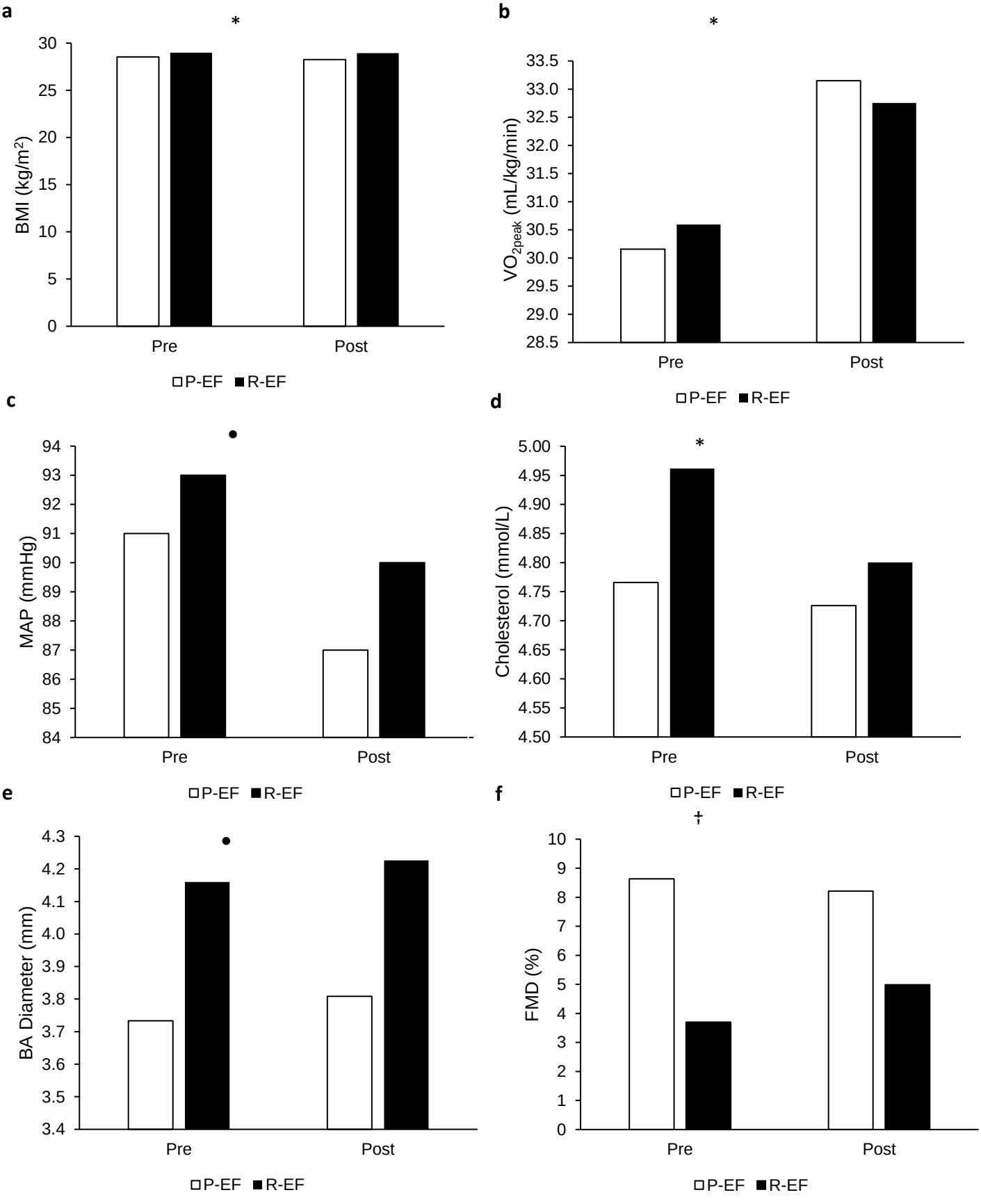

a
*
BMI (kg/m2)
30
25
20
15
10
5
0
Pre
Post
P-EF R-EF
b
*
VO2peak (mL/kg/min)
33.5
33.0
32.5
32.0
31.5
31.0
30.5
30.0
29.5
29.0
28.5
Pre
Post
P-EF R-EF
c
•
MAP (mmHg)
94
93
92
91
90
89
88
87
86
85
84
Pre
Post
P-EF R-EF
d
*
Cholesterol (mmol/L)
5.00
4.95
4.90
4.85
4.80
4.75
4.70
4.65
4.60
4.55
4.50
Pre
Post
P-EF R-EF
e
•
BA Diameter (mm)
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
Pre
Post
P-EF R-EF
f
†
FMD (%)
10
9
8
7
6
5
4
3
2
1
0
Pre
Post
P-EF R-EF

Figure 4.1 Cardiovascular risk factor responses of BMI (a), VO_{2peak} (b), Mean arterial pressure (c), Cholesterol (d), Brachial artery (BA) diameter (e) and Flow mediated dilatation (FMD%) (f) in those with endothelial function and endothelial dysfunction group following exercise training.
*denotes a significant effect of time.
·Denotes a significant effect of group and time.
†denotes a significant effect of group, time and interaction.

4.4 Discussion

The aim of the present study was to investigate whether *a priori* endothelial function status is associated with distinct training-induced improvement in traditional CV risk factors, cardiorespiratory fitness, and vascular function. To this end, analysis was performed on 338 participants who all underwent supervised exercise training across three laboratories, with pre- and post-training evaluation of endothelial function, cardiovascular risk factors and fitness. Overall, this data suggest that exercise training improved cardiorespiratory fitness, flow-mediated dilation and some (i.e. body weight, BMI, body fat percentage, waist circumference and blood pressure) but not all (i.e. fasting glucose, HDL, LDL and triglycerides) cardiovascular risk factors. After dividing the group into *a priori* reduced endothelial function (R-ED; n=215) and preserved endothelial function (P-EF; n=123), this study found comparable improvements in cardiovascular risk factors and physical fitness following exercise training in both groups. Interestingly, only those with *a priori* endothelial dysfunction demonstrated improvement in endothelial function (FMD%) after exercise training, whereas no change was found in subjects with P-EF who started with higher endothelial function, supporting previous findings (Green *et al.*, 2014*b*). Overall, this study suggests that the benefits of exercise training on many clinically important risk factors are independent of *a priori* endothelial

dysfunction status; although a period of 8-12 weeks of moderate intensity exercise may not enhance endothelial function in those with P-EF.

Previous epidemiology research has shown that moderate to vigorous physical activity is beneficial for reducing adverse outcomes, however the shape of the association is dependent upon health. A curvilinear association between healthy and individuals with CV risk factors and PA whilst a linear association was found between individuals with CVD (Bakker et al., 2021). This supports the present study's findings that individuals do benefit from physical activity however, the response may be different between those with have reduced endothelial function and preserved endothelial function. A unique aspect of this study was the classification of individuals into endothelial function or dysfunction by comparing individuals' FMD-values with recently published age- and sex- specific reference intervals for FMD (Holder et al., 2021). Sex and age specific recommendations were important to consider as higher FMD in females, but also the steeper decline in FMD with age, compared with males may relate to differences in sex hormones, especially since oestrogen has been linked to cardioprotective properties (Miller and Duckles, 2008). Adopting this approach, this dataset observed a relatively large population with *a priori* reduced endothelial function (63.6%), which is the direct consequence of the inclusion of a substantial number of training studies in clinical populations and those with CV risk factors (Table 4.1). Findings from this study are in line with our previous observations (Maiorana et al., 2003, Green et al., 2004, Green et al., 2014b) in that regular exercise training is capable of improving endothelial function, especially in those with *a priori* endothelial dysfunction (Walsh et al., 2003, Maiorana et al., 2011, Green et al., 2014b) (Table 4.3). Indeed, endothelial dysfunction is related to chronic exposure to CV risk factors (Sorensen et al., 1994, Ford et al., 2009) and CVDs (Al Suwaidi et

al., 2001, Hetzel et al., 2005) Furthermore, findings from our study are in line with our previous observations (Maiorana et al., 2003, Green et al., 2004, Green et al., 2014b) in that regular exercise training is capable of improving endothelial function, especially in those with *a priori* endothelial dysfunction (Green et al., 2003, Maiorana et al., 2011, Green et al., 2014b) (Table 3).

The key finding from this study is that exercise training is associated with improved CVD risk factors and enhanced physical fitness; an observation that is not related to *a priori* endothelial status. Previous work found that improvements in cardiovascular risk factors also occur irrespective of changes in physical fitness (Hartman et al., 2018). In fact, lower pre-training values for fitness and impaired CV risk factors or vascular function, are associated with larger training induced improvements in endothelial function in both males and females (Maiorana et al., 2003, Green et al., 2004, Green et al., 2014b). These previous studies and the results of the present study provide evidence to support the potency of supervised exercise training interventions to improve cardiovascular risk factors in those with *a priori* higher risk and/or endothelial dysfunction (Bakker et al., 2021). Indeed, those with CVD and CV risk factors typically are also characterised with endothelial dysfunction (Grover-Páez and Zavalza-Gómez, 2009, Jay Widmer and Lerman, 2014), demonstrates smaller benefits from regular physical activity in terms of relative risk reduction for all-cause mortality and cardiovascular events. Key differences relate to study design, with our study examining changes in risk factors within participants following (supervised) exercise training in groups of strictly selected and defined groups of healthy individuals, CVD risk and CVD. Accordingly, effects from our work can be directly related to exercise training. Studies adopting an epidemiological approach are strong in the volume and number of participants, but causal

links are difficult to make, whilst levels of physical activity are estimated using (subjective) questionnaires. Such differences may contribute to the distinct findings between intervention studies and epidemiological cohort observations.

This study has potential clinical implications; it further highlights the importance and benefits of exercise in healthy and CVD populations. Although those with reduced endothelial function had more cardiovascular disease risk factors at baseline, significant increase in FMD and improvements in risk factors were found in this population. Improvements in FMD are vital due to its prognostic value (Green et al., 2011), with a meta-analysis suggesting that per 1% higher FMD, the risk of experiencing a cardiovascular event is 13% lower (Inaba et al., 2010). Endothelial dysfunction is an independent predictor of future cardiac events in patients with and without established coronary artery disease (Neunteufl et al., 2000, Vita and Keaney Jr, 2002). In the current study, both groups showed improvements in multiple cardiovascular disease risk factors thus highlighting the importance of exercise training in both clinical and healthy populations. Nonetheless, it is important to highlight that individuals with endothelial dysfunction, even following the comparable improvements in cardiovascular risk factors following exercise training, presented with higher cardiovascular risk factors compared to those in the preserved endothelial function group. As this difference remained present following exercise training, this may contribute to observations from epidemiological studies, but also highlight the importance to remain physically active.

Some limitations of this study must be discussed. Firstly, controversy exists about what cut-off value represents true endothelial dysfunction, especially in relation with coronary artery endothelial function (Anderson et al., 1995). However, this study quantified presence of

endothelial dysfunction using the recently published age- and sex-based reference value data (Holder et al., 2021). Importantly, these reference values were constructed based on laboratories that adopted guideline-based approaches and similar methodology to evaluate the FMD (Anderson et al., 1995, Takase et al., 1998, Broxterman et al., 2019). Furthermore, not all FMD data was allometrically scaled due to some papers being published before allometric scaling guidelines. Whilst a strength of our study is the detailed evaluation of fitness, cardiovascular risk factors and vascular health, a limitation is that we were unable to relate these changes directly to future clinical endpoints (e.g. mortality, morbidity). Finally, our analysis only included studies in which participants completed endurance exercise or combined aerobic and resistance training, therefore results from this study cannot be extrapolated to resistance training or high intensity interval training.

4.5 Conclusion

In conclusion, this study found that regular exercise training, irrespective of the presence of *a priori* endothelial dysfunction, improves physical fitness and cardiovascular risk factors, whilst only those with *a priori* endothelial dysfunction demonstrated improvement in endothelial function. Therefore, this study further highlights the importance of exercise training, even in those with endothelial dysfunction, to improve clinically relevant markers of cardiovascular risk.

Chapter 5: The effects of acute exercise on (cerebro) vascular function in individuals with normotension and medicated hypertension.

5.1 Introduction

According to the World Health Organisation (WHO), in 2023 an estimated 1.23 billion adults aged 30-70 years had hypertension with the prevalence of hypertension increasing globally (Mills et al., 2020). One of the major global targets for noncommunicable diseases is to reduce the prevalence of hypertension by 33% between 2010 and 2030 (World Health Organisation, 2023). Since hypertension is the leading modifiable risk factor of cardiovascular and cerebrovascular disease and death worldwide, both pharmological and lifestyle interventions are needed for prevention and management (Valenzuela Ruiz et al., 2020). Regular exercise is a highly effective way to mitigate the risk of cardiovascular (Eijsvogels et al., 2016, Lee et al., 2012, Bakker et al., 2021) and cerebrovascular disease (Wang et al., 2015). Although everybody benefits from regular engagement in exercise training, the protective effects of exercise against cerebrovascular disease/events may be somewhat smaller in the presence of antihypertensive medication (*see Chapter 3*).

Exercise training is known to have beneficial effects on endothelial function, which crucially appears to be mediated by exercise-induced increases in shear stress (Tinken et al., 2010). This is seen in both peripheral vascular function (Birk et al., 2012, Green et al., 2017, Maiorana et al., 2003) and cerebrovascular function (Bliss et al., 2021). As the benefits of prolonged exercise training may be related to the repeated exposures following a single bout of exercise (Dawson et al., 2018), it is important to understand the immediate changes in blood flow and endothelial function following a single bout of acute exercise (Thijssen et al., 2018). Whilst the acute peripheral vascular responses are well reviewed (Dawson et al., 2018, Dawson et

al., 2013, Bond et al., 2015), relatively little is known about the immediate effects of exercise on cerebrovascular blood flow and function.

Cardiovascular and cerebrovascular diseases are due in part to structural and functional changes in the vasculature. FMD assessments are used to determine endothelial function in peripheral arteries (Thijssen et al., 2011, Thijssen et al., 2019). Research has tried to develop a cerebral endothelial flow mediated dilation test in humans (Carter et al., 2016, Hoiland et al., 2017). Previous research from this research group has found that cerebrovascular (ICA) and peripheral (brachial artery) artery endothelium dependent and -independent vasodilatory function are not correlated which is likely due to differences in baseline and shear stimulus (Carr et al., 2021).

Cerebral autoregulation (CA) refers to control of cerebral tissue blood flow (CBF) in response to changes in perfusion pressure. Due to the challenges of measuring intracranial pressure, CA is often described as the relationship between mean arterial pressure (MAP) and CBF. Research in individuals with hypertension and medicated hypertension suggests that the cerebral circulation preserves its ability to autoregulate, although the upper and lower limits of cerebral autoregulation are shifted to higher levels (Strandgaard and Paulson, 1995, Pavy-Le Traon et al., 2002). Cerebrovascular reactivity (CVR) is another commonly used measure of cerebrovascular function as it measures the extent of change in cerebral blood velocity in response to a stimulus and is thought to be an indication of vascular reserve and regulatory efficiency within the brain (Ito et al., 2003, Nur et al., 2009). The acute effects of exercise on CVR are not well understood. Only two studies, both performed in healthy individuals, have investigated the effects of moderate intensity continuous exercise on CVR. The studies show

conflicting findings with absolute CVR being unchanged 1-hour following thirty-minutes of moderate intensity-continuous cycling (Weston et al., 2022), whilst 45-minute moderate-continuous cycling exercise decreased CVR by 16% 1-hour post-exercise (Burma et al., 2020). A possible explanation for the differences between studies may relate to the method used to determine CVR (Koep et al., 2022). No previous studies examined the acute effects of exercise in those with cardiovascular risk, such as hypertension. This is relevant as CVR is diminished in hypertensive individuals (Settakis et al., 2003a, Settakis et al., 2003b) which may influence the acute-exercise response.

As findings from Chapter have shown that medicated hypertensive individuals may have attenuated benefits to exercise training, a potential mechanistic explanation for this observation relates to altered acute responses to exercise. Understanding the acute responses of exercise may help to understand how exercise may lead to the altered exercise-training responses. Hypertensive individuals may have different responses compared to normotensive individuals due to hypertension-induced remodelling of cerebral arteries with increases in wall thickness/lumen ratio (Baumbach and Heistad, 1989) and increased autonomic function (Grassi and Ram, 2016). Previous research has shown that the dynamics of cerebral autoregulation are well preserved in hypertensive individuals, with no difference according to the efficiency of treatment of hypertension (Pavy-Le Traon et al., 2002). Furthermore, some antihypertensive medications improve peripheral vascular function, (Koh et al., 2004, Ghiadoni et al., 2007) which may result in similar peripheral vascular function to healthy individuals.

Therefore, the purpose of this study was to examine the acute effects of moderate-intensity continuous cycling exercise on cerebrovascular function (i.e., cerebrovascular reactivity (CVR)), and specifically whether there is a difference in these responses between a control group and treated hypertensive individuals. To better understand these acute effects of exercise, we also examined resting cerebral autoregulation, CBv during exercise, in addition to the effects on post-exercise peripheral vascular function. It was hypothesised that CVR to hypercapnia would be increased post-exercise compared to baseline (Ogoh et al., 2008), with this increase attenuated in individuals who are treated for hypertension. It was also hypothesised that cerebral autoregulation, cerebral blood flow velocity during exercise and post-exercise FMD would not be different between groups.

5.2 Methods

5.2.1 Participants

Seventeen participants volunteered to participant in this study. Participants were included in the control group if they were aged between 45-65 years old, blood pressure of <130 mmHg systolic and/or <85 mmHg diastolic and has no known cardiovascular or cerebrovascular disease. Individuals were diagnosed as hypertensive by their General Practitioner and were prescribed any antihypertensive medication for at least three months were included. Individuals were excluded if they had a history of stroke (including TIAs), myocardial infarction, thrombosis, congenital heart disease, type 2 diabetes, or currently smoking or pregnant. Participant were recruited and informed of the study protocol verbally and in writing before providing written informed consent. The study was approved by Liverpool John Moores University ethics committee (approval number 23/SPS/014) and conformed to

standards set out by the Declaration of Helsinki with the exception of being registered in a database. Limited research has investigated the effect of acute exercise on cerebrovascular function (using TCD techniques) between two groups and in particular groups with cardiovascular disease or risk factors. Based on a study looking at the effects of acute exercise on MCAv in individuals with diabetes, the to achieve a power of 80% and an α level of 5% the minimal sample per group would be 4 participants (Alwatban et al., 2020). However, the two previous papers that have studied the effects of acute exercise on cerebrovascular reactivity using a repeated measures design have reported data from 9 and 10 participants (Burma et al., 2020, Weston et al., 2022). For this study, the research team will aim for 10-15 per group prior to publication.

5.2.2 Research design

Participants undertook one visit at the LJMU laboratory. Participants arrived following an overnight fast and refraining from alcohol and exercise for 24 hours and caffeine for 12 hours. Lab visits were performed in a quiet, temperature-controlled laboratory (23±1^{o}C) at a similar time of day (morning) to avoid diurnal effects. Participants rested in a supine position for ~15 minutes before resting blood pressure was taken. Approximately 5 minutes following blood pressure measurements, measures of peripheral vascular function and cerebrovascular vascular were performed. A 30-minute acute exercise bout was performed followed by 30 minutes rest in a supine position. Measures of peripheral vascular function and cerebrovascular reactivity were repeated 30 minutes following exercise cessation (Figure 5.1).

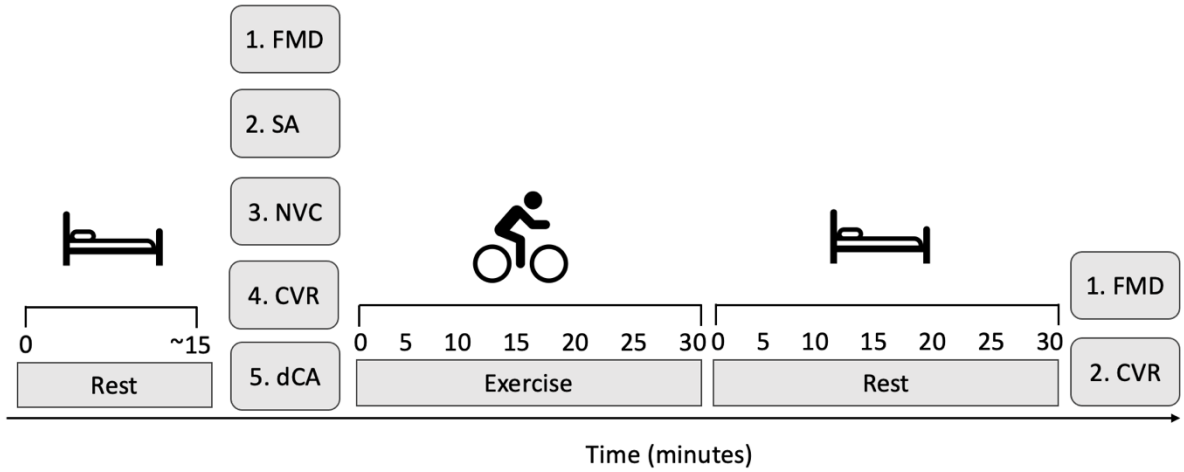


Figure 5.1. Schematic of the study design protocol. *Abbreviations: FMD, flow mediated dilation; SA, static autoregulation; NVC, neurovascular coupling; CVR, cerebrovascular reactivity; dCA, dynamic cerebral autoregulation*.

5.2.3 Experimental measures

Anthropometric measurements Participants height (SECA Stadiometer 231) and weight (SECA scales) were measured, and BMI was calculated. Automatic brachial artery blood pressure (Dinamap V100, numed health care, UK) was measured on the right arm, with measurements being repeated three times with a resting period of 2 minutes in between and subsequently values were averaged.

Peripheral vascular function Measurements of brachial artery flow mediated dilatation (FMD) were performed strictly adhering to expert consensus guidelines (Thijssen et al., 2019, Thijssen et al., 2011). This is a measure of endothelial-dependent and nitric oxide mediated function, which has been shown to be predictive of future cardiovascular risk (Inaba et al., 2010, Green et al., 2011, Ras et al., 2013). Participants rested for ≥15 minutes in the supine position. The participants' left arm was extended and positioned at an angle ~80 degree from the torso. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was

position on the forearm of the imaged arm distal to the olecranon process to provide a stimulus to forearm ischaemia. Images of the left brachial artery were acquired using high-resolution ultrasound (T3300; Terason, Burlington, MA) for one minute rest prior to cuff inflation. Analysis of brachial artery diameter was performed using custom designed edge detection and wall tracking software which is largely independent of investigator bias (Woodman et al., 2001). The cuff was inflated to supra-systolic for 5 minutes and then released. Images of the brachial artery were recorded 30 seconds before cuff deflation and for 3 minutes thereafter. The area under the curve for shear rate was calculated from the point of cuff deflation until the time of peak dilation (SR_{AUC}) (Pyke and Tschakovsky, 2007). FMD was calculated as peak artery diameter following hyperaemia, expressed as % increase using an allometric model (Atkinson et al., 2013).

Static Autoregulation Ten minutes following FMD measurements, simultaneous middle cerebral artery (MCAv) and posterior cerebra (PCAv) artery blood velocities were continuously measured through the temporal window using transcranial Doppler ultrasonography (TCD) following standardised procedures (Willie et al., 2011). Two 2-MHz Doppler probes (DWL, DiaMon, Compumedics, Singen, Germany) were adjusted until an optimal signal was identified and held in place using an adjustable head frame (DWL, DiaMon, Compumedics, Singen, Germany). Participants were instrumented with a two-way valve mouthpiece (Hans Rudolph) from which end tidal CO_2 ($P_{ET}CO_2$) was measured using a calibrated gas analyser (ML206 ADinstruments, Colorado Springs, USA). Continuous beat-by-beat blood pressure was obtained from a Finometer Pro (Finapres Amsterdam, Netherlands) on the right hand. Heart rate acquired from a five-lead electrocardiogram. All data were sampled at 50 Hz with the

data acquisition system PowerLab via the interface LabChart 8 (ADinstruments, Colorado Springs, USA).

Neurovascular coupling Two minutes following static autoregulation, neurovascular coupling measures were performed. Visual stimulation consisted of looking at a checkerboard image. A two-minute baseline of both eyes closed and eyes open was completed before five cycles of 40s of looking at the image and 20s eyes closed. MCAv and PCAv were measured throughout the test. These data were not analysed for this thesis.

Cerebrovascular reactivity to CO_2 Five to ten minutes following neurovascular coupling measures, CVR measures were performed. CO_2 reactivity was assessed whilst lying in the supine position. Bilateral MCAv was measured for the CO_2 reactivity test and to account for the influence of potential changes in mean arterial pressure on CVR outcomes from pre to post exercise, beat-by-beat blood pressure was noninvasively measured via finger plethysmography. Following a 1-minute recorded baseline, participants were switched to 5% CO_2, 21% Oxygen, and balanced nitrogen from a prefilled Douglas bag (Douglas Bag, Hans Rudolph, Oxford) for 4 minutes. Participants were instructed to breathe at their normal breathing rate. Resting MCAv, $P_{ET}CO_2$ and MAP were calculated as the mean of the minute of baseline. This response was taken as the peak response as a 30 second rolling average of MCAv. Data was extracted in 1 second time bins from LabChart. A 30 second rolling average was calculated in Microsoft Excel from the onset of gas inhalation with the peak 30 second rolling average being used to calculate CVR (Koep et al., 2022). Cerebrovascular CO_2 reactivity was calculated as both absolute and relative change from baseline MCAv per unit increase (mmHg) in P_{ETCO2}. using the equations:

$$Absolute\ CVR = \frac{\Delta MCAV}{\Delta PETCO2}$$

$$Relative\ CVR = \frac{\%\text{MCAv change from baseline}}{\Delta PETCO2}$$

Where Δ is the change from baseline to the 30 second rolling average MCAv peak. To account for the influence of MAP on MCAv, the ratio between MCAv and MAP was expressed as the cerebrovascular resistance (CBVR = MAP/MCAv). Cerebrovascular conductance (CVCi)) was then calculated (MCAv/MAP).

Cerebral dynamic autoregulation (Squat stand manoeuvres) Ten minutes following CVR, dynamic cerebral autoregulation (dCA) was assessed following guidelines by using squat-to-stands to induce transient changes in BP (Claassen et al., 2016). Participants performed low frequency squats (0.10 Hz) for five minutes. Movements were performed at 0.10Hz to create physiologically relevant changes in BP that present challenges to the autoregulatory system that are typically experienced in everyday life (Simpson and Claassen, 2018). During the manoeuvres, all participants were instructed to keep their eyes open and to avoid strenuous beathing patterns. Data were analysed using Transfer Function Analysis (TFA) in accordance with most recent guidelines (Claassen et al., 2016). Data from 5-minute recording of squat-stands manoeuvres were extracted from Labchart beat-to-beat using ECG tracing. TFA was applied using Ensemble (Version 1.0.0.28, Elucimed, Wellington, New Zealand) to calculate

associate power (normalised and absolute gain) and timing (phases) and linearity (coherence) at the point estimate of the driven frequency (0.10Hz) (Claassen et al., 2016). Gain (absolute) represents the damping effect of CA on the magnitude of BP oscillations whilst normalised gain refers to the same output as gain, except blood flow velocity values are normalised by dividing beat-to-beat values by the mean value (Van Beek et al., 2008). Phase describes the time delay between input (MAP) and output (MCAv) while coherence describes the linearity between MAP and MCAv (Van Beek et al., 2008). All TFA parameters were included for subsequent analysis as coherence exceeded 0.4. TFA parameters of BP oscillations are averaged across the very low (VLF; 0.02-0.07 Hz), low (LF; 0.07-0.2 Hz) and high (HF; 0.2-0.4) frequency domains. For dCA, forced oscillations at 0.10 Hz were employed for this study, this falls within the ranges of the LF domain therefore LF was reported as dCA is highly active within this frequency of squats (Zhang et al., 1998).

Acute exercise Participants performed 30 minutes of exercise on a cycle ergometer (Corvial, Lode, Netherlands) at 50-60% HR_{max} [220-(age x 0.7)] at 60-70 rpm. During the exercise bout MCAv, $P_{ET}CO_2$, HR (Polar, FT1, Finland) and rating of perceived exertion (RPE, Borg Scale) was recorded every 10 minutes during the exercise bout.

5.4.2 Statistical analysis

Analysis was performed using SPSS (Version 29; SPSS Inc, Chicago, IL). Baseline characteristics between the control group and medicated hypertension group was analysed using an independent samples t-test. A two-way mixed design ANOVA was used to evaluate group differences in (cerebro)vascular function (FMD and absolute/relative CVR) pre- and 30-minute post exercise, with group (control and medicated hypertension) and time (pre and 30 min post

exercise) used as main effects. A two-way mixed design ANOVA was used to evaluate the potential impact of group (and medicated hypertension) on changes in MAP, MCAv, HR, RPE during exercise (0,5,10,15,20,25,30 minutes). Given the possible change in baseline brachial artery diameter and post-deflation shear rate area under the curve, acute changes in FMD% were also analysed using allometric scaling methods (Atkinson et al., 2013). Statistical significance was delimited at $P<0.05$ and exact P values are cited.

5.3 Results

5.3.1 Participants

A total of 17 participants were included in this study, 10 participants (6 male and 4 female) were classified in the control group and 7 participants (5 male and 2 female) were diagnosed with hypertension and were prescribed antihypertensive medication which are listed in Table 5.1.

Table 5.1. Baseline characteristics for the normotension and medicated hypertension group. Values are means ± SD.

Baseline characteristics	Control (4 females, 6 males)	Medicated hypertension (2 females, 5 males)	*P* value
Age (years)	54 ± 7	55 ± 6	0.618
Height (cm)	170 ± 14	168 ± 9	0.103
Weight (kg)	73.02 ± 15.22	87.34 ± 14.11	0.873
Body mass index (kg/m^2)	25.17 ± 3.97	30.74 ± 7.30	0.081
Resting heart rate (bmp)	64 ± 13	67 ± 9	0.193
Systolic blood pressure (mmHg)	128 ± 9	136 ± 13	0.326
Diastolic blood pressure (mmHg)	79 ± 10	81 ± 12	0.455
Mean arterial pressure (mmHg)	99 ± 8	103 ± 7	0.930
Medications			
N = 2	Calcium channel blocker		
N = 1	ACE inhibitors		
N = 1	ARB blocker		
N = 1	ACE inhibitor and calcium channel blocker		
N =2	β-blocker and ACE inhibitor		

Abbreviations; ACE inhibitor, Angiotensin-converting enzyme; ARB blocker, Angiotensin receptor blockers.

5.3.2 Resting conditions

Baseline characteristics. There was no significant difference in age, height, weight, BMI, SBP, DBP and MAP (taken from automatic blood pressure cuff) between the normotensive control and medicated hypertension group (all *P*>0.05) (Table 5.1).

Baseline haemodynamics. There was no significant difference in MAP, MCAv, $PETCO_2$, cerebrovascular resistance, SBP, DBP between the control and medicated hypertension group (all $P>0.05$) (Table 5.2).

Table 5.2 Baseline haemodynamics (taken during measurements for static autoregulation) for the control and medicated hypertension group. Values are means± SD.

	Control	Medicated hypertension	*P* value
Resting Data			
MAP (mmHg)	71 ± 10	72 ± 7	0.519
MCAv ($cm.s^{-1}$)	58.18 ± 11.42	49.21 ± 15.08	0.238
$P_{ET}CO_2$ (mmHg)	43.88 ± 5.51	39.97 ± 5.33	0.907
CVRi	0.59 ± 0.15	0.522 ± 0.19	0.93
CVCi	0.59 ± 0.16	0.54 ± 0.18	0.555
SBP (mmHg)	128 ± 9	136 ± 13	0.933
DBP (mmHg)	79 ± 10	81 ± 9	0.292

Abbreviations; MAP, mean arterial pressure; MCAv, middle cerebral artery blood velocity, $P_{ET}CO_2$, partial pressure of end tidal carbon dioxide; CVRi, cerebrovascular resistance index, CVCi, cerebral vascular conductance indexe, SBP, systolic blood pressure; DBP, diastolic blood pressure.

Cerebrovascular function. For static autoregulation, baseline blood pressure power was significantly higher in the medicated hypertensive group across all frequencies ($P<0.05$) for spontaneous oscillations of MCAv (Table 5.3). Baseline blood pressure power (power spectral density) was only significantly different between groups in the high frequency for PCAv ($P=0.034$). Transfer function analysis demonstrated a significantly greater low frequency gain in the control group ($P=0.001$) whilst there was no significant difference in gain, normalised gain, coherence, and phase between the groups (Table 5.3). Regarding dynamic autoregulation, parameters of cerebral autoregulation (low frequency gain, normalised gain, phase, and coherence) were not significantly different between the medicated hypertensive group and the control group ($P>0.05$, Table 5.4).

Table 5.3 Power spectral and transfer function analysis of cerebral autoregulation during spontaneous changes in BP and CBFv.

	Control	Medicated hypertension	*P Values*
Power Spectrum			
Baseline MCAv power (cm/s^2)			
VLF (10/7)	10.86 ± 9.69	6.48 ± 5.96	0.259
LF (10/7)	6.09 ± 6.00	7.69 ± 11.51	0.282
HF (10/7)	4.85 ± 7.19	8.08 ± 15.57	0.139
Baseline BP power ($mmHg^2$)			
VLF (10/7)	16.19 ± 9.23	31.39 ± 33.13	**0.008**
LF (10/7)	10.75 ± 9.22	25.81 ± 30.30	**0.009**
HF (10/7)	6.37 ± 8.23	22.47 ± 30.90	**0.005**
Transfer Function			
Spontaneous Oscillations			
VLF gain ($cm \cdot s \cdot mmHg$) (10/5)	0.72 ± 0.32	0.54 ± 0.18	0.333
VLF Normalised gain ($\%.mmHg^{-1}$) (10/5)	1.18 ± 0.28	1.43 ± 0.28	0.302
VLF phase (radians) (10/5)	0.82 ± 0.70	0.19 ± 0.74	0.967
VLF coherence (10/7)	0.57 ± 0.15	0.44 ± 0.14	0.730
LF gain ($cm \cdot s \cdot mmHg$) (10/6)	0.72 ± 0.18	0.68 ± 0.19	0.938
LF Normalised gain ($\%.mmHg^{-1}$) (10/6)	1.18 ± 0.28	1.42 ± 0.28	0.865
LF coherence (10/7)	0.70 ± 0.21	0.59 ± 0.27	0.716
LF phase (radians) (10/6)			0.414
HF gain ($cm \cdot s \cdot mmHg$) (10/6)	1.45 ± 1.79	0.71 ± 0.28	0.197

HF Normalised gain (%.mmHg^{-1}) (10/6)	2.36 ± 2.73	1.46 ± 0.37	0.183
HF coherence (10/7)	0.60 ± 0.29	0.57 ± 0.29	0.605
HF phase (radians) (10/6)	0.35 ± 0.68	-0.05 ± 1.15	0.539
Baseline PCAv power (cm/s^2)			
VLF (5/6)	5.43 ± 3.28	5.80 ± 6.91	0.454
LF (5/6)	3.31 ± 2.70	6.24 ± 9.44	0.185
HF (5/6)	2.3 ± 3.12	7.12 ± 13.28	0.127
Baseline BP power (mmHg^2)			
VLF (5/6)	13.14 ± 6.56	21.02 ± 20.37	0.188
LF (5/6	10.94 ± 10.70	16.75 ± 20.29	0.212
HF (5/6)	7.00 ± 9.07	21.15 ± 33.64	**0.034**
Transfer Function			
Spontaneous Oscillations			
VLF gain (cm·s·mmHg) (5/4)	0.44 ± 0.79	0.43 ± 0.17	0.107
VLF Normalised gain (%.mmHg^{-1}) (5/4)	0.99 ± 0.36	1.21 ± 0.21	0.478
VLF phase (radians) (5/4)	1.24 ± 0.61	0.30 ± 0.76	0.681
VLF coherence (5/6)	0.48 ± 0.10	0.43 ± 0.14	0.549
LF gain (cm·s·mmHg) (5/6)	0.54 ± 0.05	0.53 ± 0.12	**0.001**
LF Normalised gain (%.mmHg^{-1}) (5/6)	1.22 ± 0.24	1.39 ± 0.18	0.272
LF phase (radians) (5/6)	0.66 ± 0.55	0.35 ± 0.26	0.330
LF coherence (5/6)	0.63 ± 0.26	0.64 ± 0.17	0.270
HF gain (cm·s·mmHg) (4/6)	0.54 ± 0.05	0.53 ± 0.12	0.121

HF Normalised gain (%.mmHg^{-1}) (4/6)	1.11 ± 0.15	1.29 ± 0.48	0.094
HF phase (radians) (4/6)	0.28 ± 0.45	0.39 ± 0.63	0.737
HF coherence (5/6)	0.59 ± 0.32	0.56 ± 0.26	0.295

Data presented as mean±SD. Abbreviations; VLF, very low frequency; LF, low frequency; HF, high frequency; MCAv, middle cerebral artery velocity; BP, blood pressure

Table 5.4 Power spectrum densities of forced oscillations in mean arterial pressure and cerebral blood flow velocity during squat-stand manoeuvres (0.10Hz, low frequency).

	Control	Medicated hypertension	*P* value
Power Spectrum			
BP power (mmHg^2)	390.16 ± 177.25	534.88 ± 282.93	0.310
Baseline MCAv power (cm/sec^2)	336.30 ±182.5	130.94 ± 69.91	**0.048**
Transfer Function			
LF gain (cm·s·mmHg)	0.87 ± 0.32	0.54 ± 0.16	0.245
LF Normalised gain (%.mmHg^{-1})	1.35 ± 0.28	1.35 ± 0.35	0.798
LF coherence	0.73 ± 0.16	0.74 ± 0.24	0.243
LF phase (radians)	0.46 ± 0.37	0.18 ± 0.36	0.918

Data presented as means ± SD. Abbreviations; LF, very low frequency; LF, low frequency; HF, high frequency; MCAv, middle cerebral artery velocity; BP, blood pressure

5.3.3 During exercise

MCAv ($F_{2.386,4.825}$, P= 0.016), Heart rate ($F_{3.130, 71.787}$, P<0.001) and RPE ($F_{2.808,90.103}$, P<0.001) all increased during the exercise bout with a main effect of time (Figure 5.2) but there was no significant group or interaction effect. There was no effect of group, time or interaction on MAP and $PETCO_2$ during the exercise bout all P>0.05.

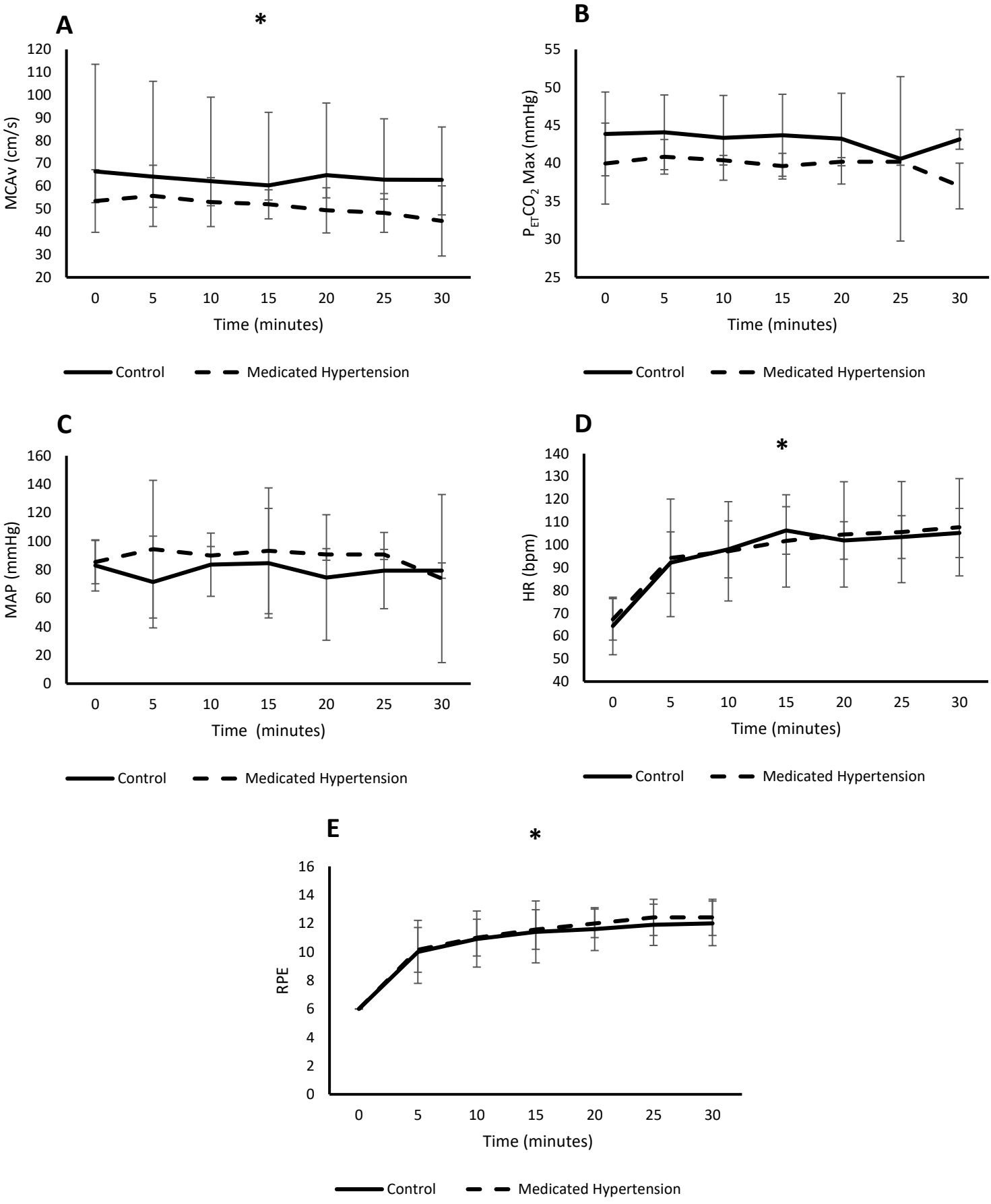

A
*
MCAv (cm/s)
120
110
100
90
80
70
60
50
40
30
20
0
5
10
15
20
25
30
Time (minutes)
Control
Medicated Hypertension
B
$P_{ET}CO_2$ Max (mmHg)
55
50
45
40
35
30
25
0
5
10
15
20
25
30
Time (minutes)
Control
Medicated Hypertension
C
MAP (mmHg)
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (minutes)
Control
Medicated Hypertension
D
*
HR (bpm)
140
130
120
110
100
90
80
70
60
50
40
0
5
10
15
20
25
30
Time (minutes)
Control
Medicated Hypertension
E
*
RPE
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
Time (minutes)
Control
Medicated Hypertension

Figure 5.2: Middle cerebral artery velocity (MCAv) (a), $P_{ET}CO_2$ (b), MAP (c), heart rate (d), RPE (e) in response to moderate intensity cycling in individuals in the group and medicated hypertension group. *Error bars represent SD. * indicated a significant effect of time P<0.05. Abbreviations; MCAv, Middle cerebral artery velocity, $P_{ET}CO_2$, end tidal volume of CO_2; MA, mean arterial pressure; HR, heart rate; bpm, beats per minute; RPE, rate of perceived exertion.*

- *0 minutes represents starting values whilst seated on the cycle ergometer.*

5.3.4 The effects of acute exercise on cerebral and peripheral vascular function

Cerebrovascular function. There was a significant interaction effect between groups for resting MCAv ($F_{1.00,13.00}$ = 6.31, *P*=0.026). The control group showed a reduction in resting MCAv (8.98 cm/s) from pre-exercise to 30-minute post exercise, whilst the medicated hypertensive group showed an increase in resting MCAv (4.34 cm/s) from pre to post exercise (Table 5.5). Upon correction for differences in blood pressure, we found no significant effect of group (control and medicated hypertension), time (pre to post exercise) or interaction for cerebrovascular conductance (all *P*>0.05) (Table 5.5).

There was a significant interaction effect ($F_{1,13}$= 4.801, P=0.02) between group and the change across time for CVR. Post-hoc analyses revealed that absolute CVR decreased by 0.6 cm/s/mmHg in the control group, but increased by 1.03 cm/s/mmHg in the medicated hypertensive group (Table 5, Figure 5.3).

Table 5.5 Cerebrovascular reactivity to 5% carbon dioxide.

Variable	Control Pre exercise	Control 30 min post exercise	Medicated Hypertension Pre exercise	Medicated Hypertension 30 min post exercise	Time	Group	Time*Condition
Baseline							
MCA_V (cm/s)	67.21 ± 14.19	58.23 ± 14.92	48.62 ± 11.86	52.96 ± 7.85	0.397	0.103	**0.026***
$PETCO_{2max}$ (mmHg)	44.29 ± 5.54	43.92 ± 5.64	40.38 ± 3.96	41.08 ± 4.45	0.862	0.231	0.589
MAP (mmHg)	80 ± 21	85 ± 23	85 ± 19	81 ± 31	0.933	0.960	0.548
CBVR	1.25 ± 0.42	1.51 ± 0.44	1.82 ± 0.42	1.62 ± 0.79	0.812	0.212	0.081
CVCi	0.92 ± 0.42	0.71 ± 0.19	0.59 ± 0.18	0.77 ± 0.42	0.895	0.355	0.107
30s rolling average							
MCA (cm/s)	87.44 ± 19.55	73.73 ± 19.24	64.93 ± 17.22	73.37 ± 21.04	0.468	0.273	0.08
$PETCO_2$ (mmHg)	52.03 ± 4.29	51.32 ± 4.29	48.65 ± 5.06	48.12 ± 4.76	0.158	0.197	0.843
MAP (mmHg)	94 ± 17	90 ± 24	98 ± 12	94 ± 26	0.584	0.714	0.952
Absolute CVR (cm/s/mmHg)	2.77 ± 1.24	2.17 ± 0.67	1.85 ± 0.60	2.88 ± 1.76	0.572	0.834	**0.047***
Relative CVR (%/mmHg)	4.01 ± 1.22	3.96 ± 1.67	3.86 ± 0.99	5.20 ± 2.59	0.328	0.398	0.297
Absolute CVR_{CO2MAP} ($cm.s,mmHg^{-1}.mmHg^{-1}$)	0.81 ± 1.31	0.50 ± 0.78	0.60 ± 1.37	0.50 ± 1.13	0.701	0.831	0.844
Relative CVR_{CO2MAP} ($\%cm.s/mmHg^{-1}.mmHg^{-1}$	0.95 ± 1.43	0.50 ± 0.74	0.79 ± 1.60	0.50 ± 1.13	0.514	0.883	0.889
CVRi	1.13 ± 0.30	1.21 ± 0.32	1.26 ± 0.78	1.15 ± 0.84	0.890	0.889	0.267
CVCi	0.93 ± 0.20	0.64 ± 0.07	0.75 ± 0.33	0.64 ± 0.04	0.183	0.541	0.540

Abbreviations; MCAv, middle cerebral artery velocity; $P_{ET}CO_2$, partial pressure of end tidal carbon dioxide; MAP mean arterial pressure; CVRi cerebrovascular resistance index; CVCi, conductance index to carbon dioxide; CVR cerebrovascular reactivity; CVR_{CO2MAP} mean arterial pressure reactivity to carbon dioxide.
**Indicated significance P<0.05*

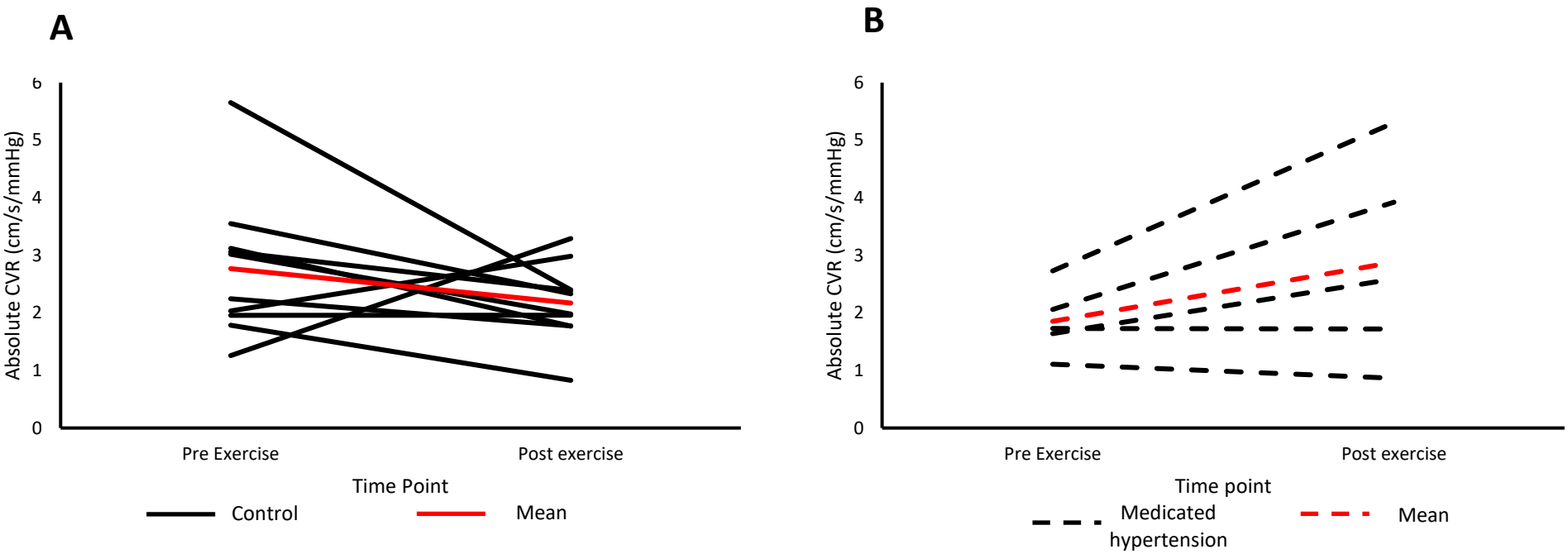


Figure 5.3. Individual data points (black lines) and mean (red line) for absolute CVR pre and post exercise for control (A) and medicated hypertensive (B) groups.

Peripheral vascular function There was no significant effect of group (control and medicated hypertension), time (pre to post exercise) or interaction effect on FMD (Table 5.6).

Table 5.6 Vascular function for participants with normotension and medicated hypertension

	Control Pre exercise	Control 30 min post exercise	Medicated hypertension Pre exercise	Medicated hypertension 30 min post exercise	*Group*	*Time*	*Interaction*
Baseline Diameter (mm)	3.70 ± 0.54	3.77 ± 0.57	4.41 ± 0.91	4.29 ± 1.03	0.108	0.781	0.309
Peak Diameter (mm)	3.95 ± 0.54	4.02 ± 0.58	4.63 ± 0.80	4.53 ± 0.94	0.096	0.867	0.368
FMD (%)	6.75 ± 2.75	6.59 ± 3.19	5.59 ± 3.83	6.32 ± 3.76	0.647	0.644	0.466
FMD (mm)	0.25 ± 0.09	0.24 ± 0.11	0.22 ± 0.12	0.24 ± 0.11	0.141	0.929	0.639
Time to Peak (s)	48.30 ± 29.83	38.09 ± 18.24	55.86 ± 22.83	52.26 ± 8.03	0.186	0.371	0.665
Shear AUC (10^3)	16488.83 ± 8058.94	13366.77 ± 6563.705	12460.41 ± 8956.656	17579.18 ± 7486.338	0.959	0.644	0.094

Abbreviations: Shear AUC; Shear rate area under the curve, FMD; Flow mediated dilatation.

5.4 Discussion

The aim of the current study was to investigate the acute effects of moderate-intensity continuous cycling on cerebrovascular function, but also to examine whether there is a difference in responses between a control group and treated hypertensive individuals. This study found that individuals who are treated for hypertension demonstrated a distinct response for post exercise levels of MCAv and absolute CVR compared to the control group. The differences in responses between the two groups are unlikely related to baseline autoregulation or blood flow changes during exercise, as both groups showed similarity in these responses. This study also found no change in peripheral vascular function in either group following acute exercise. Overall, findings from this study suggest that individuals treated for hypertension have a different post-exercise cerebrovascular response.

In the present study cerebrovascular reactivity decreased following acute exercise in healthy individuals. Only two previous studies, both performed in healthy individuals, have examined the effects of acute exercise on CVR. Burma and colleagues examined 45-minute moderate-continuous cycling exercise (at ~50% heart-rate reserve) and reported a decreased relative hypercapnic slope (7% CO_2 closed circuit rebreathing) by 20% immediately following exercise and 16% 1 hour post-exercise in healthy individuals (Burma et al., 2020). This finding is comparable to the control group within our study, which showed a small decrease (0.6 cm/s/mmHg) in absolute CVR 30 minutes post moderate intensity cycling. Conversely, another study found that 30 minutes of moderate intensity continuous exercise did not change absolute CVR (6% CO_2 for 4 minutes) 1 hour post-exercise cessation (Weston et al., 2022). The differences in outcomes between studies may relate to the method used to assess CVR when comparing open circuit breathing (Weston et al., 2022) or 7% CO_2 closed circuit

rebreathing (Burma et al., 2020). There are recent concerns regarding the variability or changes in MCAv during open circuit hypercapnic challenges. (Koep et al., 2021, Burley et al., 2020). CVR in the present study was analysed using a reliable approach from Koep et al (2022). In addition, there may be a dynamic post-exercise response, as reported in peripheral vascular function (Dawson et al., 2013), and as such the timing of assessments post-exercise may influence the findings. Further work is needed to examine post-exercise responses in CVR in healthy individuals.

A key finding in this study is the different post-exercise response for CVR in medicated hypertensive individuals compared to control. The increase in CVR following acute exercise in medicated hypertensive individuals may be attributed to the effects of antihypertensive medication. Mean absolute CVR increased in individuals who are treated for hypertensive following acute exercise, whilst in controls CVR decreased. As this study observed no difference at baseline in static and dynamic autoregulation (gain, phase or coherence) between groups, and no difference in cerebral perfusion or exercise haemodynamics (MCAv, MAP, $P_{ET}CO_2$, HR) at rest or during exercise between healthy individuals and those with medicated hypertension, it is unlikely that differences in post-exercise responses are due to altered cerebral autoregulation at baseline. More specifically, the differences in post exercise response in CVR between the medicated hypertensive group and healthy individuals may be influenced by the effects of exercise on vascular dynamics and autonomic regulation. The Selfish Brain hypothesis of hypertension suggests that that brainstem hypoperfusion triggers increased sympathetic nervous system activity and blood pressure as a mechanism to maintain cerebral perfusion (Hart, 2016). Individuals with hypertension have altered autonomic nervous activity, with an increased sympathetic output and decreased

parasympathetic tone, compared to healthy individuals (Grassi and Ram, 2016). Acute exercise can further modulate autonomic function, increasing sympathetic activity and decreasing parasympathetic activity (Robinson et al., 1966, Ekblom et al., 1972, Fagraeus and Linnarsson, 1976). In medicated hypertensive individuals, this shift in autonomic balance following exercise may lead to a more pronounced increase in CVR, reflecting enhanced sympathetic-mediated vasodilation but it currently unknown what effects increased sympathetic nervous system activation has on the cerebrovasculature.

This study also found no difference in resting peripheral vascular function between the normotensive and medicated hypertensive group. Previous work found that FMD was lower in individuals with essential hypertension compared to normotensives despite good blood pressure control (Furumoto et al., 2002). Given that blood pressure has been shown to be negatively associated with FMD in unmedicated individuals (Holder et al., 2021), the use of antihypertension medication may explain why there is no difference in peripheral vascular function between the two groups. Previous work has demonstrated that angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) improve FMD (Koh et al., 2004, Ghiadoni et al., 2007). Furthermore, the individuals in the control group in the present study were older and could have been pre-hypertensive, which may offer a potential explanation for the lack of a difference between groups.

This study demonstrated that acute exercise did not affect FMD in either group. There is variability among the literature examining the effects of acute exercise on vascular function. Measurements taken 30-60 minutes post exercise have reported conflicting findings with some studies reporting an increase (Goel et al., 2007, Harris et al., 2008, Harvey et al., 2005, Johnson et al., 2012, Hanson and Casey, 2023), decrease (Harris et al., 2008, Jurva et al., 2006,

Rognmo et al., 2008, Caldwell et al., 2023) or no change (Harvey et al., 2005, Rognmo et al., 2008, Johnson et al., 2012) in FMD following exercise. Studies that have taken multiple FMD measures after exercise training have shown that the timing of the postexercise FMD assessment can alter the findings (Dawson et al., 2013). Exercise intensity can also influence post exercise FMD (Dawson et al., 2013). Serval studies (Dawson et al., 2013, Dawson et al., 2018, Birk et al., 2012, Johnson et al., 2012, Katayama et al., 2013) but not all (Iwamoto et al., 2018, Dawson et al., 2008) have reported that acute endurance exercise leads to a decrease in FMD when performed at higher intensities, therefore our moderate intensity exercise may not have been sufficient to elicit a decrease.

Increase in cerebrovascular function post-acute exercise was not mirrored by changes in peripheral vascular function. This supports previous research that reported no significant correlation between resting peripheral and cerebral shear-mediated endothelial function at rest (Carr et al., 2020). Findings from the present study support the idea that peripheral and cerebrovascular function (measured in the MCA) responds differently to acute exercise which has also been demonstrated in work examining the acute effects of HIIE on cerebrovascular and peripheral vascular function (Weston et al., 2014). Non-exercise interventions have also shown peripheral and cerebrovascular function measures to respond differently; sugar sweetened-beverage consumption increased brachial artery FMD but did not change CVR in adolescents (Koep et al., 2021). Collectively, these data provide further support that findings from the periphery cannot be extrapolated to cerebrovascular reactivity when measured using TCD.

Whilst the relevance of acute exercise responses are not fully understood, previous research has shown that acute peripheral vascular responses to exercise stimulus may predict vascular

adaptation (Dawson et al., 2018). Dawson et al found a significant positive correlation between the immediate exercise-induced change (post–pre) in FMD and the change in FMD after training (Dawson et al., 2018). The training-induced change in FMD was positively and significantly correlated to acute exercise-induced changes in mean shear. However, correlation does not imply causation and it is currently unknown if the drop in FMD is related to the transient period of risk for cardiovascular events that has been previously reported for one hour following exercise (Whang et al., 2006, Smyth et al., 2016). Understanding the acute effects of exercise may predict future clinical cardioprotective responses (Thijssen et al., 2018). To date it is unknown what an increase, decrease or no change in (cerebro) vascular parameters following exercise means for future cardiovascular risk. Interestingly, in *Chapter 3*, it was found that individuals who were prescribed antihypertensive medication may also demonstrate impaired cardioprotective effects of physical activity on the risk of stroke, compared to normotensive individuals. However, it cannot be speculated that the different association on stroke risk is due to differences in acute exercise responses of cerebrovascular reactivity between the control and medicated hypertensive group, further research is warranted.

There are several strengths to this study including that FMD was analysed according to published guidelines in which FMD being scaled allometrically (Atkinson et al., 2013) and CVR was analysed using a reliable approach from an open circuit CO_2 breathing test (Koep et al., 2022). However, this study does present with limitations; the first notable limitation was the small sample size. The use of TCD as a surrogate measure of CBF presents with challenges as this method does not allow for directly measured changes in vessel diameter in response to changes in $P_{ET}CO_2$. Research demonstrates that MCAv diameter to remain constant in

response to modest changes in CO_2 (± 5 mmHg) but this remains debated (Brothers and Zhang, 2016, Hoiland and Ainslie, 2016) as well as during acute moderate changes in BP (Giller et al., 1993, Serrador et al., 2000). Furthermore, as measurements have only been taken at one time point following exercise (30 minutes), this means we are not examining the time-course of potential post exercise changes. Measurements at more time points need to be taken to examine the full time-course changes. Furthermore, VO_{2max} was not measured which may influence cerebral blood flow and CVR (Barnes et al., 2013, Intzandt et al., 2020, Thomas et al., 2013) although this effect is not clear.

5.5 Conclusion

To summarise, findings from this study suggest that acute cerebrovascular responses following a bout of moderate intensity exercise may be different between healthy individuals and medicated hypertensives which was shown by a reduction in absolute CVR in the control group and increases in medicated hypertensives. The results need to be interpreted with caution due to the small sample size but warrant future studies to understand the time-course and potential implications of responses to cerebrovascular function in individuals with treated hypertension.

Chapter 6: Synthesis of Findings

6.1 Aims of Thesis

The overarching aim of this thesis was to further investigate the heterogeneity of cardiovascular responses to exercise training. For this purpose, a range of clinical conditions, interventions and assessments of cardiovascular health were included in the thesis. The work described in the present thesis was designed to examine the interaction between cardiovascular risk factors and exercise on the cardioprotective effects associated with regular exercise. In order to examine this topic, *Chapter 3* investigated the impact of hypertension (a leading cardiovascular risk factor) on the dose-response association between regular physical activity and cardiovascular events (stroke) and explored whether medication altered this relationship. Secondly, this thesis investigated whether *a priori* endothelial dysfunction is associated with distinct exercise training-induced improvements in traditional cardiovascular disease risk factors, cardiopulmonary fitness, or vascular function. Finally, *Chapter 5* aimed to investigate the potential mechanistic explanations on the impact of antihypertensive medication use on vascular responses to acute exercise.

6.2 Summary of Major findings

The novel work undertaken in this thesis has generated new knowledge which could inform future clinical practice. The main findings of this thesis are:

1. Regular physical activity reduces stroke risk in the total population. The dose response association does not differ between normotensive and hypertensive individuals. However, the use of antihypertensive medication may be associated with a smaller benefit of regular physical activity on stroke risk (chapter 3).

2. The benefits of exercise training on cardiovascular risk factors seems similarly present across multiple clinical groups and does not seem to be affected by *a priori* endothelial function status (chapter 4).
3. Acute exercise leads to immediate changes in cerebral blood flow velocity and cerebrovascular function following exercise, with medicated hypertensive individuals demonstrating distinct acute effects of moderate intensity exercise on cerebrovascular function and cerebral blood velocity compared to controls (chapter 5).

6.3 General discussion of major findings

6.3.1 A priori heath status does not alter the cardioprotective effect of increased physical activity.

This thesis aimed to investigate what factors may alter the cardioprotective effects of exercise. Whilst increased PA is accepted as a lifestyle intervention to improve cardiovascular health in most populations, the heterogeneity of the response and the mechanisms underpinning improved cardiovascular health are still not fully known. The interaction between cardiovascular risk factors and endothelial function is complex, with changes in one impacting the other. However, it is possible that there are independent and possibly additive effects of endothelial dysfunction and CVD risk factors following increased PA/exercise training. It is widely acknowledged that regular PA is strongly associated with a reduced risk of noncommunicable diseases and mortality (Eijsvogels et al., 2016, Lee et al., 2012). Data from the general population indicate that the benefits of PA on mortality and morbidity follow a curvilinear dose–response relationship (Eijsvogels et al., 2016, Moore et al., 2012, Kraus et al., 2019, Arem et al., 2015). However, individuals with established CVD patients show conflicting results as some studies found a linear association between PA and mortality

reductions (Moholdt et al., 2008, Stewart et al., 2017, Jeong et al., 2017) whereas others demonstrate the presence of a reverse J-shaped or U-shaped relationship (Mons et al., 2014, Wannamethee et al., 2000, Williams and Thompson, 2014, Keteyian et al., 2012). The cardiovascular health status may influence the benefits of MVPA as larger volumes of MVPA are linked to greater health benefits in healthy participants compared to individuals without CVD (Jeong et al., 2019, Bakker et al., 2021). Presence of cardiovascular risk factors has also been shown to alter the association between MVPA and incident major adverse cardiovascular events and mortality, as a curvilinear association was found in healthy individuals and individuals with cardiovascular disease risk factors, whilst CVD patients demonstrated a linear relationship (Bakker et al., 2021). A novel aspect of this thesis is the focus on the relationship between MVPA and stroke risk, whilst investigating the effect of a singular cardiovascular risk factor on this dose response association between MVPA and stroke (i.e., hypertension, *Chapter 3*). Findings from *Chapter 3* and *Chapter 4* reinforcing importance of regular physical activity, regardless of *a priori* health status, due to its cardioprotective effects.

6.3.2 Medication use may alter the cardioprotective effect of acute exercise and exercise training.

Whilst it was found that exercise training was beneficial in hypertensive individuals to reduce stroke risk, there is still variation in individual responses. As antihypertensive medication is prescribed to individuals with hypertension when lifestyle interventions have not reduced blood pressure, it is important to understand the impact of antihypertensive medication use on exercise training responses. Findings of *Chapter 3* and *5* suggested that, although exercise training has benefits in most individuals, the use of medication may alter both the acute effects of exercise (*Chapter 5*) as well as the cardioprotective effect of regular exercise

(Chapter 3). There is no consensus among the literature on the impact of medication use on the cardioprotective benefits of exercise. The influence of antihypertensive medication is likely to be multifactorial due to different antihypertensive medications affecting different pathophysiological pathways.

There is limited research that has investigated the impact of ACE inhibitor on exercise training functional responses, with the majority of research investigating the impact in older individuals with functional impairment. Twenty weeks of ACE inhibitor treatment alone (compared to placebo) has been shown to improve 6-minute walking distance in elderly people with functional impairment (Sumukadas et al., 2007) and heart failure (Hutcheon et al., 2002) whilst others have shown no improvement in physical function (Cesari et al., 2010, Zi et al., 2003). A two-year cohort of older individuals with hypertension demonstrated that exercise is an effective strategy to reach hypertensive and functional status goals, independently of the ACE inhibitor therapy (Baptista et al., 2018). However, chronic treatment of ACE inhibitor alone reduced upper body strength and aerobic endurance (measured by senior fitness test battery) (Baptista et al., 2018) while others did not find any association between ACE inhibitor treatment and markers of physical performance (Cesari et al., 2010, Spira et al., 2016) or exercise-induced adaptations (Sumukadas et al., 2014). An 8-week randomised control trial of normotensive untrained participants receiving either ACE inhibitor or placebo found that high-intensity physical activity increases whole-body maximal oxygen uptake and skeletal muscle endurance independent of ACE inhibitor treatment in normotensive adults. However, the administration of ACE inhibitor impairs the exercise-induced increase in lean mass and left atrial volume and compromises total haemoglobin mass (Sjúrðarson et al., 2022). On the other hand, older individuals with mild to moderate

functional impairment following a 12- month exercise intervention which involved strength, flexibility, and balance training, reported more pronounced training-induced improvements (following a 12-month exercise intervention) in walking speed and mobility (measured using Short Physical Performance Battery test) in older individuals with mild to moderate functional impairment receiving ACE inhibitors compared to nonusers (Buford et al., 2012). Within this study, individuals taking ACE inhibitors alongside other antihypertensive medication were categorised within the ACE inhibitor group therefore results need to be interpreted with caution. More research is warranted to understand the impact of ACE inhibitors on exercise induced adaptation in order to understand why we have preliminary found that the use of antihypertensive medication may be associated with smaller cardioprotective benefits when combined with exercise. The majority of research has investigated the impact of ACE inhibitors on exercise-induced adaptations in older individuals with functional impairment, more research is warranted to investigate this impact in individuals with hypertension in order to clearly understand the impact that ACE inhibitors may have.

Angiotensin type-1 receptor (AT_1) and bradykinin receptor B2 are widely expressed (Deminice et al., 2020, Figueroa et al., 1996, Matsumoto et al., 2000, Minshall et al., 1995), and their activation partially mediates adaptations to exercise training (Barauna et al., 2008, Massidda et al., 2014). ACE inhibitors have a range of effects including reduced circulating angiotensin II and increased plasma bradykinin concentration (Su et al., 1999) therefore, ACE inhibitors may alter exercise adaptations. ACE inhibitors inhibit the conversion of angiotensin I to angiotensin II which indirectly impacts the impact of AT_1 receptor signalling. Reduction of angiotensin II results in vasodilation of the blood vessels (Sudhir et al., 1996). ACE inhibition has been demonstrated to partially normalise impaired vasodilation after long-term but not

short-term administration (Drexler et al., 1989, Wilson and Ferraro, 1985). The increased vasodilation may be beneficial in improving blood flow to active muscles (thus enhancing oxygen delivery and nutrient delivery) meaning that those taking this medication may experience improved exercise tolerance due to enhanced muscle perfusion. Improvements in leg blood flow after ACE inhibition parallel increases in exercise capacity in heart failure patients (Drexler et al., 1989). On the other hand, the reduced vasoconstriction may alter the redistribution of blood flow to active muscles and non-exercising tissue during exercise. Angiotensin II is also involved in cardiac remodelling via activation of AT_1 receptors, consequently taking ACE inhibitors has been shown to impair cardiac hypertrophy (Barauna et al., 2008). Whilst this may be beneficial in some cardiovascular conditions, it may impact cardiac adaptations that normally occur in response to exercise-induced stress, potentially altering the beneficial effects of exercise on cardiac structure and function. Overall, ACE inhibitor treatment may alter exercise-induced adaptations via multiple different pathways including reducing circulating angiotensin II and increased plasma bradykinin.

Beta blockers and calcium channel blockers are also commonly prescribed to individuals with hypertension. β-blockade has been found to compromise exercise performance, impair thermoregulation during exercise (Gordon and Duncan, 1991) as well as attenuate the exercise effectiveness on lowering lipids (Duncan et al., 1989) and can adversely affect or attenuate the acute response to exercise and trainability to aerobic exercise (Pollock et al., 1991). These effects of beta-blockers may theoretically attenuate the exercise-induced hermetic stimuli and diminish the ability to benefit and adapt to exercise training. This is supported by studies examining responses during submaximal exercise, as the use of beta-blockers alone or in combination with other antihypertensive medications was associated

with lower SBP, DBP and HR (Kokkinos et al., 2006). In a 6-week exercise training study (5 days/week, 45 min/day, at least 75% peak heart rate), 39 men were assigned oral propranolol, atenolol or matched placebo. Peak oxygen consumption increased significantly with training in all three groups. Similarly, peak work rate and duration of work increased in all 3 groups. Thus, cardiovascular training effects can be produced in healthy men despite the presence of beta blockers (Savin et al., 1985). Sixteen weeks of aerobic exercise training in healthy individuals, 17 to 34 years of age, receiving either placebo, propranolol (160 mg/day) and atenolol (100 mg/day). All groups reduced their submaximal steady-state heart rates consequent to training; submaximal oxygen uptake was slightly reduced; submaximal stroke volume was increased only in the placebo and atenolol groups; submaximal cardiac output was generally lower; and arterial-mixed venous oxygen difference was increased after training and maximal treadmill time were increased in all three groups after training (Wilmore et al., 1985). Furthermore, in a randomised control trial on cardiovascular effects of an endurance training programme in elderly hypertensives with or without beta-blocker were randomly assigned to sedentary activity or a heart rate controlled twelve-week treadmill exercise programme. In the exercise group, the training significantly decreased systolic and diastolic 24-hour ABP, blood pressure on exertion (100 W) and increased endothelium-dependent vasodilation (flow-mediated vasodilation, FMD) and physical performance both in the presence and absence of beta-blockade. Mean training heart rate was significantly lower in the patients on beta-blockers. These findings suggest that endurance training evokes comparable cardiovascular benefits in the presence and absence of beta-blockade including a marked improvement of endothelial function (Westhoff et al., 2007). Collectively, the highlighted exercise training studies demonstrate that despite the presence of beta-blockers, cardiovascular adaptations can still occur following exercise training.

Furthermore, another explanation for the difference in normotensive and medicated hypertensives may be due to the pathophysiology of hypertension. Specifically, those with hypertension require relatively greater increases in arterial pressure, heart rate, and sympathetic efferent activity during exercise compared to normotensive individuals (Anderson et al., 1989, Aoki et al., 1983, Floras and Hara, 1993). Such excessive elevations in blood pressure and sympathetic activity may attenuate the efficiency of acute responses to exercise, but also has potential detrimental effects. Combined, such effects may attenuate adaptation to exercise training. For example, elevated sympathetic tone and endothelial dysfunction may contribute to increased vascular resistance (due to impaired vasodilation), potentially impacting blood flow regulation and increasing the workload on the heart, as appropriate systemic vascular function during exercise is essential for counteracting excessive rises in arterial blood pressure and decreasing left ventricular afterload (Munir et al., 2008). Individuals with hypertension have demonstrated an impairment in exercise induced vasodilation (Miyai et al., 2000, Stewart et al., 2004) which elevate the ratio of pulsatile pressure to pulsatile flow and cause a greater rise in systolic BP during exercise. (Schultz and Sharman, 2014). Impaired vasodilation may also limit blood flow to working muscles during exercise which may result in reduced exercise capacity and endurance in hypertensive individuals compared to normotensive individuals. Systemic vascular resistance measured during exercise was the strongest predictor of future CV events, irrespective of BP at rest or during exercise (Fagard et al., 1996). Consequently, this may explain why those with hypertension may show an altered response to exercise compared to healthy individuals. However, there is a lack of studies directly examining the influence of the pathophysiology of hypertension on exercise induced adaptations.

Disease severity (which links into endothelial health and risk factors) may offer a potential explanation for the differences seen between individuals with normotension and medicated hypertension. Previous research has suggested that those with established CVD may not benefit to the same extent as healthy individuals (Stewart et al., 2017, Jeong et al., 2017, Bakker et al., 2021). Studies have found that the magnitude of endothelial dysfunction increases in proportion with the accumulation of CV risk factors (Hashimoto et al., 2000). Chapter 3 is a large epidemiology study from data collected in The Netherlands. General practitioners in the Netherlands prescribe antihypertensive medication to individuals based on a SCORE model (which includes sex, age, smoking status, BP, cholesterol) (Piepoli et al., 2016). Depending on the SCORE, general practitioners will start treating the patient with medication, with low scores receiving only lifestyle advice and higher scores receiving medication, Therefore, it is possible that the hypertensive individuals who were prescribed antihypertensive medication may have a greater number of risk factors to untreated hypertensives, which could explain why findings of this thesis have demonstrated that individuals who are prescribed antihypertensive medication have an altered cardioprotective response compared to healthy individuals.

6.3.3 The role of the endothelium in the response to exercise training.

The heterogeneity in individuals' response to increased physical activity may also relate to the initial health of the cardiovascular system. More specifically, it is possible that have a 'dysfunctional' endothelium may impact on its ability to positively responded to increased physical activity and exercise training. Early animal studies have found that removing the endothelium abolished the ability of the arteries for acute (Berdeaux et al., 1994) and chronic

(Langille and O'Donnell, 1986) adaptation. Also in humans, damage to the endothelial function impairs vasodilatory function (Tryfonos et al., 2020, Dawson et al., 2010). Nonetheless, it is well established that exercise provides cardioprotective effects and is effective in the primary and secondary prevention of CVD. Benefits of exercise training are explained through improvements in traditional cardiovascular disease risk factors, e.g., hypertension (Haung et al., 2013), obesity (Yung et al., 2009), diabetes (MacLeod et al., 2013), cholesterol (Dunn et al., 1997, Kodama et al., 2007), and cardiorespiratory fitness (Blair et al., 1995), but also seem related to improvements in vascular health, including endothelial function (Green et al., 2008, Tinken et al., 2010, Ashor et al., 2015, Green et al., 2017, Pedralli et al., 2020). The endothelium is central to acute and chronic adaptations of the heart and arteries to exercise training (Green et al., 2017) with damage to the endothelial function has been shown to impair dilator function (Tryfonos et al., 2020, Dawson et al., 2010). Individuals with CVD demonstrate poor endothelial function carrying a prognostic relevance for the risk of ischemic and coronary events. The impaired endothelial function in CVD patients may contribute to a smaller hemodynamic stimulus (i.e., small increase shear stress) and subsequently, smaller adaptive response. However, findings from Chapter 4 question whether the *a priori* endothelial dysfunction indeed leads to an attenuated effect. First, *Chapter 4* found that those with lower a prior endothelial dysfunction demonstrated greater adaptations in vascular function following exercise training. This finding is in line with previous observations (Maiorana *et al.*, 2003; Green *et al.*, 2004, 2014*b*) in that regular exercise training is capable of improving endothelial function, especially in those with *a priori* endothelial dysfunction (Walsh *et al.*, 2003; Maiorana *et al.*, 2011; Green *et al.*, 2014*b*) which may relate the greater capacity to improve function when it starts at a lower baseline.

Individuals' cardiovascular health status has also been shown to alter the dose-response relationship between MVPA and incident morbidity and mortality (Bakker et al., 2021). A novel part of *Chapter 4* of this thesis was that I was able to categorise individuals as reduced endothelial function and preserved endothelial function based on recently published age and sex-based reference value data (Holder et al., 2021). *Chapter 4* found comparable improvements in cardiovascular risk factors and physical fitness in response to exercise training in both groups highlighting that exercise training is beneficial and has cardioprotective effects in both healthy and individuals with endothelial dysfunction. However, only those with *a priori* reduced endothelial function demonstrated improvement in endothelial function (FMD%) following exercise training which may therefore suggest that exercise is more beneficial in those with *a priori* endothelial dysfunction. Furthermore, endothelial dysfunction can also lead to vascular remodelling resulting in narrowing of the vessel diameter and increasing artery thickness. Regular exercise training has been shown to increase artery diameter and reduce arterial wall thickness, contributing to improved vascular health and may contribute to the cardioprotective effect exercise in healthy individuals and those with cardiovascular risk (Thijssen et al., 2012). *Chapter 4* does not measure changes in artery diameter or wall thickness pre and post exercise training which may provide an incomplete assessment of the impact of exercise training in determining overall vascular health and cardiovascular risk. Future work is needed to look at structural adaptations alongside the functional adaptations.

6.3.4 Acute mechanisms that may affect the cardioprotective effect of exercise training.

As with exercise training responses, acute exercise responses to a given intervention shows a large heterogeneity in response. As chronic benefits of exercise may be related to the repeated acute responses following a single bout of exercise (Dawson et al., 2018) understanding the acute effects of exercise on endothelial (thus (cerebro) vascular) function is important (Thijssen et al., 2018). During acute exercise, the vasculature is exposed to multiple physical stimuli during exercise including increases in shear stress (Niebauer and Cooke, 1996, Tinken et al., 2009), blood pressure (Okamoto et al., 2006, Okamoto et al., 2008) and compressive forces all of which can independently influence endothelial function. Therefore, understanding the acute effects of exercise on peripheral and cerebrovascular function in may have offered an explanation to why some individuals show smaller benefits of regular physical activity compared to others. Findings from *Chapter 5* have suggested that both control healthy individuals and individuals who are treated for hypertension present a similar baseline peripheral vascular function and a similar response in peripheral vascular function post-acute exercise. However, differences are apparent in the acute response cerebrovascular function following acute exercise. Individuals who are medicated for hypertension demonstrated elevated MCAv for longer following exercise. In addition, individuals with normotension demonstrated reduction in absolute CVR whereas increases were shown in medicated hypertensives.

The mechanisms underpinning differences in post exercise response in CVR between the medicated hypertensive group and healthy individuals may be influenced by the effects of exercise on vascular dynamics and autonomic regulation. Individuals with hypertension have

altered autonomic nervous activity compared to healthy individuals (Grassi and Ram, 2016). Acute exercise can further modulate autonomic function, increasing sympathetic activity and decreasing parasympathetic activity (Robinson et al., 1966, Ekblom et al., 1972, Fagraeus and Linnarsson, 1976). In medicated hypertensive individuals, this shift in autonomic balance following exercise may lead to a more pronounced increase in CVR, reflecting enhanced sympathetic-mediated vasodilation. In contrast, healthy individuals may exhibit a decrease in CVR due to a different autonomic response pattern or a more rapid restoration of baseline autonomic balance post-exercise. However, it can only be speculated as post exercise measures of autonomic function were not measured. The difference in responses to acute exercise between the two groups may begin to offer an explanation as to why individuals who are medicated for hypertension demonstrate a different dose response association (*Chapter 4),* however further research is warranted.

It is currently unknown what the acute responses on cerebrovascular function mean in relation to chronic adaptation or acute transient risk. Previous research has suggested that acute peripheral responses to exercise predict chronic adaptation (Dawson et al., 2018) therefore understanding the acute effects of exercise may predict future clinical cardioprotective responses (Thijssen et al., 2018). Acute responses to exercise may also represent a transient period of cardiovascular event risk up to one hour post exercise but the magnitude of short term relative risk varied across studies (Mittleman et al., 1993, Siscovick et al., 1984, Whang et al., 2006, Albert et al., 2000, Willich et al., 1993, Smyth et al., 2016). However, it is currently unknown what an increase, decrease or no-change in cerebrovascular function following acute exercise means for a transient risk in stroke.

6.4 Methodological considerations and limitations

There are a number of strengths in the methodology of this thesis. The participants recruited for *Chapter 3* give a good representation of individuals in the UK, Netherlands and Australia in both healthy individuals, those with established CVD and those with cardiovascular risk factors. Based on this, the findings of *Chapter 3* are applicable to a wide cohort and not limited to one participant group. Individuals recruited for *Chapter 4* give an excellent representation of individuals living in the Netherlands and include large numbers of individuals with normotension (n=78,309) and hypertension (n=61,621).

The laboratory-based studies included in this thesis (including the studies used in the secondary analysis for *chapter 3*) were performed under strict inclusion and exclusion criteria in addition to control of diet and exercise prior to laboratory visits. Measurements were performed adhering to the most recent published guidelines for each measurement. FMD were performed according to the latest peer reviewed consensus guidelines, and analysed using custom-designed edge-detection and wall tracking analysis software which maximised the accuracy, validity and prognostic value of FMD outcomes. FMD data was also analysed according to published guidelines in which FMD being scaled allometrically (Atkinson et al., 2013). dCA measurements and TFA analysis were all performed following cerebral autoregulation network recommendations (Claassen, 2016). Within the literature, there are many different methods used to measure and analyse cerebrovascular reactivity. Analysis was also performed according to a recommendation following research suggesting that the most reliable interpretation of CVR data is an absolute CVR calculated as either a 30s or a 1s peak (Koep et al., 2022).

Despite these methodological strengths, there are a number of limitations that need to be noted. In *Chapter 3* measurements of MVPA were self-reported which may have resulted in overestimation of MVPA levels (Celis-Morales et al., 2012). The major methodological limitation is the use of TCD to measure CBF and cerebrovascular function. TCD provides measurement of CBFv as an index of CBF but it doesn't not provide any information regarding changes in blood vessel diameter which can affect measurement accuracy of CBF with TCD (de Heus et al., 2018). However, research has suggested that MCAv Is a reliable index of CBF if the isonated vessel maintains a constant diameter across time and experimental conditions (Ainslie and Hoiland, 2014). Research has shown that dilation of extra-cranial arteries matches dilation in the cerebrovasculature (Smith et al., 2019). Assessments of internal carotid arteries was employed during the CO_2 reactivity test; however, it was difficult to obtain clear images of the internal carotid on every individual and given the small sample size, we would have been statistically under powered to preform analysis on the images obtained. The use of additional imaging techniques such as functional magnetic resonance imaging and arterial spin labelling have been used to detect changes in global and regional perfusion, however their temporal resolution is currently too low to assess the dynamics of dCA (Heus et al., 2019) such as during exercise and they are either expensive or invasive techniques. The MCA diameter is unlikely to change under resting conditions or during moderate changes in BP (Serrador et al., 2000), similar to that induced during repeated squat stands manoeuvres. MCA diameter alterations in responses to changes in blood CO_2 remains a highly controversial topic (Brothers and Zhang, 2016, Hoiland and Ainslie, 2016). Studies have highlighted that MCA diameter may remain constant during modest changes in $P_{ET}CO_2$ but no definitive threshold has yet been described (Verbree et al., 2014). Based on this uncertainty, it is

important to acknowledge that the MCAv data during hypercapnia may have underestimated flow as a result of potential MCA diameter changes.

6.5 Summary

Overall, the thesis findings reinforce the cardioprotective effects of exercise in healthy individuals and those with cardiovascular risk factors. Our findings demonstrate that exercise training is beneficial on many clinically important cardiovascular risk factors which is independent of *a priori* health status as those with endothelial dysfunction and hypertension demonstrate a similar response to exercise training and regular physical activity compared to individuals with endothelial function and normotension. However, the use of antihypertensive medication may alter the cardioprotective effects of exercise as benefits of regular MVPA on stroke risk appears to only be stronger in non-medicated individuals with hypertension. This thesis offers primarily findings that suggest that individuals who are taking antihypertensive medication may demonstrate an altered cerebrovascular response to acute exercise compared to normotensive individuals which may begin to explain why those with antihypertensive medication may attenuate the association between long term PA and stroke risk. More research is warranted to understand the mechanisms on vascular responses to exercise which may underpin this altered response.

6.6 Recommendations for clinical practice and future studies

The findings of this thesis further highlight the importance of exercise training for cardioprotection. However, findings from *Chapter 3* and *Chapter 5* highlight that the use of antihypertensive medications may interfere with the effects of exercise, related to acute

exercise responses (Chapter 5) up to the ultimate health benefits (Chapter 3). Several potential areas for future research have emerged following the researched detailed in this thesis.

1. Findings reinforce the importance in exercise in the total population including those with cardiovascular disease risk factors. Collectively, findings of this thesis suggest that PA recommendations should not follow a 'one-guideline-fits-all' approach as the use of antihypertensive medication may influence the responses to exercise. This highlights the need to investigate further prescription of personal exercise training programmes. A large, randomised control trial comparing the efficacy of different types of exercise (e.g., aerobic training, resistance training, combined training) among individuals with cardiovascular disease risk factors, stratified by antihypertensive medication use. Assess outcomes such as blood pressure control, cardiovascular fitness, muscle strength, and medication side effects.
2. Further investigation is needed to understand the potential mechanisms that explain why individuals who are treated for hypertension demonstrate an altered response to exercise and physical activity compared to healthy individuals. Future research should investigate the specific pathophysiology pathways affected by antihypertensive medication use that may impair exercise induced adaptations which is dependent upon which antihypertensive medication that is prescribed. Animal model studies could be used to investigate specific molecular pathways affected by different antihypertensive medications in relation to exercise-induced adaptations. This could involve studying changes in gene expression, protein signalling pathways, mitochondrial function, or oxidative stress markers in response to medication treatment and exercise stimuli. In humans, a randomised control trial comparing the

effects of different classes of antihypertensive medications on exercise-induced adaptations. Participants could be randomized to receive different antihypertensive medications or placebo, and their exercise performance and physiological responses (e.g., cardiovascular function, muscle metabolism) could be assessed before and after a structured exercise intervention.

3. As prescribing antihypertensive medication may impact stroke risk reduction, future research should be focused on investigating how much of the benefit from regular exercise training and physical activity does antihypertensive medication take away and whether it is worth to stop taking the antihypertensive medication if the benefits of exercise outweigh the benefits of medication itself. A longitudinal database study, similar to the study presented in *Chapter 4,* should be conducted cohorts of individuals with hypertension from different countries who are stratified by physical activity levels and antihypertensive medication. The study should analyse the incidence of stroke and other cardiovascular events in relation to levels of physical activity, medication adherence and any alterations in antihypertensive medication use (i.e. discontinuation or dosage adjustments).
4. Based on the work in *chapter 5*, further research is warranted to understand the time course response to acute exercise in individuals who are treated for hypertension and whether this alters exercise training responses and stroke risk reduction and whether acute exercise responses predict long term adaptation.

Chapter 8: Supplementary Material

Supplemental Table 3.1 STROBE Checklist.
Strengthening the Reporting of Observational Studies in Epidemiology.

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Title and abstract paragraph 2 abstract
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction, paragraph 1-3
Objectives	3	State specific objectives, including any prespecified hypotheses	Introduction, paragraph 3
Methods			
Study design	4	Present key elements of study design early in the paper	Study population, paragraph 1
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Study population , paragraph 1
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	Study population, paragraph 1 and Clinical outcomes, paragraph 1 -
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods, all paragraphs
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods, all paragraphs
Bias	9	Describe any efforts to address potential sources of bias	Statistical analyses, all paragraphs
Study size	10	Explain how the study size was arrived at	Study population, paragraph 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Statistical analyses, paragraph 2
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	Statistical analyses, all paragraphs Statistical analyses, paragraph 2 to 4 Statistical analyses, paragraph 3 Clinical outcomes, paragraph 1 Statistical analyses, paragraph 4
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility,	Study population (methods), paragraph 1

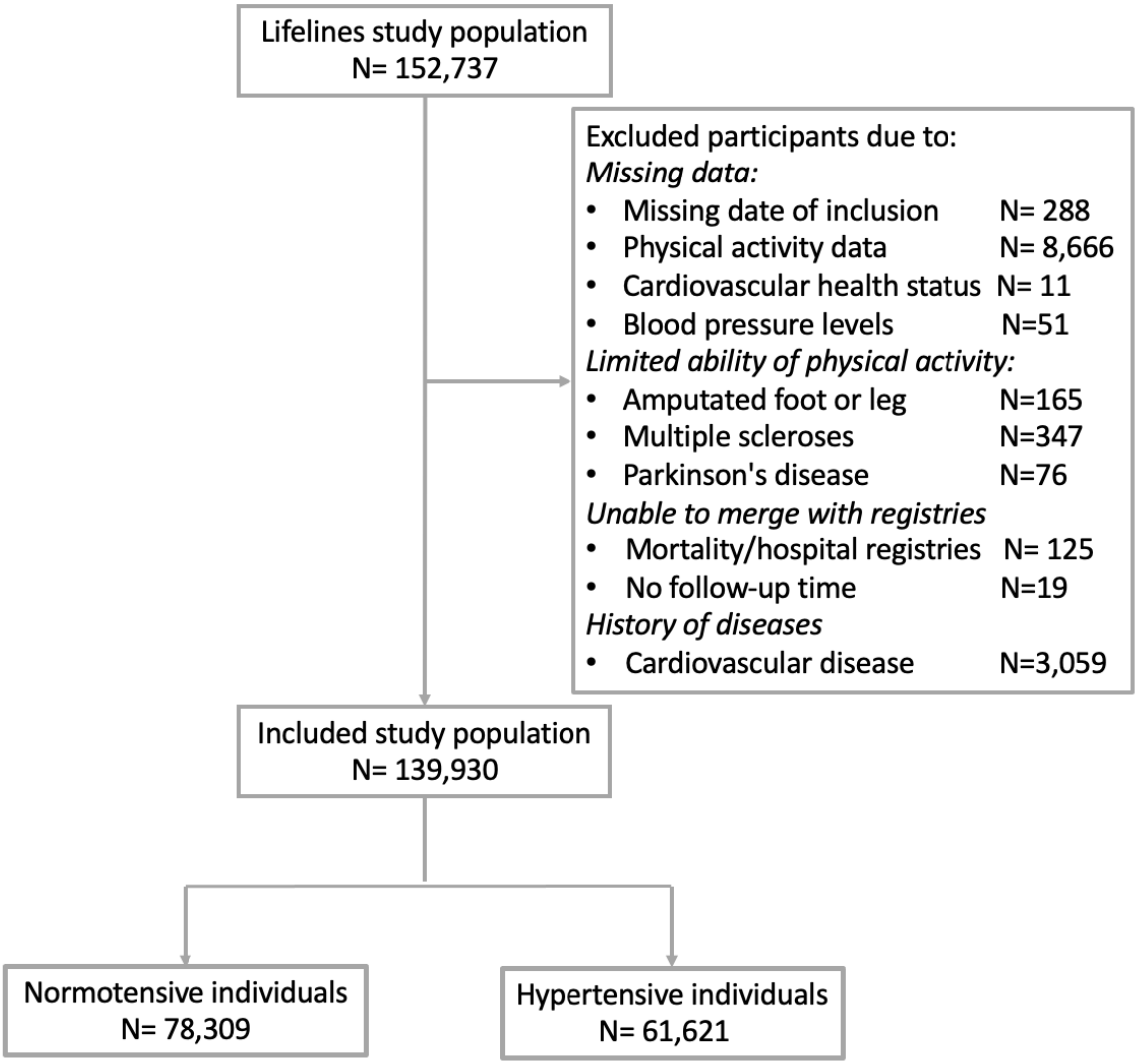
			confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	and Study population (results), paragraph 1 Study population (methods), paragraph 1 and Study population (results), paragraph 1 and S1 fig
Descriptive data		14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	Study population (results), paragraph 1 and table 1 - Clinical outcomes, paragraph 1
Outcome data		15*	Report numbers of outcome events or summary measures over time	Clinical outcomes, paragraph 1
Main results	16		(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Health benefits of MVPA to Dose-response relationship of domain-specific MVPA and Tables 2, S2 t/m S12 Statistical analyses paragraph 2 -
Other analyses	17		Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Health benefits of MVPA, paragraphs 2 and 3
Discussion				
Key results	18		Summarise key results with reference to study objectives	Discussion, paragraph 1
Limitations	19		Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Strengths and limitations, paragraph 1
Interpretation	20		Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Cardiovascular health status and MVPA benefits to Leisure versus non-leisure MVPA
Generalisability	21		Discuss the generalisability (external validity) of the study results	Cardiovascular health status and MVPA benefits to Strengths and limitations
Other information				
Funding	22		Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Title page, funding/support

*Give information separately for exposed and unexposed groups.

Supplemental table 4.2. Baseline table of individuals with hypertension

General characteristics	Hypertensives without medication (N=48,452)	Hypertensives with medication (N=13,169)	P-value
Sex (male)	27,584 (57%)	5,394 (41%)	<0.001
Age	46.43 (11.90)	56.16 (10.95)	<0.001
Income x 1000/year	27 [25.00, 30.00]	26.80 [24.90, 29.70]	<0.001
Education level			<0.001
low	15797 (33%)	5,946 (47%)	
moderate	18620 (39%)	4,049 (32%)	
high	12943 (27%)	2,695 (21%)	
BMI (median [IQR])	26.40 [24.20, 29.10]	27.90 [25.40, 31.10]	<0.001
Lifestyle characteristics			
Smoking status			<0.001
Never	21,246 (44%)	5,082 (39%)	
Previous	16,474 (34%)	6,143 (47%)	
Current	10,256 (21%)	1,849 (14%)	
Alcohol consumption (high)	11,824 (26%)	2,722 (21%)	<0.001
Medication use			
Antiplatelet	47 (0.1%)	56 (0.4%)	<0.001
Anti-hypertensive	0 (0.0%)	7,371 (56%)	<0.001
Anti-coagulant	175 (0.4%)	328 (3%)	<0.001
Acetylsalicylic acid	549 (1%)	1,160 (9%)	<0.001
Beta-blocker	0 (0.0%)	5,378 (41%)	<0.001
Calcium antagonists	0 (0.0%)	1,983(15%)	<0.001
Diuretics	0 (0.0%)	4,689 (36%)	<0.001
Statins	1886 (4%)	2,814 (21%)	<0.001
Alternative cholesterol lowering medication	95 (0.2%)	119 (0.9%)	<0.001
Anti-diabetics	592 (1%)	1,004 (8%)	<0.001
Health characteristics			
Diagnosed hypertension	0 (0.0%)	12,291 (93%)	<0.001
Diagnosed hypercholesterolemia	7,657 (16%)	3,974 (30%)	<0.001
Diagnosed diabetes	1,142 (2%)	1,428 (11%)	<0.001
Systolic blood pressure	136.00 [131.00, 143.00]	135.00 [125.00, 146.00]	<0.001
Diastolic blood pressure	81.00 [75.00, 86.00]	78.00 [72.00, 85.00]	<0.001

Total cholesterol	5.20 [4.60, 5.90]	5.20 [4.50, 5.90]	<0.001
LDL cholesterol	3.44 (0.92)	3.36 (0.93)	<0.001
HDL cholesterol	1.40 [1.20, 1.70]	1.40 [1.10, 1.60]	<0.001
Triglycerides	1.11 [0.80, 1.58]	1.26 [0.91, 1.76]	<0.001
Renal function	96.41 [86.10, 100.00]	88.45 [76.94, 97.76]	<0.001



Supplemental Figure 4.1. Flow chart of included study population.

Supplemental Table 4.1 Methods of FMD, Cardiopulmonary fitness test, blood sample analysis and body composition in the included studies.

Study	FMD	Cardiopulmonary fitness test	Blood samples	Body Fat
Tinken 2008	10 MHz multifrequency linear array probe Aspen, Acuson Mountain view, CA, USA.	Treadmill Initial workload of 6 hm-1 and stepwise increments in speed (speed increased by 2km^{-1} every 2 min until 16km-1) and slope (2% every minute when 16km h^{-1} is reached) until volitional exhaustion. Breath-by-breath analysis (Medgraphics CPX/D and Ultima CardiO_2 systems, MN, USA). Oxygen consumption was recorded during the final 40 s of each stage of the test and expressed relative to body weight (ml kg^{-1} min^{-1}). Peak oxygen consumption was calculated as the highest consecutive 10 s period of gas exchange data occurring in the last minute before volitional exhaustion, which generally occurred due to leg fatigue or breathlessness.		DEXA (Hologic, QDR Series Discovery A, Bedford, MA, USA)
Black 2009	10 MHz multifrequency linear array probe Aspen, Acuson Mountain view, CA, USA.	Exercise testing was undertaken on a treadmill ergometer (H/P/Cosmos, Pulsar 4.0, Nussdorf-Traunstein, Germany), with the initial workload set at 4 km/h at 5% gradient and stepwise increments in speed and grade every 3 min until volitional exhaustion. The volume of oxygen consumed during exercise was directly calculated from minute ventilation, measured using a pneumotach and simultaneous breath-by-breath analysis of expired gas fractions (Medgraphics CPX/D and Ultima CardiO_2 systems). Gas analyzers and flow probes were calibrated before each test. Oxygen consumption was recorded during the final 40 s of each stage of the test and expressed as maximal oxygen consumption ($\dot{V}o_{2\,max}$) relative to body weight (in ml·kg^{-1}·min^{-1}). $\dot{V}o_{2\,max}$ was calculated as the highest		Dexa (Hologic, QDR Series Discovery A, Bedford, MA, USA)

		consecutive 10-s period of gas exchange data occurring in the last minute before volitional exhaustion, which generally occurred due to leg fatigue or breathlessness.	
Birk 2012	10-MHz multifrequency linear array probes, attached to high-resolution ultrasound machines with identical settings (T3000; Terason, Burlington, MA)	80% maximal heart rate (80%HR_{max}) was used to determine exercise training which was performed on a bike.	
Pugh 2016	High-resolution ultrasound (Terason, t3000, Aloka, Burlington, MA, USA)	Treadmill ergometer, initially 2.7 km h^{-1} at 5° gradient, with step-wise increments every 1 min. VO_{2peak} was calculated from expired gas (Oxycon Pro, Jaegar, Hoechberg, Germany) as the highest consecutive 15 s period of oxygen uptake in the last min before exhaustion.	
Sprung 2013	A 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Siemens Medical Solutions, Malvern, PA).	Exercise testing was undertaken on a Treadmill ergometer using the a modified Bruce protocol . After a 2-min warm-up at 2.2 km·h^{-1} on a flat gradient, the initial workload was set at 2.7 km·h^{-1} at 5° grade. Thereafter, stepwise increments in speed and grade were made every minute. $\dot{V}O_{2peak}$ was calculated from expired gas fractions (Oxycon Pro, Jaegar, Germany) as the highest consecutive 15-s period of data in the last minute before volitional exhaustion.	Samples were analyzed using the Olympus AU2700 analyzer (Beckman Coulter (UK) Ltd., Buckinghamshire, UK) with standard proprietary reagents as follows: glucose with hexokinase, total cholesterol, and HDL with cholesterol esterase/oxidase, triglyceride with glycerol

			kinase, and ALT with IFCC kinetic UV (without pyridoxal phosphate activation). LDL was calculated according to the Friedwald formula
Buckley 2018	Images of the left brachial artery were acquired using high-resolution ultrasound (T3300; Terason, Burlington, MA).	Maximal oxygen consumption ($VO2max^{-2}$) was estimated via the submaximal Astrand-Rhyming cycle ergometer protocol. A cycle ergometer test of aerobic fitness. The subject cycles at 50 rev mm^{-1} for 6 min at a work load set at a level related to the sex and condition of the subject (unconditioned males, 50–100 watts, unconditioned females 50–75 watts, conditioned mates 100–150 watts, and conditioned females 75–110 watts). Participants' heart rate was taken in the last 10 s of each of the two final minutes of exercise. The average of these two figures, corrected for the subject's age, was used to estimate a maximal oxygen uptake (VO_2max), The estimate is based on the assumption that subjects of the same age have a similar maximal heart rate.	
Buckley 2020	Images of the left brachial artery were acquired using high-resolution ultrasound (T3300; Terason, Burlington, MA).	Maximal oxygen consumption ($VO2max^{-2}$) was estimated via the submaximal Astrand-Rhyming cycle ergometer protocol. A cycle ergometer test of aerobic fitness. The subject cycles at 50 rev mm^{-1} for 6 min at a work load set at a level related to the sex and condition of the subject (unconditioned males, 50–100 watts, unconditioned females 50–75 watts, conditioned mates 100–150 watts, and conditioned females 75–110 watts). Participants'	

		heart rate was taken in the last 10 s of each of the two final minutes of exercise. The average of these two figures, corrected for the subject's age, was used to estimate a maximal oxygen uptake (VO_2max), The estimate is based on the assumption that subjects of the same age have a similar maximal heart rate.	
Miller 2022	Images of the left brachial artery were acquired using high-resolution ultrasound (T3300; Terason, Burlington, MA).	An incremental maximal exercise test was performed on a treadmill (Pulsar 4.0, HP Cosmos, Germany). After a 5-min warm-up (self-paced between 6 and 8 km.h^{-1}), participants started the test at treadmill speed 8 km.h^{-1} and 1% gradient. Every 3 min the treadmill speed increased by 2 km.h^{-1} until 16 km.h^{-1}. Thereafter the treadmill speed remained the same and the incline gradient increased by 2% until volitional exhaustion. Breath by breath expired gases were measured (Oxycon Pro, Jaeger, Germany) for oxygen consumption (ml.kg.min–^{1}) and data were averaged over 15 s blocks. Maximum oxygen consumption was calculated as the highest consecutive 15-s period of gas exchange data occurring in the final minute before volitional exhaustion. Heart rate (HR) was measured continuously using short range telemetry (FT1, Polar, Finland) alongside perceived exertion at each exercise stage.	
Maxwell 2021	Images of the left brachial artery were acquired using high-resolution ultrasound (T3300; Terason, Burlington, MA).	Exercise testing was undertaken on a treadmill (H/P Cosmos, Pulsar 4.0, Nussdorf-Traunstein, Germany) using a modified Bruce protocol. Following a 5-min warm-up period at a self-selected speed, the protocol begins with a 2-min stage at 2.2 km/h on a flat gradient, followed by 2 min at 2.7 km/h at a 5% gradient. Subsequently, stepwise increments in speed and gradient are applied every minute until volitional	Blood samples were obtained from the antecubital vein via standard venepuncture technique (Vacutainers Systems, Becton–Dickinson). All samples were collected into vacutainers containing

		exhaustion. Breath-by-breath expired gases were continuously monitored (Oxycon Pro, Jaeger, Hochberg Germany) for oxygen consumption (ml/kg/min) and were averaged over 15 s. Peak oxygen uptake was calculated from the highest consecutive 15-s period of expired gas fractions.	a polymer gel for serum separation. Centrifugation for 10 min at 1000 *g* at 4 °C was applied and samples were stored at − 80 °C for subsequent analysis. Plasma glucose was determined spectrophotometrically using commercially available kits (Randox Laboratories, Antrim, UK) with each sample analysed in duplicate.
Thijssen 2007	Echo Doppler device (Megas; Esaote,Firenze, Italy) with a 5–7.5 MHz broadband lineararray transducer.	Incremental maximal exercise-test on a cycling ergometer (Lode, Angio300, Groningen, the Nether-lands) using a multistage protocol (workload increased by 10 W L)1, starting at 10 W, until exhaustion). Oxygen consumption was measured continuously using a gas-analyzer (Jaeger Benelux BV, Breda, the Nether-lands). Maximal oxygen consumption (VO2max) was calculated as the mean of the last minute of the test.	
Schreuder 2014a	A 10 MHz multifrequency linear-array probe attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA, USA).	Incremental maximal exercise test on a bicycle ergometer (Lode; Excalibur, Groningen, The Netherlands). These tests started at a power output of 10 W for 1 min, and power output increased by 10 W min^{-1} until exhaustion. Subjects were instructed to maintain a cadence of between 60 and 80 r.p.m. during the test. We continuously recorded oxygen consumption (in millilitres of O_2 per kilogram per minute), ventilation (in litres per minute), respiratory quotient (Oxycon IV; Jaeger, Hoechberg, Germany) and heart rate (in beats per minute).	

Schreuder 2014b	A 10 MHz multifrequency linear-array probe attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA, USA).	Incremental cycle exercise test on a cycle ergometer (Lode, Excalibur, Groningen, the Netherlands). These tests started at a power output of 10 W for 1 min, and power output increased by 10 W min^{-1} until exhaustion. Subjects were instructed to maintain a cadence of between 60 and 80 r.p.m. during the test. We continuously recorded oxygen consumption (in millilitres of O_2 per kilogram per minute), ventilation (in litres per minute), respiratory quotient (Oxycon IV; Jaeger, Hoechberg, Germany) and heart rate (in beats per minute).	Venous blood sample was taken for assessment of fasting glucose, insulin, total cholesterol, HDL, LDL and triglycerides. (no info on analysis.	
Poelkens	A 10 MHz multifrequency linear-array probe attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA, USA).	Women performed a maximal exercise test on an electrically braked leg cycling ergometer (Angio 300, Lode, Excalibur Sport, Groningen, the Netherlands) using an incremental protocol to assess their cardiorespiratory fitness level. Workload increased by 10 W·min^{-1}, starting at 10 W, until exhaustion. A gas analyzer was used to measure oxygen consumption continuously (Jaeger Benelux BV, Breda, the Netherlands). Maximal oxygen consumption ($\dot{V}O_{2max}$**)** was analyzed as the mean of the last minute of the exercise bout. During the test, heart rate was measured continuously.		Fasting glucose levels were determined using standard laboratory techniques, and fasting insulin levels were determined with an electrochemiluminescence immunoassay (ECLIA) on the E170 module of a modular analytics EVO analyzer (Roche, Mannheim, Germany)
Scholten 2012	who used a 10-MHz multifrequency linear array probe attached to a high resolution ultrasound machine (T3000; Terason	Exercise testing was undertaken on a cycle ergometer (Excalibur Sport; Lode BV, Groningen, The Netherlands). The initial workload set at 10W W for 1 minute followed by 10-W increments every minute until complete exhaustion. Breath-by-breath oxygen uptake was measured with spiroergometric equipment (Quark CPET, Cosmed, Milan, Italy). Heart rate and rhythm were continuously recorded by 3-lead echocardiography.	Venous blood samples were taken from the antecubital vein and analyzed for metabolic parameters: glucose, insulin, total cholesterol, high- and low-density lipoproteins, and triglycerides (Aeroset; Abbott Laboratories).	

	Corporation, Burlington, MA).		
Brenda 2015	10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Terason T3000, Burlington, MA, USA).	An incremental maximal cycling test was performed on a cycle ergometer (Ergoline, Ergoselect 200k, Bitz, Germany). Subjects were instructed to pedal (>60rpm) whilst workload was increased 10–15 Watt/min, depending on the expected physical fitness of the participant (based on sex, age, height, and previous results on exercise testing). During exercise, breath-by-breath gas analysis was recorded continuously (LabManager V5.32.0). Peak oxygen uptake (VO_{2peak}) was defined as the highest oxygen uptake (30-second average).	
Green 2003	A 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Aspen; Acuson, CA)	A graded maximal exercise test that was performed on an electronically braked bicycle ergometer (Orival 400, Lode). Initial resistance was set between 20 and 60 W and increased in 20- to 25-W increments, depending on subject ability, every 3 min until fatigue or termination, according to standard indications for stopping an exercise test. Volumes of oxygen consumed ($\dot{V}o_2$) and carbon dioxide produced ($\dot{V}co_2$) during exercise were calculated from minute ventilation and measured by using mass flow ventilometry and simultaneous mixing chamber analysis of expired gas fractions. Gas analyzers and flow probes were calibrated before each test. $\dot{V}o_2$ and $\dot{V}co_2$ (expressed in l/min and $ml \cdot kg^{-1} \cdot min^{-1}$) were recorded during the final 40 s of each stage of the test. $\dot{V}o_{2\ peak}$ was calculated as the average of the two highest consecutive 20-s periods of gas exchange data occurring in the last minute before volitional exhaustion, which was generally due to leg fatigue or breathlessness.	Skinfolds were measured using spring-loaded calipers (Harpenden) at eight standard sites: triceps, biceps, subscapulare, supraspinale, iliocristale, midabdominal, anterior thigh, and medial calf. All sites were measured in triplicate, with the median score recorded. Muscle girths were similarly recorded at the following standard sites using an anthropometric steel tape (Lufkin): relaxed arm, flexed arm, waist, hip, thigh, and the waist-to-hip ratio (waist/hip) was calculated.

Naylor 2006	A 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Aspen; Acuson, Mountain View, CA).	Exercise testing was undertaken on a modified Concept II rowing ergometer (Concept Inc., Vermont). The goal was to complete the 2000 m as quickly as possible, and involved a maximal effort from start to finish. A custom-built gas analysis system was used to determine $\dot{V}O_2$. Subjects were connected to a Hans Rudolph 2700 respiratory valve and inspired volume was measured during the test by a Morgan ventilometer (Mark II 225A), which was calibrated before testing using a five-point calibration procedure spanning the physiological range. Expired air passed through 35-mm Collins tubing into a 4-L mixing chamber. A small sample was directed to an Applied Electrochemistry S-3A oxygen analyzer and an Applied Electrochemistry CD-3A analyzer for determination of fractions of O_2 and CO_2 in expired air. The sample first passed through a thermoelectric cooling chamber for the removal of water vapor. A three-point calibration procedure spanning the physiological range of respiratory gases was applied before and after each test. Custom designed software was used to integrate the various inputs and to determine $\dot{V}O_2$. Data was collected over 30-s epochs with the two highest consecutive epochs taken as the $\dot{V}O_{2max}$.	
Haynes 2021	A 10- to 15-MHz multifrequency linear array probe, attached to a high-resolution	12-lead ECG stress test supervised by a qualified medical practitioner, which also permitted analysis of aerobic fitness via maximal oxygen consumption (VO_2).	

	ultrasound machine (T3200; Terason, Burlington, MA).

Supplemental Table 3.1 STROBE Checklist.
Strengthening the Reporting of Observational Studies in Epidemiology.

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Title and abstract paragraph 2 abstract
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction, paragraph 1-3
Objectives	3	State specific objectives, including any prespecified hypotheses	Introduction, paragraph 3
Methods			
Study design	4	Present key elements of study design early in the paper	Study population, paragraph 1
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Study population , paragraph 1
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	Study population, paragraph 1 and Clinical outcomes, paragraph 1 -
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods, all paragraphs
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods, all paragraphs
Bias	9	Describe any efforts to address potential sources of bias	Statistical analyses, all paragraphs
Study size	10	Explain how the study size was arrived at	Study population, paragraph 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Statistical analyses, paragraph 2
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	Statistical analyses, all paragraphs Statistical analyses, paragraph 2 to 4 Statistical analyses, paragraph 3 Clinical outcomes, paragraph 1 Statistical analyses, paragraph 4
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility,	Study population (methods), paragraph 1

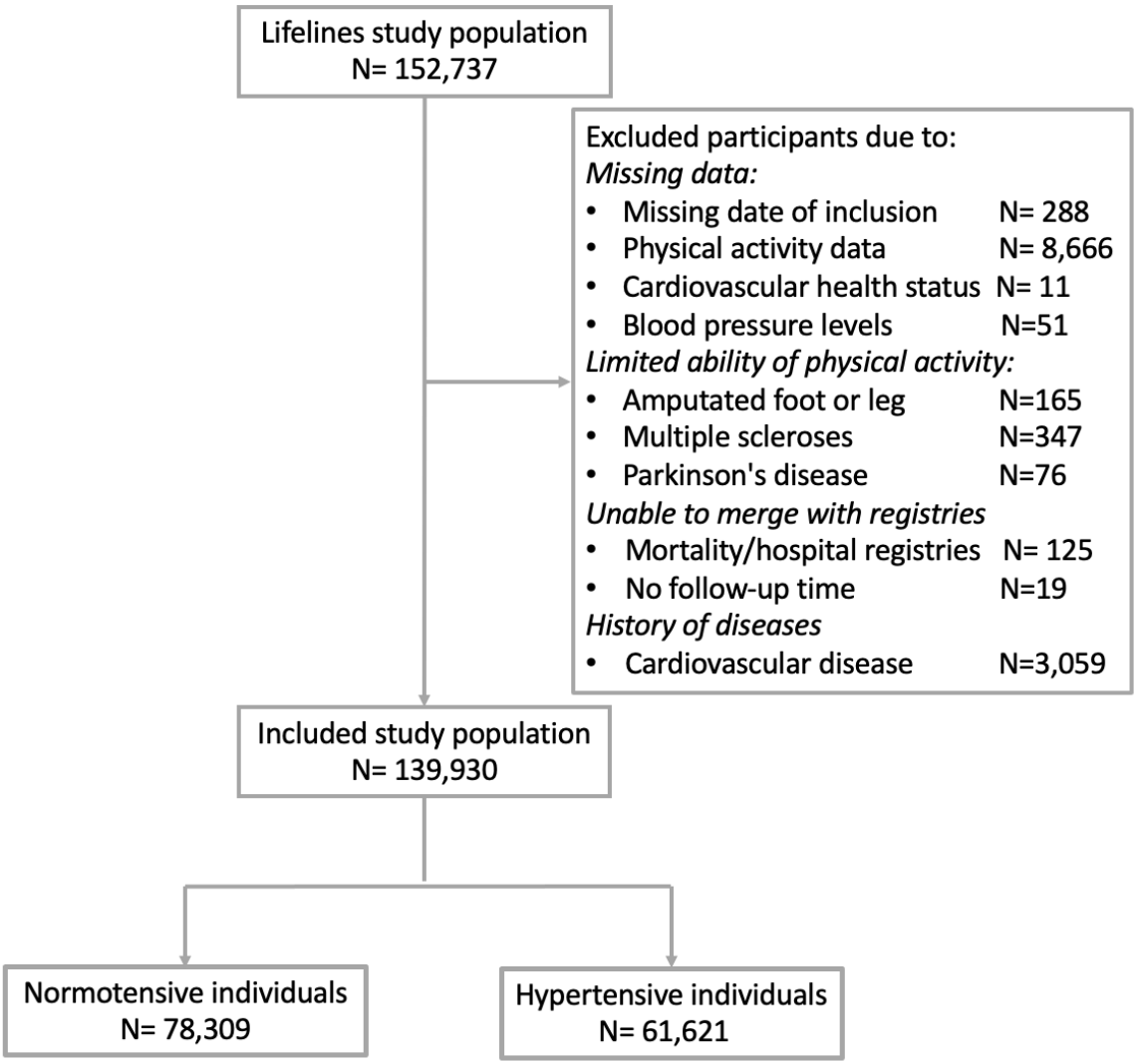
			confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	and Study population (results), paragraph 1 Study population (methods), paragraph 1 and Study population (results), paragraph 1 and S1 fig
Descriptive data		14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	Study population (results), paragraph 1 and table 1 - Clinical outcomes, paragraph 1
Outcome data		15*	Report numbers of outcome events or summary measures over time	Clinical outcomes, paragraph 1
Main results	16		(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Health benefits of MVPA to Dose-response relationship of domain-specific MVPA and Tables 2, S2 t/m S12 Statistical analyses paragraph 2 -
Other analyses	17		Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Health benefits of MVPA, paragraphs 2 and 3
Discussion				
Key results	18		Summarise key results with reference to study objectives	Discussion, paragraph 1
Limitations	19		Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Strengths and limitations, paragraph 1
Interpretation	20		Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Cardiovascular health status and MVPA benefits to Leisure versus non-leisure MVPA
Generalisability	21		Discuss the generalisability (external validity) of the study results	Cardiovascular health status and MVPA benefits to Strengths and limitations
Other information				
Funding	22		Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Title page, funding/support

*Give information separately for exposed and unexposed groups.

Supplemental table 4.2. Baseline table of individuals with hypertension

General characteristics	Hypertensives without medication (N=48,452)	Hypertensives with medication (N=13,169)	P-value
Sex (male)	27,584 (57%)	5,394 (41%)	<0.001
Age	46.43 (11.90)	56.16 (10.95)	<0.001
Income x 1000/year	27 [25.00, 30.00]	26.80 [24.90, 29.70]	<0.001
Education level			<0.001
low	15797 (33%)	5,946 (47%)	
moderate	18620 (39%)	4,049 (32%)	
high	12943 (27%)	2,695 (21%)	
BMI (median [IQR])	26.40 [24.20, 29.10]	27.90 [25.40, 31.10]	<0.001
Lifestyle characteristics			
Smoking status			<0.001
Never	21,246 (44%)	5,082 (39%)	
Previous	16,474 (34%)	6,143 (47%)	
Current	10,256 (21%)	1,849 (14%)	
Alcohol consumption (high)	11,824 (26%)	2,722 (21%)	<0.001
Medication use			
Antiplatelet	47 (0.1%)	56 (0.4%)	<0.001
Anti-hypertensive	0 (0.0%)	7,371 (56%)	<0.001
Anti-coagulant	175 (0.4%)	328 (3%)	<0.001
Acetylsalicylic acid	549 (1%)	1,160 (9%)	<0.001
Beta-blocker	0 (0.0%)	5,378 (41%)	<0.001
Calcium antagonists	0 (0.0%)	1,983(15%)	<0.001
Diuretics	0 (0.0%)	4,689 (36%)	<0.001
Statins	1886 (4%)	2,814 (21%)	<0.001
Alternative cholesterol lowering medication	95 (0.2%)	119 (0.9%)	<0.001
Anti-diabetics	592 (1%)	1,004 (8%)	<0.001
Health characteristics			
Diagnosed hypertension	0 (0.0%)	12,291 (93%)	<0.001
Diagnosed hypercholesterolemia	7,657 (16%)	3,974 (30%)	<0.001
Diagnosed diabetes	1,142 (2%)	1,428 (11%)	<0.001
Systolic blood pressure	136.00 [131.00, 143.00]	135.00 [125.00, 146.00]	<0.001
Diastolic blood pressure	81.00 [75.00, 86.00]	78.00 [72.00, 85.00]	<0.001
Total cholesterol	5.20 [4.60, 5.90]	5.20 [4.50, 5.90]	<0.001

LDL cholesterol	3.44 (0.92)	3.36 (0.93)	<0.001
HDL cholesterol	1.40 [1.20, 1.70]	1.40 [1.10, 1.60]	<0.001
Triglycerides	1.11 [0.80, 1.58]	1.26 [0.91, 1.76]	<0.001
Renal function	96.41 [86.10, 100.00]	88.45 [76.94, 97.76]	<0.001



Supplemental Figure 4.1. Flow chart of included study population.

Supplemental Table 5.1 Cerebrovascular reactivity to 5% CO_2. Absolute and relative cerebrovascular reactivity were calculated from MCAv at 1s peak, 30s rolling average and 2:30-3:00min following gas inhalation.

Variable	Control Pre exercise	Control 30 min post exercise	Medicated Hypertension Pre exercise	Medicated Hypertension Post 30 min post exercise	Time	Group	Time*Condition
Baseline							
MCA_V (cm/s)	67.21 ± 14.19	58.23 ± 14.92	48.62 ± 11.86	52.96 ± 7.85	0.397	0.103	**0.026**
$PETCO_{2max}$ (mmHg)	44.29 ± 5.54	43.92 ± 5.64	40.38 ± 3.96	41.08 ± 4.45	0.862	0.231	0.589
MAP (mmHg)	80 ± 21	85 ± 23	85 ± 19	81 ± 31	0.933	0.960	0.548
CBVR	1.25 ± 0.42	1.51 ± 0.44	1.82 ± 0.42	1.62 ± 0.79	0.812	0.212	0.081
CVCi	0.92 ± 0.42	0.71 ± 0.19	0.59 ± 0.18	0.77 ± 0.42	0.895	0.355	0.107
1 second peak							
MCAv (cm/s)	94.32 ± 21.64	78.67 ± 21.80	73.21 ± 15.53	76.06 ± 14.07	0.120	0.265	0.032
$PETCO_2$ (mmHg)	51.83 ± 4.10	49.89 ± 5.17	48. 90 ± 5.18	48.44 ± 4.64	0.140	0.393	0.355
MAP (mmHg)	98 ± 14	94 ± 28	101 ± 12	98 ± 25	0.696	0.728	0.928
Absolute CVR (cm/s/mmHg)	3.99 ± 1.87	4.17 ± 2.59	2.87 ± 0.88	3.20 ± 0.94	0.711	0.217	0.929
Relative CVR (%/mmHg)	4.19 ± 7.92	2.50 ± 3.44	1.38 ± 1.38	1.16 ± 1.57	0.577	0.456	0.665
Absolute CVR_{CO2MAP} ($cm.s,mmHg^{-1}.mmHg^{-1}$)	2.59 ± 3.80	2.40 ± 3.54	1.13 ± 1.14	0.94 ± 1.28	0.814	0.423	1.00
Relative CVR_{CO2MAP} ($\%cm.s/mmHg^{-1}.mmHg^{-1}$)	5.83 ± 2.15	6.91 ± 3.25	6.23 ± 2.75	5.97 ± 1.08	0.715	0.766	0.546

CBVR	1.08 ± 0.30	1.20 ± 0.35	1.38 ± 0.23	1.38 ± 0.54	0.561	0.199	0.599
CVCi	0.98 ± 0.22	0.91 ± 0.31	0.74 ± 0.13	0.84 ± 0.44	0.872	0.333	0.295
30s rolling average							
MCA (cm/s)	87.44 ± 19.55	73.73 ± 19.24	64.93 ± 17.22	73.37 ± 21.04	0.468	0.273	0.08
$PETCO_2$ (mmHg)	52.03 ± 4.29	51.32 ± 4.29	48.65 ± 5.06	48.12 ± 4.76	0.158	0.197	0.843
MAP (mmHg)	94 ± 17	90 ± 24	98 ± 12	94 ± 26	0.584	0.714	0.952
Absolute CVR (cm/s/mmHg)	2.77 ± 1.24	2.17 ± 0.67	1.85 ± 0.60	2.88 ± 1.76	0.572	0.834	**0.047**
Relative CVR (%/mmHg)	4.01 ± 1.22	3.96 ± 1.67	3.86 ± 0.99	5.20 ± 2.59	0.328	0.398	0.297
Absolute CVR_{CO2MAP} ($cm.s,mmHg^{-1}.mmHg^{-1}$)	0.81 ± 1.31	0.50 ± 0.78	0.60 ± 1.37	0.50 ± 1.13	0.701	0.831	0.844
Relative CVR_{CO2MAP} ($\%cm.s/mmHg^{-1}.mmHg^{-1}$	0.95 ± 1.43	0.50 ± 0.74	0.79 ± 1.60	0.50 ± 1.13	0.514	0.883	0.889
CBVR	1.13 ± 0.30	1.21 ± 0.32	1.26 ± 0.78	1.15 ± 0.84	0.890	0.889	0.267
CVCi	0.93 ± 0.20	0.75 ± 0.33	0.66 ± 0.17	0.32 ± 0.37	0.051	**0.011**	0.512
2:30-3:00							
MCAv (cm/s)	82.17 ± 18.63	70.28 ± 17.97	64.06 ± 15.97	70.25 ± 11.96	0.432	0.316	**0.023**
$PETCO_2$ (mmHg)	51.75 ± 3.98	51.33 ± 4.31	48.44 ± 5.35	48.67 ± 4.55	0.823	0.233	0.418
MAP (mmHg)	93 ± 12	93 ± 24	97 ± 15	97 ± 25	0.951	0.697	0.951

Absolute CVR (cm/s/mmHg)	1.95 ± 0.90	1.88 ± 0.61	1.84 ± 0.40	2.32 ± 0.70	0.377	0.623	0.242
Relative CVR (%/mmHg)	2.85 ± 1.12	0.50 ± 0.71	0.81 ± 0.73	0.86 ± 0.76	0.307	0.004	0.980
Absolute CVR_{CO2MAP} ($cm.s,mmHg^{-1}.mmHg^{-1}$)	0.51 ± 0.58	0.48 ± 0.75	0.70 ± 0.67	0.68 ± 0.55	0.434	0.686	0.442
Relative CVR_{CO2MAP} ($\%cm.s/mmHg^{-1}.mmHg^{-1}$)	0.61 ± 0.72	0.23 ± 0.38	0.43 ± 0.42	0.41 ± 0.35	0.484	0.628	0.496
CBVR	1.17 ± 0.29	1.24 ± 0.33	1.29 ± 0.86	1.18 ± 0.82	0.770	0.920	0.279
CVCi	0.89 ± 0.20	0.75 ± 0.33	0.67 ± 0.21	0.32 ± 0.36	0.018	0.60	0.390

Chapter 9: References

AASLID, R. 1986. Transcranial Doppler examination techniques. *Transcranial doppler sonography.* Springer.

AASLID, R., LINDEGAARD, K.-F., SORTEBERG, W. & NORNES, H. 1989. Cerebral autoregulation dynamics in humans. *Stroke,* 20**,** 45-52.

AGARWAL, N. & CARARE, R. O. 2021. Cerebral vessels: an overview of anatomy, physiology, and role in the drainage of fluids and solutes. *Frontiers in neurology,* 11**,** 611485.

AINSLIE, P. N., BARACH, A., MURRELL, C., HAMLIN, M., HELLEMANS, J. & OGOH, S. 2007. Alterations in cerebral autoregulation and cerebral blood flow velocity during acute hypoxia: rest and exercise. *American Journal of Physiology-Heart and Circulatory Physiology,* 292**,** H976-H983.

AINSLIE, P. N., COTTER, J. D., GEORGE, K. P., LUCAS, S., MURRELL, C., SHAVE, R., THOMAS, K. N., WILLIAMS, M. J. & ATKINSON, G. 2008. Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. *The Journal of physiology,* 586**,** 4005-4010.

AINSLIE, P. N. & DUFFIN, J. 2009. Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 296**,** R1473-R1495.

AINSLIE, P. N. & HOILAND, R. L. 2014. Transcranial Doppler ultrasound: valid, invalid, or both? : American Physiological Society Bethesda, MD.

AINSWORTH, B. E., HASKELL, W. L., HERRMANN, S. D., MECKES, N., BASSETT JR, D. R., TUDOR-LOCKE, C., GREER, J. L., VEZINA, J., WHITT-GLOVER, M. C. & LEON, A. S. 2011. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Medicine & science in sports & exercise,* 43**,** 1575-1581.

AKAZAWA, N., CHOI, Y., MIYAKI, A., SUGAWARA, J., AJISAKA, R. & MAEDA, S. 2012. Aerobic exercise training increases cerebral blood flow in postmenopausal women. *Artery Research,* 6**,** 124-129.

AL SUWAIDI, J., HIGANO, S. T., HOLMES, D. R., LENNON, R. & LERMAN, A. 2001. Obesity is independently associated with coronary endothelial dysfunction in patients with normal or mildly diseased coronary arteries. *Journal of the American College of Cardiology,* 37**,** 1523-1528.

ALBERT, C. M., MITTLEMAN, M. A., CHAE, C. U., LEE, I.-M., HENNEKENS, C. H. & MANSON, J. E. 2000. Triggering of sudden death from cardiac causes by vigorous exertion. *New England Journal of Medicine,* 343**,** 1355-1361.

ALISTAIR, D. G. 2002. Hypertensive cerebral small vessel disease and stroke. *Brain pathology,* 12**,** 358-370.

ALKAITIS, M. S. & CRABTREE, M. J. 2012. Recoupling the cardiac nitric oxide synthases: tetrahydrobiopterin synthesis and recycling. *Current heart failure reports,* 9**,** 200-210.

ALVES, A. J., VIANA, J. L., CAVALCANTE, S. L., OLIVEIRA, N. L., DUARTE, J. A., MOTA, J., OLIVEIRA, J. & RIBEIRO, F. 2016. Physical activity in primary and secondary prevention of cardiovascular disease: Overview updated. *World journal of cardiology,* 8**,** 575.

ANDERSON, E. A., SINKEY, C., LAWTON, W. & MARK, A. 1989. Elevated sympathetic nerve activity in borderline hypertensive humans. Evidence from direct intraneural recordings. *Hypertension,* 14**,** 177-183.

ANDERSON, T. J., UEHATA, A., GERHARD, M. D., MEREDITH, I. T., KNAB, S., DELAGRANGE, D., LIEBERMAN, E. H., GANZ, P., CREAGER, M. A. & YEUNG, A. C. 1995. Close relation of

endothelial function in the human coronary and peripheral circulations. *Journal of the American College of Cardiology,* 26**,** 1235-1241.

AOKI, K., SATO, K., KONDO, S., CHAE-BOK, P. & YAMAMOTO, M. 1983. Increased response of blood pressure to rest and handgrip in subjects with essential hypertension. *Japanese circulation journal,* 47**,** 802-809.

AREM, H., MOORE, S. C., PATEL, A., HARTGE, P., DE GONZALEZ, A. B., VISVANATHAN, K., CAMPBELL, P. T., FREEDMAN, M., WEIDERPASS, E. & ADAMI, H. O. 2015. Leisure time physical activity and mortality: a detailed pooled analysis of the dose-response relationship. *JAMA internal medicine,* 175**,** 959-967.

ASHBY, J. W. & MACK, J. J. 2021. Endothelial control of cerebral blood flow. *The American Journal of Pathology,* 191**,** 1906-1916.

ASHOR, A. W., LARA, J., SIERVO, M., CELIS-MORALES, C., OGGIONI, C., JAKOVLJEVIC, D. G. & MATHERS, J. C. 2015. Exercise modalities and endothelial function: a systematic review and dose–response meta-analysis of randomized controlled trials. *Sports medicine,* 45**,** 279-296.

ASHTON, R. E., TEW, G. A., ANING, J. J., GILBERT, S. E., LEWIS, L. & SAXTON, J. M. 2020. Effects of short-term, medium-term and long-term resistance exercise training on cardiometabolic health outcomes in adults: systematic review with meta-analysis. *British journal of sports medicine,* 54**,** 341-348.

ASMAR, R. G., TOPOUCHIAN, J. A., BENETOS, A., SAYEGH, F. A., MOURAD, J.-J. & SAFAR, M. E. 1997. Non-invasive evaluation of arterial abnormalities in hypertensive patients. *Journal of Hypertension,* 15**,** S99-S107.

ATKINSON, G., BATTERHAM, A. M., THIJSSEN, D. H. & GREEN, D. J. 2013. A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. *Journal of hypertension,* 31**,** 287-291.

ATTWELL, D., BUCHAN, A. M., CHARPAK, S., LAURITZEN, M., MACVICAR, B. A. & NEWMAN, E. A. 2010. Glial and neuronal control of brain blood flow. *Nature,* 468**,** 232-243.

BAILEY, D. M., EVANS, K. A., MCENENY, J., YOUNG, I. S., HULLIN, D. A., JAMES, P. E., OGOH, S., AINSLIE, P. N., LUCCHESI, C. & ROCKENBAUER, A. 2011. Exercise-induced oxidative–nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood–brain barrier leakage. *Experimental physiology,* 96**,** 1196-1207.

BAILEY, T. G., CABLE, N. T., MILLER, G., SPRUNG, V., LOW, D. & JONES, H. 2016. Repeated warm water immersion induces similar cerebrovascular adaptations to 8 weeks of moderate-intensity exercise training in females. *International journal of sports medicine***,** 757-765.

BAKKER, E. A., LEE, D.-C., HOPMAN, M. T., OYMANS, E. J., WATSON, P. M., THOMPSON, P. D., THIJSSEN, D. H. & EIJSVOGELS, T. M. 2021. Dose–response association between moderate to vigorous physical activity and incident morbidity and mortality for individuals with a different cardiovascular health status: A cohort study among 142,493 adults from the Netherlands. *PLoS medicine,* 18**,** e1003845.

BAMFORD, J., SANDERCOCK, P., DENNIS, M., WARLOW, C. & BURN, J. 1991. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *The Lancet,* 337**,** 1521-1526.

BAPTISTA, L. C., MACHADO-RODRIGUES, A. M., VERISSIMO, M. T. & MARTINS, R. A. 2018. Exercise training improves functional status in hypertensive older adults under angiotensin converting enzymes inhibitors medication. *Experimental gerontology,* 109**,** 82-89.

BARAUNA, V. G., MAGALHAES, F. C., KRIEGER, J. E. & OLIVEIRA, E. M. 2008. AT1 receptor participates in the cardiac hypertrophy induced by resistance training in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 295**,** R381-R387.

BARNES, J. N., TAYLOR, J. L., KLUCK, B. N., JOHNSON, C. P. & JOYNER, M. J. 2013. Cerebrovascular reactivity is associated with maximal aerobic capacity in healthy older adults. *Journal of applied physiology,* 114**,** 1383-1387.

BAUMBACH, G. L. & HEISTAD, D. D. 1988. Cerebral circulation in chronic arterial hypertension. *Hypertension,* 12**,** 89-95.

BAUMBACH, G. L. & HEISTAD, D. D. 1989. Remodeling of cerebral arterioles in chronic hypertension. *Hypertension,* 13**,** 968-972.

BAYLISS, W., HILL, L. & GULLAND, G. L. 1895. On intra-cranial pressure and the cerebral circulation: Part I. Physiological; Part II. Histological. *The Journal of physiology,* 18**,** 334.

BAYS, H. E., TAUB, P. R., EPSTEIN, E., MICHOS, E. D., FERRARO, R. A., BAILEY, A. L., KELLI, H. M., FERDINAND, K. C., ECHOLS, M. R. & WEINTRAUB, H. 2021. Ten things to know about ten cardiovascular disease risk factors. *American journal of preventive cardiology,* 5**,** 100149.

BECKMAN, J. S. & KOPPENOL, W. H. 1996. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *American Journal of Physiology-cell physiology,* 271**,** C1424-C1437.

BEEVERS, G., LIP, G. Y. & O'BRIEN, E. 2001. The pathophysiology of hypertension. *Bmj,* 322**,** 912-916.

BERDEAUX, A., GHALEH, B., DUBOIS-RANDE, J. L., VIGUE, B., DRIEU LA ROCHELLE, C., HITTINGER, L. & GIUDICELLI, J. F. 1994. Role of vascular endothelium in exercise-induced dilation of large epicardial coronary arteries in conscious dogs. *Circulation,* 89**,** 2799-2808.

BERTSCH, K., HAGEMANN, D., HERMES, M., WALTER, C., KHAN, R. & NAUMANN, E. 2009. Resting cerebral blood flow, attention, and aging. *Brain research,* 1267**,** 77-88.

BILLINGER, S. A., WHITAKER, A. A., MORTON, A., KAUFMAN, C. S., PERDOMO, S. J., WARD, J. L., EICKMEYER, S. M., BAI, S. X., LEDBETTER, L. & ABRAHAM, M. G. 2021. Pilot study to characterize middle cerebral artery dynamic response to an acute bout of moderate intensity exercise at 3-and 6-months Poststroke. *Journal of the American Heart Association,* 10**,** e017821.

BIRK, G., DAWSON, E., BATTERHAM, A., ATKINSON, G., CABLE, T., THIJSSEN, D. & GREEN, D. 2012. Effects of exercise intensity on flow mediated dilation in healthy humans. *International journal of sports medicine***,** 409-414.

BLAIR, S. N., KOHL, H. W., BARLOW, C. E., PAFFENBARGER, R. S., GIBBONS, L. W. & MACERA, C. A. 1995. Changes in physical fitness and all-cause mortality: a prospective study of healthy and unhealthy men. *Jama,* 273**,** 1093-1098.

BLISS, E. S., WONG, R. H., HOWE, P. R. & MILLS, D. E. 2021. Benefits of exercise training on cerebrovascular and cognitive function in ageing. *Journal of Cerebral Blood Flow & Metabolism,* 41**,** 447-470.

BOEHME, A. K., ESENWA, C. & ELKIND, M. S. 2017. Stroke risk factors, genetics, and prevention. *Circulation research,* 120**,** 472-495.

BOLÍVAR, J. J. 2013. Essential hypertension: an approach to its etiology and neurogenic pathophysiology. *International journal of hypertension,* 2013.

BOND, B., HIND, S., WILLIAMS, C. A. & BARKER, A. R. 2015. The acute effect of exercise intensity on vascular function in adolescents.

BOULANGER, C. M. 2016. Endothelium. *Arteriosclerosis, thrombosis, and vascular biology,* 36**,** e26-e31.

BRAZ, I. D. & FISHER, J. P. 2016. The impact of age on cerebral perfusion, oxygenation and metabolism during exercise in humans. *The Journal of physiology,* 594**,** 4471-4483.

BRIET, M. & SCHIFFRIN, E. L. 2013. Vascular actions of aldosterone. *Journal of vascular research,* 50**,** 89-99.

BROTHERS, R. M. & ZHANG, R. 2016. CrossTalk opposing view: The middle cerebral artery diameter does not change during alterations in arterial blood gases and blood pressure. *The Journal of physiology,* 594**,** 4077.

BROWN, A. M. & RANSOM, B. R. 2007. Astrocyte glycogen and brain energy metabolism. *Glia,* 55**,** 1263-1271.

BROXTERMAN, R. M., WITMAN, M. A., TRINITY, J. D., GROOT, H. J., ROSSMAN, M. J., PARK, S.-Y., MALENFANT, S., GIFFORD, J. R., KWON, O. S. & PARK, S. H. 2019. Strong relationship between vascular function in the coronary and brachial arteries: a clinical coming of age for the updated flow-mediated dilation test? *Hypertension,* 74**,** 208-215.

BUFORD, T. W., MANINI, T. M., HSU, F. C., CESARI, M., ANTON, S. D., NAYFIELD, S., STAFFORD, R. S., CHURCH, T. S., PAHOR, M. & CARTER, C. S. 2012. Angiotensin-converting enzyme inhibitor use by older adults is associated with greater functional responses to exercise. *Journal of the American Geriatrics Society,* 60**,** 1244-1252.

BULL, F. C., AL-ANSARI, S. S., BIDDLE, S., BORODULIN, K., BUMAN, M. P., CARDON, G., CARTY, C., CHAPUT, J.-P., CHASTIN, S. & CHOU, R. 2020. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *British journal of sports medicine,* 54**,** 1451-1462.

BURLEY, C. V., LUCAS, R. A., WHITTAKER, A. C., MULLINGER, K. & LUCAS, S. J. 2020. The CO2 stimulus duration and steady-state time point used for data extraction alters the cerebrovascular reactivity outcome measure. *Experimental physiology,* 105**,** 893-903.

BURMA, J. S., MACAULAY, A., COPELAND, P., KHATRA, O., BOULIANE, K. J. & SMIRL, J. D. 2020. Comparison of cerebrovascular reactivity recovery following high-intensity interval training and moderate-intensity continuous training. *Physiological Reports,* 8**,** e14467.

CALDWELL, J. T., FENN, S. A., BEKKEDAL, L. M., DODGE, C. & MULLER-DELP, J. 2023. Preexercise intermittent passive stretching and vascular function after treadmill exercise. *Journal of Applied Physiology,* 135**,** 786-794.

CAMPBELL, A., GRACE, F., RITCHIE, L., BEAUMONT, A. & SCULTHORPE, N. 2019. Long-term aerobic exercise improves vascular function into old age: a systematic review, meta-analysis and meta regression of observational and interventional studies. *Frontiers in physiology***,** 31.

CAPILDEO, R., HABERMAN, S. & ROSE, F. C. 1978. The definition and classification of stroke: a new approach. *QJM: An International Journal of Medicine,* 47**,** 177-196.

CARR, J. M., CALDWELL, H. G. & AINSLIE, P. N. 2021. Cerebral blood flow, cerebrovascular reactivity and their influence on ventilatory sensitivity. *Experimental Physiology,* 106**,** 1425-1448.

CARR, J. M., HOILAND, R. L., CALDWELL, H. G., COOMBS, G. B., HOWE, C. A., TREMBLAY, J. C., GREEN, D. J. & AINSLIE, P. N. 2020. Internal carotid and brachial artery shear-dependent vasodilator function in young healthy humans. *The Journal of physiology,* 598**,** 5333-5350.

CARTER, H. H., ATKINSON, C. L., HEINONEN, I. H., HAYNES, A., ROBEY, E., SMITH, K. J., AINSLIE, P. N., HOILAND, R. L. & GREEN, D. J. 2016. Evidence for shear stress–mediated dilation of the internal carotid artery in humans. *Hypertension,* 68**,** 1217-1224.

CELERMAJER, D. S., SORENSEN, K. E., GOOCH, V. M., SPIEGELHALTER, D., MILLER, O., SULLIVAN, I., LLOYD, J. & DEANFIELD, J. 1992. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *The lancet,* 340**,** 1111-1115.

CELERMAJER, D. S., SORENSEN, K. E., SPIEGELHALTER, D. J., GEORGAKOPOULOS, D., ROBINSON, J. & DEANFIELD, J. E. 1994. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *Journal of the American College of Cardiology,* 24**,** 471-476.

CELIS-MORALES, C. A., PEREZ-BRAVO, F., IBAÑEZ, L., SALAS, C., BAILEY, M. E. & GILL, J. M. 2012. Objective vs. self-reported physical activity and sedentary time: effects of measurement method on relationships with risk biomarkers. *PloS one,* 7**,** e36345.

CESARI, M., PEDONE, C., INCALZI, R. A. & PAHOR, M. 2010. ACE-inhibition and physical function: results from the Trial of Angiotensin-Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors (TRAIN) study. *Journal of the American Medical Directors Association,* 11**,** 26-32.

CHEN, J.-Y., YE, Z.-X., WANG, X.-F., CHANG, J., YANG, M.-W., ZHONG, H.-H., HONG, F.-F. & YANG, S.-L. 2018. Nitric oxide bioavailability dysfunction involves in atherosclerosis. *Biomedicine & Pharmacotherapy,* 97**,** 423-428.

CHRISSOBOLIS, S., MILLER, A. A., DRUMMOND, G. R., KEMP-HARPER, B. K. & SOBEY, C. G. 2011. Oxidative stress and endothelial dysfunction in cerebrovascular disease. *Frontiers in Bioscience-Landmark,* 16**,** 1733-1745.

CHRISSOBOLIS, S. & SOBEY, C. G. 2006. Recent evidence for an involvement of rho-kinase in cerebral vascular disease. *Stroke,* 37**,** 2174-2180.

CLAASSEN, J. A. 2016. The plateau phase is a slippery slope: raising blood pressure may lower brain perfusion. *The Journal of Physiology,* 594**,** 2783.

CLAASSEN, J. A., MEEL-VAN DEN ABEELEN, A. S., SIMPSON, D. M., PANERAI, R. B. & NETWORK, I. C. A. R. 2016. Transfer function analysis of dynamic cerebral autoregulation: a white paper from the International Cerebral Autoregulation Research Network. *Journal of Cerebral Blood Flow & Metabolism,* 36**,** 665-680.

CLAASSEN, J. A., THIJSSEN, D. H., PANERAI, R. B. & FARACI, F. M. 2021. Regulation of cerebral blood flow in humans: physiology and clinical implications of autoregulation. *Physiological reviews,* 101**,** 1487-1559.

COSIO-LIMA, L. M., THOMPSON, P. D., REYNOLDS, K. L., HEADLEY, S. A., WINTER, C. R., MANOS, T., LAGASSE, M. A., TODOROVICH, J. R. & GERMAIN, M. 2006. The acute effect of aerobic exercise on brachial artery endothelial function in renal transplant recipients. *Preventive cardiology,* 9**,** 211-214.

COWLEY JR, A. W., LIARD, J. F. & GUYTON, A. C. 1973. Role of the baroreceptor reflex in daily control of arterial blood pressure and other variables in dogs. *Circulation research,* 32**,** 564-576.

CZOSNYKA, M., SMIELEWSKI, P., KIRKPATRICK, P., MENON, D. K. & PICKARD, J. D. 1996. Monitoring of cerebral autoregulation in head-injured patients. *Stroke,* 27**,** 1829-1834.

DAHLÖF, B. 2007. Prevention of stroke in patients with hypertension. *The American journal of cardiology,* 100**,** S17-S24.

DAVIGNON, J. & GANZ, P. 2004. Role of endothelial dysfunction in atherosclerosis. *Circulation,* 109**,** III-27-III-32.

DAWSON, E. A., CABLE, N. T., GREEN, D. J. & THIJSSEN, D. H. 2018. Do acute effects of exercise on vascular function predict adaptation to training? *European journal of applied physiology,* 118**,** 523-530.

DAWSON, E. A., GREEN, D. J., TIMOTHY CABLE, N. & THIJSSEN, D. H. 2013. Effects of acute exercise on flow-mediated dilatation in healthy humans. *Journal of applied physiology,* 115**,** 1589-1598.

DAWSON, E. A., RATHORE, S., CABLE, N. T., WRIGHT, D. J., MORRIS, J. L. & GREEN, D. J. 2010. Impact of introducer sheath coating on endothelial function in humans after transradial coronary procedures. *Circulation: Cardiovascular Interventions,* 3**,** 148-156.

DAWSON, E. A., WHYTE, G. P., BLACK, M. A., JONES, H., HOPKINS, N., OXBOROUGH, D., GAZE, D., SHAVE, R. E., WILSON, M. & GEORGE, K. P. 2008. Changes in vascular and cardiac function after prolonged strenuous exercise in humans. *Journal of applied physiology,* 105**,** 1562-1568.

DE HEUS, R. A., DE JONG, D. L., SANDERS, M. L., VAN SPIJKER, G. J., OUDEGEEST-SANDER, M. H., HOPMAN, M. T., LAWLOR, B. A., OLDE RIKKERT, M. G. & CLAASSEN, J. A. 2018. Dynamic regulation of cerebral blood flow in patients with Alzheimer disease. *Hypertension,* 72**,** 139-150.

DE SILVA, T. M. & FARACI, F. M. 2013. Effects of angiotensin II on the cerebral circulation: role of oxidative stress. *Frontiers in physiology,* 3**,** 484.

DEMINICE, R., HYATT, H., YOSHIHARA, T., OZDEMIR, M., NGUYEN, B., LEVINE, S. & POWERS, S. 2020. Human and rodent skeletal muscles express angiotensin II type 1 receptors. *Cells,* 9**,** 1688.

DIBONA, G. F. & ESLER, M. 2010. Translational medicine: the antihypertensive effect of renal denervation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 298**,** R245-R253.

DICKERMAN, R., MCCONATHY, W., SMITH, G., EAST, J. & RUDDER, L. 2000. Middle cerebral artery blood flow velocity in elite power athletes during maximal weight-lifting. *Neurological research,* 22**,** 337-340.

DICKINSON, C. & THOMSON, A. 1959. Vertebral and internal carotid arteries in relation to hypertension and cerebrovascular disease. *The Lancet,* 274**,** 46-48.

DICKINSON, C. J. 2001. Why are strokes related to hypertension? Classic studies and hypotheses revisited. *Journal of hypertension,* 19**,** 1515-1521.

DIMEO, F., PAGONAS, N., SEIBERT, F., ARNDT, R., ZIDEK, W. & WESTHOFF, T. H. 2012. Aerobic exercise reduces blood pressure in resistant hypertension. *Hypertension,* 60**,** 653-658.

DREXLER, H., BANHARDT, U., MEINERTZ, T., WOLLSCHLÄGER, H., LEHMANN, M. & JUST, H. 1989. Contrasting peripheral short-term and long-term effects of converting enzyme inhibition in patients with congestive heart failure. A double-blind, placebo-controlled trial. *Circulation,* 79**,** 491-502.

DUNCAN, J. J., VAANDRAGER, H., FARR, J. E., KOHL, H. W. & GORDON, N. F. 1989. Effect of Intrinsic Sympathomimetic Activity on Serum Lipids During Exercise Training in Hypertensive Patients Receiving Chronic β-Blocker Therapy. *Journal of Cardiopulmonary Rehabilitation and Prevention,* 9**,** 110-114.

DUNN, A. L., MARCUS, B. H., KAMPERT, J. B., GARCIA, M. E., KOHL III, H. W. & BLAIR, S. N. 1997. Reduction in cardiovascular disease risk factors: 6-month results from ProjectActive. *Preventive medicine,* 26**,** 883-892.

DURAND, M. J. & GUTTERMAN, D. D. 2013. Diversity in mechanisms of endothelium-dependent vasodilation in health and disease. *Microcirculation,* 20**,** 239-247.

DURIER, S., FASSOT, C., LAURENT, S., BOUTOUYRIE, P., COUETIL, J.-P., FINE, E., LACOLLEY, P., DZAU, V. J. & PRATT, R. E. 2003. Physiological genomics of human arteries: quantitative relationship between gene expression and arterial stiffness. *Circulation,* 108**,** 1845-1851.

EARLY, K. S., STEWART, A., JOHANNSEN, N., LAVIE, C. J., THOMAS, J. R. & WELSCH, M. 2017. The effects of exercise training on brachial artery flow-mediated dilation: a meta-analysis. *Journal of Cardiopulmonary Rehabilitation and Prevention,* 37**,** 77-89.

EDVINSSON, L. & KRAUSE, D. N. 1993. Cerebral blood flow and metabolism. *(No Title)*.

EDWARDS, D. G., SCHOFIELD, R. S., LENNON, S. L., PIERCE, G. L., NICHOLS, W. W. & BRAITH, R. W. 2004. Effect of exercise training on endothelial function in men with coronary artery disease. *The American journal of cardiology,* 93**,** 617-620.

EDWARDS, M. R., MARTIN, D. H. & HUGHSON, R. L. 2002. Cerebral hemodynamics and resistance exercise. *Medicine & Science in Sports & Exercise,* 34**,** 1207-1211.

EELEN, G., DE ZEEUW, P., SIMONS, M. & CARMELIET, P. 2015. Endothelial cell metabolism in normal and diseased vasculature. *Circulation research,* 116**,** 1231-1244.

EIJSVOGELS, T. M., MOLOSSI, S., LEE, D.-C., EMERY, M. S. & THOMPSON, P. D. 2016. Exercise at the extremes: the amount of exercise to reduce cardiovascular events. *Journal of the American College of Cardiology,* 67**,** 316-329.

EKBLOM, B., GOLDBARG, A., KILBOM, Å. & ÅSTRAND, P. 1972. Effects of atropine and propranolol on the oxygen transport system during exercise in man. *Scandinavian journal of clinical and laboratory investigation,* 30**,** 35-42.

ENDEMANN, D. H. & SCHIFFRIN, E. L. 2004. Endothelial Dysfunction. *Journal of the American Society of Nephrology,* 15**,** 1983-1992.

ESLER, M., STRAZNICKY, N., EIKELIS, N., MASUO, K., LAMBERT, G. & LAMBERT, E. 2006. Mechanisms of sympathetic activation in obesity-related hypertension. *Hypertension,* 48**,** 787-796.

FAGARD, R. H., PARDAENS, K., STAESSEN, J. A. & THIJS, L. 1996. Prognostic value of invasive hemodynamic measurements at rest and during exercise in hypertensive men. *Hypertension,* 28**,** 31-36.

FAGRAEUS, L. & LINNARSSON, D. 1976. Autonomic origin of heart rate fluctuations at the onset of muscular exercise. *Journal of Applied Physiology,* 40**,** 679-682.

FANTINI, S., SASSAROLI, A., TGAVALEKOS, K. T. & KORNBLUTH, J. 2016. Cerebral blood flow and autoregulation: current measurement techniques and prospects for noninvasive optical methods. *Neurophotonics,* 3**,** 031411-031411.

FARACI, F. M. & HEISTAD, D. D. 1990. Regulation of large cerebral arteries and cerebral microvascular pressure. *Circulation research,* 66**,** 8-17.

FARSIDFAR, F., KASIKCIOGLU, E., OFLAZ, H., KASIKCIOGLU, D., MERIC, M. & UMMAN, S. 2008. Effects of different intensities of acute exercise on flow-mediated dilatation in patients with coronary heart disease. *International journal of cardiology,* 124**,** 372-374.

FICZERE, A., VALIKOVICS, A., FÜLESDI, B., JUHÁSZ, A., CZURIGA, I. & CSIBA, L. 1997. Cerebrovascular reactivity in hypertensive patients: a transcranial Doppler study. *Journal of clinical ultrasound,* 25**,** 383-389.

FIERSTRA, J., SOBCZYK, O., BATTISTI-CHARBONNEY, A., MANDELL, D., POUBLANC, J., CRAWLEY, A., MIKULIS, D., DUFFIN, J. & FISHER, J. 2013. Measuring cerebrovascular reactivity: what stimulus to use? *The Journal of physiology,* 591**,** 5809-5821.

FIGUEROA, C. D., DIETZE, G. & MÜLLER-ESTERL, W. 1996. Immunolocalization of bradykinin B2 receptors on skeletal muscle cells. *Diabetes,* 45**,** S24-S28.

FIUZA-LUCES, C., SANTOS-LOZANO, A., JOYNER, M., CARRERA-BASTOS, P., PICAZO, O., ZUGAZA, J. L., IZQUIERDO, M., RUILOPE, L. M. & LUCIA, A. 2018. Exercise benefits in cardiovascular disease: beyond attenuation of traditional risk factors. *Nature Reviews Cardiology,* 15**,** 731-743.

FLEMING, M. F. 2004. Screening and brief intervention in primary care settings. *Alcohol Research & Health,* 28**,** 57.

FLORAS, J. S. & HARA, K. 1993. Sympathoneural and haemodynamic characteristics of young subjects with mild essential hypertension. *Journal of hypertension,* 11**,** 647-655.

FOLKOW, B. 1982. Physiological aspects of primary hypertension. *Physiological reviews,* 62**,** 347-504.

FORD, M. A., MCCONNELL, J. P., LAVI, S., RIHAL, C. S., PRASAD, A., SANDHU, G. S., HARTMAN, S. J., LERMAN, L. O. & LERMAN, A. 2009. Coronary artery endothelial dysfunction is positively correlated with low density lipoprotein and inversely correlated with high density lipoprotein subclass particles measured by nuclear magnetic resonance spectroscopy. *Atherosclerosis,* 207**,** 111-115.

FOROUZANFAR, M. H., AFSHIN, A., ALEXANDER, L. T., ANDERSON, H. R., BHUTTA, Z. A., BIRYUKOV, S., BRAUER, M., BURNETT, R., CERCY, K., CHARLSON, F. J., COHEN, A. J., DANDONA, L., ESTEP, K., FERRARI, A. J., FROSTAD, J. J., FULLMAN, N., GETHING, P. W., GODWIN, W. W., GRISWOLD, M., HAY, S. I., KINFU, Y., KYU, H. H., LARSON, H. J., LIANG, X., LIM, S. S., LIU, P. Y., LOPEZ, A. D., LOZANO, R., MARCZAK, L., MENSAH, G. A., MOKDAD, A. H., MORADI-LAKEH, M., NAGHAVI, M., NEAL, B., REITSMA, M. B., ROTH, G. A., SALOMON, J. A., SUR, P. J., VOS, T., WAGNER, J. A., WANG, H., ZHAO, Y., ZHOU, M., AASVANG, G. M., ABAJOBIR, A. A., ABATE, K. H., ABBAFATI, C., ABBAS, K. M., ABD-ALLAH, F., ABDULLE, A. M., ABERA, S. F., ABRAHAM, B., ABU-RADDAD, L. J., ABYU, G. Y., ADEBIYI, A. O., ADEDEJI, I. A., ADEMI, Z., ADOU, A. K., ADSUAR, J. C., AGARDH, E. E., AGARWAL, A., AGRAWAL, A., KIADALIRI, A. A., AJALA, O. N., AKINYEMIJU, T. F., AL-ALY, Z., ALAM, K., ALAM, N. K. M., ALDHAHRI, S. F., ALDRIDGE, R. W., ALEMU, Z. A., ALI, R., ALKERWI, A. A., ALLA, F., ALLEBECK, P., ALSHARIF, U., ALTIRKAWI, K. A., MARTIN, E. A., ALVIS-GUZMAN, N., AMARE, A. T., AMBERBIR, A., AMEGAH, A. K., AMINI, H., AMMAR, W., AMROCK, S. M., ANDERSEN, H. H., ANDERSON, B. O., ANTONIO, C. A. T., ANWARI, P., ÄRNLÖV, J., ARTAMAN, A., ASAYESH, H., ASGHAR, R. J., ASSADI, R., ATIQUE, S., AVOKPAHO, E. F. G. A., AWASTHI, A., QUINTANILLA, B. P. A., AZZOPARDI, P., BACHA, U., et al. 2016. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet,* 388**,** 1659-1724.

FÖRSTERMANN, U. 2006. Endothelial NO synthase as a source of NO and superoxide. *European Journal of Clinical Pharmacology,* 62**,** 5-12.

FÖRSTERMANN, U. & SESSA, W. C. 2012. Nitric oxide synthases: regulation and function. *European heart journal,* 33**,** 829-837.

FOUNDATIO, N. K. 2002. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis. ,* 39**,** 266.

FOUNTAIN, J. H., KAUR, J. & LAPPIN, S. L. 2023. Physiology, renin angiotensin system. *StatPearls [Internet].* StatPearls Publishing.

FUJISHIMA, S., OHYA, Y., SUGIMORI, H., KITAYAMA, J., KAGIYAMA, S., IBAYASHI, S., ABE, I. & FUJISHIMA, M. 2001. Transcranial Doppler sonography and ambulatory blood pressure monitoring in patients with hypertension. *Hypertension Research,* 24**,** 345-351.

FURCHGOTT, R. F. & ZAWADZKI, J. V. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *nature,* 288**,** 373-376.

FURUMOTO, T., SAITO, N., DONG, J., MIKAMI, T., FUJII, S. & KITABATAKE, A. 2002. Association of cardiovascular risk factors and endothelial dysfunction in Japanese hypertensive patients: implications for early atherosclerosis. *Hypertension Research,* 25**,** 475-480.

FYHRQUIST, F. & SAIJONMAA, O. 2008. Renin-angiotensin system revisited. *Journal of internal medicine,* 264**,** 224-236.

GALLO, G., VOLPE, M. & SAVOIA, C. 2022. Endothelial dysfunction in hypertension: current concepts and clinical implications. *Frontiers in Medicine,* 8**,** 798958.

GARLAND, C. J., HILEY, C. R. & DORA, K. A. 2011. EDHF: spreading the influence of the endothelium. *British journal of pharmacology,* 164**,** 839-852.

GHIADONI, L., VERSARI, D., MAGAGNA, A., KARDASZ, I., PLANTINGA, Y., GIANNARELLI, C., TADDEI, S. & SALVETTI, A. 2007. Ramipril dose-dependently increases nitric oxide availability in the radial artery of essential hypertension patients. *Journal of hypertension,* 25**,** 361-366.

GHITESCU, L. & ROBERT, M. 2002. Diversity in unity: the biochemical composition of the endothelial cell surface varies between the vascular beds. *Microscopy research and technique,* 57**,** 381-389.

GILLER, C. A., BOWMAN, G., DYER, H., MOOTZ, L. & KRIPPNER, W. 1993. Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery,* 32**,** 737-742.

GOEL, R., MAJEED, F., VOGEL, R., CORRETTI, M. C., WEIR, M., MANGANO, C., WHITE, C., PLOTNICK, G. D. & MILLER, M. 2007. Exercise-induced hypertension, endothelial dysfunction, and coronary artery disease in a marathon runner. *The American journal of cardiology,* 99**,** 743-744.

GONZALES, J. U., THOMPSON, B. C., THISTLETHWAITE, J. R. & SCHEUERMANN, B. W. 2011. Association between exercise hemodynamics and changes in local vascular function following acute exercise. *Applied Physiology, Nutrition, and Metabolism,* 36**,** 137-144.

GONZALEZ-GUERRA, A., ROCHE-MOLINA, M., GARCÍA-QUINTÁNS, N., SÁNCHEZ-RAMOS, C., MARTÍN-PÉREZ, D., LYTVYN, M., DE NICOLÁS-HERNÁNDEZ, J., RIVERA-TORRES, J., ARROYO, D. F. & SANZ-ROSA, D. 2021. Sustained Elevated Blood Pressure Accelerates Atherosclerosis Development in a Preclinical Model of Disease. *International Journal of Molecular Sciences,* 22**,** 8448.

GORDON, N. F. & DUNCAN, J. J. 1991. Effect of beta-blockers on exercise physiology: implications for exercise training. *Medicine and science in sports and exercise,* 23**,** 668-676.

GRASSI, G., MARK, A. & ESLER, M. 2015. The sympathetic nervous system alterations in human hypertension. *Circulation research,* 116**,** 976-990.

GRASSI, G. & RAM, V. S. 2016. Evidence for a critical role of the sympathetic nervous system in hypertension. *Journal of the American Society of Hypertension,* 10**,** 457-466.

GRASSI, G., SERAVALLE, G. & QUARTI-TREVANO, F. 2010. The 'neuroadrenergic hypothesis' in hypertension: current evidence. *Experimental physiology,* 95**,** 581-586.

GREEN, D. J., DAWSON, E. A., GROENEWOUD, H. M., JONES, H. & THIJSSEN, D. H. 2014a. Is flow-mediated dilation nitric oxide mediated? A meta-analysis. *Hypertension,* 63**,** 376-382.

GREEN, D. J., EIJSVOGELS, T., BOUTS, Y. M., MAIORANA, A. J., NAYLOR, L. H., SCHOLTEN, R. R., SPAANDERMAN, M. E., PUGH, C. J., SPRUNG, V. S. & SCHREUDER, T. 2014b. Exercise training and artery function in humans: nonresponse and its relationship to cardiovascular risk factors. *Journal of Applied Physiology,* 117**,** 345-352.

GREEN, D. J., HOPMAN, M. T., PADILLA, J., LAUGHLIN, M. H. & THIJSSEN, D. H. 2017. Vascular adaptation to exercise in humans: role of hemodynamic stimuli. *Physiological reviews,* 97**,** 495-528.

GREEN, D. J., JONES, H., THIJSSEN, D., CABLE, N. & ATKINSON, G. 2011. Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? *Hypertension,* 57**,** 363-369.

GREEN, D. J., MAIORANA, A., O'DRISCOLL, G. & TAYLOR, R. 2004. Effect of exercise training on endothelium-derived nitric oxide function in humans. *The Journal of physiology,* 561**,** 1-25.

GREEN, D. J., O'DRISCOLL, G., JOYNER, M. J. & CABLE, N. T. 2008. Exercise and cardiovascular risk reduction: time to update the rationale for exercise? *Journal of applied physiology,* 105**,** 766-768.

GREEN, D. J. & SMITH, K. J. 2018. Effects of exercise on vascular function, structure, and health in humans. *Cold Spring Harbor perspectives in medicine,* 8**,** a029819.

GREEN, D. J., WALSH, J. H., MAIORANA, A., BEST, M. J., TAYLOR, R. R. & O'DRISCOLL, J. G. 2003. Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. *American Journal of Physiology-Heart and Circulatory Physiology,* 285**,** H2679-H2687.

GROVER-PÁEZ, F. & ZAVALZA-GÓMEZ, A. B. 2009. Endothelial dysfunction and cardiovascular risk factors. *Diabetes research and clinical practice,* 84**,** 1-10.

GUESSOUM, O., DE GOES MARTINI, A., SEQUEIRA-LOPEZ, M. L. S. & GOMEZ, R. A. 2021. Deciphering the identity of renin cells in health and disease. *Trends in molecular medicine,* 27**,** 280-292.

GUO, X., ZHANG, X., GUO, L., LI, Z., ZHENG, L., YU, S., YANG, H., ZHOU, X., ZHANG, X. & SUN, Z. 2013. Association between pre-hypertension and cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *Current hypertension reports,* 15**,** 703-716.

GUPTA, A., CHAZEN, J. L., HARTMAN, M., DELGADO, D., ANUMULA, N., SHAO, H., MAZUMDAR, M., SEGAL, A. Z., KAMEL, H. & LEIFER, D. 2012. Cerebrovascular reserve and stroke risk in patients with carotid stenosis or occlusion: a systematic review and meta-analysis. *Stroke,* 43**,** 2884-2891.

HADI, H. A., CARR, C. S. & AL SUWAIDI, J. 2005. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. *Vascular health and risk management,* 1**,** 183.

HAMER, M., INGLE, L., CARROLL, S. & STAMATAKIS, E. 2012. Physical activity and cardiovascular mortality risk: possible protective mechanisms? *Medicine & science in sports & exercise,* 44.

HANSON, B. E. & CASEY, D. P. 2023. Intermittent versus continuous handgrip exercise and peripheral endothelial function: impact of shear rate fluctuations. *Journal of Applied Physiology,* 135**,** 892-901.

HARRIS, R. A., PADILLA, J., HANLON, K. P., RINK, L. D. & WALLACE, J. P. 2008. The flow-mediated dilation response to acute exercise in overweight active and inactive men. *Obesity,* 16**,** 578-584.

HARRISON, D. G., COFFMAN, T. M. & WILCOX, C. S. 2021. Pathophysiology of hypertension: the mosaic theory and beyond. *Circulation research,* 128**,** 847-863.

HART, E. C. 2016. Human hypertension, sympathetic activity and the selfish brain. *Experimental Physiology,* 101**,** 1451-1462.

HARTMAN, Y. A., HOPMAN, M. T., SCHREUDER, T. H., VERHEGGEN, R. J., SCHOLTEN, R. R., OUDEGEEST-SANDER, M. H., POELKENS, F., MAIORANA, A. J., NAYLOR, L. H. & WILLEMS, P. H. 2018. Improvements in fitness are not obligatory for exercise training-induced improvements in CV risk factors. *Physiological reports,* 6**,** e13595.

HARTWICH, D., FOWLER, K. L., WYNN, L. J. & FISHER, J. P. 2010. Differential responses to sympathetic stimulation in the cerebral and brachial circulations during rhythmic handgrip exercise in humans. *Experimental physiology,* 95**,** 1089-1097.

HARVEY, P. J., MORRIS, B. L., KUBO, T., PICTON, P. E., SU, W. S., NOTARIUS, C. F. & FLORAS, J. S. 2005. Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women. *Journal of hypertension,* 23**,** 285-292.

HASHIMOTO, M., KOZAKI, K., ETO, M., AKISHITA, M., AKO, J., IIJIMA, K., KIM, S., TOBA, K., YOSHIZUMI, M. & OUCHI, Y. 2000. Association of coronary risk factors and endothelium-dependent flow-mediated dilatation of the brachial artery. *Hypertension Research,* 23**,** 233-238.

HELLSTROM, G., FISCHER-COLBRIE, W., WAHLGREN, N. & JOGESTRAND, T. 1996. Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise. *Journal of Applied Physiology,* 81**,** 413-418.

HERNANZ, R., BRIONES, A. M., SALAICES, M. & ALONSO, M. J. 2014. New roles for old pathways? A circuitous relationship between reactive oxygen species and cyclo-oxygenase in hypertension. *Clinical science,* 126**,** 111-121.

HETZEL, J. R., BALLETSHOFER, B., RITTIG, K., WALCHER, D., KRATZER, W., HOMBACH, V., HÄRING, H.-U., KOENIG, W. & MARX, N. 2005. Rapid effects of rosiglitazone treatment on endothelial function and inflammatory biomarkers. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 25**,** 1804-1809.

HIGASHI, Y., SASAKI, S., KURISU, S., YOSHIMIZU, A., SASAKI, N., MATSUURA, H., KAJIYAMA, G. & OSHIMA, T. 1999. Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. *Circulation,* 100**,** 1194-1202.

HIGUCHI, S., OHTSU, H., SUZUKI, H., SHIRAI, H., FRANK, G. D. & EGUCHI, S. 2007. Angiotensin II signal transduction through the AT1 receptor: novel insights into mechanisms and pathophysiology. *Clinical science,* 112**,** 417-428.

HISHAM, N. F. & BAYRAKTUTAN, U. 2013. Epidemiology, pathophysiology, and treatment of hypertension in ischaemic stroke patients. *Journal of stroke and cerebrovascular diseases,* 22**,** e4-e14.

HIURA, M., MIZUNO, T. & FUJIMOTO, T. Cerebral oxygenation in the frontal lobe cortex during incremental exercise tests: the regional changes influenced by volitional exhaustion. Oxygen Transport to Tissue XXXI, 2010. Springer, 257-263.

HOILAND, R. L. & AINSLIE, P. N. 2016. CrossTalk proposal: The middle cerebral artery diameter does change during alterations in arterial blood gases and blood pressure. *The Journal of physiology,* 594**,** 4073.

HOILAND, R. L., SMITH, K. J., CARTER, H. H., LEWIS, N. C., TYMKO, M. M., WILDFONG, K. W., BAIN, A. R., GREEN, D. J. & AINSLIE, P. N. 2017. Shear-mediated dilation of the internal carotid artery occurs independent of hypercapnia. *American Journal of Physiology-Heart and Circulatory Physiology,* 313**,** H24-H31.

HOLDER, S. M., BRUNO, R. M., SHKREDOVA, D. A., DAWSON, E. A., JONES, H., HOPKINS, N. D., HOPMAN, M. T., BAILEY, T. G., COOMBES, J. S. & ASKEW, C. D. 2021. Reference intervals for brachial artery flow-mediated dilation and the relation with cardiovascular risk factors. *Hypertension,* 77**,** 1469-1480.

HOPKINS, N. D., DENGEL, D. R., STRATTON, G., KELLY, A. S., STEINBERGER, J., ZAVALA, H., MARLATT, K., PERRY, D., NAYLOR, L. H. & GREEN, D. J. 2015. Age and sex relationship with flow-mediated dilation in healthy children and adolescents. *Journal of Applied Physiology,* 119**,** 926-933.

HUANG, G., SHI, X., GIBSON, C. A., HUANG, S. C., COUDRET, N. A. & EHLMAN, M. C. 2013. Controlled aerobic exercise training reduces resting blood pressure in sedentary older adults. *Blood pressure,* 22**,** 386-394.

HUTCHEON, S., GILLESPIE, N., CROMBIE, I., STRUTHERS, A. & MCMURDO, M. 2002. Perindopril improves six minute walking distance in older patients with left ventricular systolic dysfunction: a randomised double blind placebo controlled trial. *Heart,* 88**,** 373-377.

INABA, Y., CHEN, J. A. & BERGMANN, S. R. 2010. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *The international journal of cardiovascular imaging,* 26**,** 631-640.

INTZANDT, B., SABRA, D., FOSTER, C., DESJARDINS-CRÉPEAU, L., HOGE, R. D., STEELE, C. J., BHERER, L. & GAUTHIER, C. J. 2020. Higher cardiovascular fitness level is associated with lower cerebrovascular reactivity and perfusion in healthy older adults. *Journal of Cerebral Blood Flow & Metabolism,* 40**,** 1468-1481.

ITO, H., KANNO, I., IBARAKI, M., HATAZAWA, J. & MIURA, S. 2003. Changes in human cerebral blood flow and cerebral blood volume during hypercapnia and hypocapnia measured by positron emission tomography. *Journal of Cerebral Blood Flow & Metabolism,* 23**,** 665-670.

IVEY, F. M., RYAN, A. S., HAFER-MACKO, C. E. & MACKO, R. F. 2011. Improved cerebral vasomotor reactivity after exercise training in hemiparetic stroke survivors. *Stroke,* 42**,** 1994-2000.

IWAMOTO, E., BOCK, J. M. & CASEY, D. P. 2018. High-intensity exercise enhances conduit artery vascular function in older adults. *Medicine and science in sports and exercise,* 50**,** 124.

JACKSON, W. F. 2005. Potassium channels and proliferation of vascular smooth muscle cells. Am Heart Assoc.

JARVIS, S. S., LEVINE, B. D., PRISK, G. K., SHYKOFF, B. E., ELLIOTT, A. R., ROSOW, E., BLOMQVIST, C. G. & PAWELCZYK, J. A. 2007. Simultaneous determination of the accuracy and precision of closed-circuit cardiac output rebreathing techniques. *Journal of applied physiology,* 103**,** 867-874.

JAY WIDMER, R. & LERMAN, A. 2014. Endothelial dysfunction and cardiovascular disease. *Global Cardiology Science and Practice,* 2014**,** 43.

JENNINGS, J., MULDOON, M., RYAN, C., PRICE, J., GREER, P., SUTTON-TYRRELL, K., VAN DER VEEN, F. & MELTZER, C. 2005. Reduced cerebral blood flow response and compensation among patients with untreated hypertension. *Neurology,* 64**,** 1358-1365.

JEONG, H. G., KIM, D. Y., KANG, D. W., KIM, B. J., KIM, C. K., KIM, Y., YANG, W., PARK, E. S. & LEE, S. H. 2017. Physical Activity Frequency and the Risk of Stroke: A Nationwide Cohort Study in Korea. *Journal of the American Heart Association,* 6.

JEONG, S.-W., KIM, S.-H., KANG, S.-H., KIM, H.-J., YOON, C.-H., YOUN, T.-J. & CHAE, I.-H. 2019. Mortality reduction with physical activity in patients with and without cardiovascular disease. *European heart journal,* 40**,** 3547-3555.

JOHANSSON, B. B. 1999. Hypertension mechanisms causing stroke. *Clinical and experimental pharmacology and physiology,* 26**,** 563-565.

JOHNSON, B. D., PADILLA, J. & WALLACE, J. P. 2012. The exercise dose affects oxidative stress and brachial artery flow-mediated dilation in trained men. *European journal of applied physiology,* 112**,** 33-42.

JONES, W. J., WILLIAMS, L. S., BRUNO, A. & BILLER, J. Hypertension and cerebrovascular disease. Seminars in cerebrovascular diseases and stroke, 2003. Elsevier, 144-154.

JORFELDT, L., JUHLIN-DANNFELT, A., PERNOW, B. & WASSEN, E. 1978. Determination of human leg blood flow: a thermodilution technique based on femoral venous bolus injection. *Clinical Science and Molecular Medicine,* 54**,** 517-523.

JOSEPH, G., MAROTT, J. L., TORP-PEDERSEN, C., BIERING-SØRENSEN, T., NIELSEN, G., CHRISTENSEN, A.-E., JOHANSEN, M. B., SCHNOHR, P., SOGAARD, P. & MOGELVANG, R. 2019. Dose-Response Association Between Level of Physical Activity and Mortality in Normal, Elevated, and High Blood Pressure. *Hypertension,* 74**,** 1307-1315.

JOYNER, M. J. & GREEN, D. J. 2009. Exercise protects the cardiovascular system: effects beyond traditional risk factors. *The Journal of physiology,* 587**,** 5551-5558.

JUNEJO, R. T., BRAZ, I. D., LUCAS, S. J., VAN LIESHOUT, J. J., LIP, G. Y. & FISHER, J. P. 2019. Impaired cerebrovascular reactivity in patients with atrial fibrillation. *Journal of the American College of Cardiology,* 73**,** 1230-1232.

JURVA, J. W., PHILLIPS, S. A., SYED, A. Q., SYED, A. Y., PITT, S., WEAVER, A. & GUTTERMAN, D. D. 2006. The effect of exertional hypertension evoked by weight lifting on vascular endothelial function. *Journal of the American College of Cardiology,* 48**,** 588-589.

KANNEL, W. B., WOLF, P. A., MCGEE, D. L., DAWBER, T. R., MCNAMARA, P. & CASTELLI, W. P. 1981. Systolic blood pressure, arterial rigidity, and risk of stroke: the Framingham study. *Jama,* 245**,** 1225-1229.

KATAYAMA, K., FUJITA, O., IEMITSU, M., KAWANO, H., IWAMOTO, E., SAITO, M. & ISHIDA, K. 2013. The effect of acute exercise in hypoxia on flow-mediated vasodilation. *European journal of applied physiology,* 113**,** 349-357.

KETEYIAN, S. J., LEIFER, E. S., HOUSTON-MILLER, N., KRAUS, W. E., BRAWNER, C. A., O'CONNOR, C. M., WHELLAN, D. J., COOPER, L. S., FLEG, J. L. & KITZMAN, D. W. 2012. Relation between volume of exercise and clinical outcomes in patients with heart failure. *Journal of the American College of Cardiology,* 60**,** 1899-1905.

KETY, S. S., HAFKENSCHIEL, J. H., JEFFERS, W. A., LEOPOLD, I. H. & SHENKIN, H. A. 1948. The blood flow, vascular resistance, and oxygen consumption of the brain in essential hypertension. *The Journal of clinical investigation,* 27**,** 511-514.

KETY, S. S. & SCHMIDT, C. F. 1948. The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure and normal values. *The Journal of clinical investigation,* 27**,** 476-483.

KIELY, D. K., WOLF, P. A., CUPPLES, L. A., BEISER, A. S. & KANNEL, W. B. 1994. Physical activity and stroke risk: the Framingham Study. *American journal of epidemiology,* 140**,** 608-620.

KIM, Y., SHARP, S., HWANG, S. & JEE, S. H. 2019. Exercise and incidence of myocardial infarction, stroke, hypertension, type 2 diabetes and site-specific cancers: prospective cohort study of 257 854 adults in South Korea. *BMJ open,* 9**,** e025590.

KIM, Y.-S., KROGH-MADSEN, R., RASMUSSEN, P., PLOMGAARD, P., OGOH, S., SECHER, N. H. & VAN LIESHOUT, J. J. 2007. Effects of hyperglycemia on the cerebrovascular response to rhythmic handgrip exercise. *American Journal of Physiology-Heart and Circulatory Physiology,* 293**,** H467-H473.

KISSNER, R., NAUSER, T., BUGNON, P., LYE, P. G. & KOPPENOL, W. H. 1997. Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis. *Chemical research in toxicology,* 10**,** 1285-1292.

KLEINLOOG, J. P., MENSINK, R. P., IVANOV, D., ADAM, J. J., ULUDAĞ, K. & JORIS, P. J. 2019. Aerobic exercise training improves cerebral blood flow and executive function: a randomized, controlled cross-over trial in sedentary older men. *Frontiers in aging neuroscience,* 11**,** 333.

KOCH, A., IVERS, M., GEHRT, A., SCHNOOR, P., RUMP, A. & RIECKERT, H. 2005. Cerebral autoregulation is temporarily disturbed in the early recovery phase after dynamic resistance exercise. *Clinical Autonomic Research,* 15**,** 83-91.

KODAMA, S., SAITO, K., TANAKA, S., MAKI, M., YACHI, Y., ASUMI, M., SUGAWARA, A., TOTSUKA, K., SHIMANO, H. & OHASHI, Y. 2009. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *Jama,* 301**,** 2024-2035.

KOEP, J. L., BARKER, A. R., BANKS, R., BANGER, R. R., LESTER, A., SANSUM, K. M., WESTON, M. E. & BOND, B. 2021. The acute and postprandial effects of sugar moiety on vascular and metabolic health outcomes in adolescents. *Applied Physiology, Nutrition, and Metabolism,* 46**,** 906-914.

KOEP, J. L., WESTON, M. E., BARKER, A. R., BAILEY, T. G., COOMBES, J. S., LESTER, A. & BOND, B. 2022. The within-and between-day reliability of cerebrovascular reactivity using traditional and novel analytical approaches. *Experimental Physiology,* 107**,** 29-41.

KOH, K. K., HAN, S. H., CHUNG, W.-J., AHN, J. Y., JIN, D. K., KIM, H. S., PARK, G. S., KANG, W. C., AHN, T. H. & SHIN, E. K. 2004. Comparison of effects of losartan, irbesartan, and candesartan on flow-mediated brachial artery dilation and on inflammatory and thrombolytic markers in patients with systemic hypertension. *The American journal of cardiology,* 93**,** 1432-1435.

KOKKINOS, P., CHRYSOHOOU, C., PANAGIOTAKOS, D., NARAYAN, P., GREENBERG, M. & SINGH, S. 2006. Beta-blockade mitigates exercise blood pressure in hypertensive male patients. *Journal of the American College of Cardiology,* 47**,** 794-798.

KONO, Y., KAWAJIRI, H., KAMISAKA, K., KAMIYA, K., AKAO, K., ASAI, C., INUZUKA, K. & YAMADA, S. 2015. Predictive impact of daily physical activity on new vascular events in patients with mild ischemic stroke. *International Journal of Stroke,* 10**,** 219-223.

KRAUS, W. E., POWELL, K. E., HASKELL, W. L., JANZ, K. F., CAMPBELL, W. W., JAKICIC, J. M., TROIANO, R. P., SPROW, K., TORRES, A. & PIERCY, K. L. 2019. Physical activity, all-cause

and cardiovascular mortality, and cardiovascular disease. *Medicine and science in sports and exercise,* 51**,** 1270.

LANDERS-RAMOS, R. Q., DONDERO, K., IMERY, I., REVEILLE, N., ZABRISKIE, H. A. & DOBROSIELSKI, D. A. 2023. Influence of cardiorespiratory fitness and body composition on resting and post-exercise indices of vascular health in young adults. *Sports Medicine and Health Science*.

LANG, E. W., LAGOPOULOS, J., GRIFFITH, J., YIP, K., MUDALIAR, Y., MEHDORN, H. M. & DORSCH, N. W. 2003. Noninvasive cerebrovascular autoregulation assessment in traumatic brain injury: validation and utility. *Journal of neurotrauma,* 20**,** 69-75.

LANGILLE, B. L. & O'DONNELL, F. 1986. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science,* 231**,** 405-407.

LASSEN, N. A. 1959. Cerebral blood flow and oxygen consumption in man. *Physiological reviews,* 39**,** 183-238.

LAURENT, S., BOUTOUYRIE, P. & LACOLLEY, P. 2005. Structural and genetic bases of arterial stiffness. *Hypertension,* 45**,** 1050-1055.

LAW, M. R., MORRIS, J. K. & WALD, N. 2009. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *Bmj,* 338.

LEE, C. D., FOLSOM, A. R. & BLAIR, S. N. 2003a. Physical activity and stroke risk: a meta-analysis. *Stroke,* 34**,** 2475-2481.

LEE, I.-M., SESSO, H. D., OGUMA, Y. & PAFFENBARGER JR, R. S. 2003b. Relative intensity of physical activity and risk of coronary heart disease. *Circulation,* 107**,** 1110-1116.

LEE, I.-M., SHIROMA, E. J., LOBELO, F., PUSKA, P., BLAIR, S. N. & KATZMARZYK, P. T. 2012. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *The lancet,* 380**,** 219-229.

LEUNG, F. P., YUNG, L. M., LAHER, I., YAO, X., CHEN, Z. Y. & HUANG, Y. 2008. Exercise, vascular wall and cardiovascular diseases: an update (Part 1). *Sports Medicine,* 38**,** 1009-1024.

LEWINGTON, S., CLARKE, R., QIZILBASH, N., PETO, R. & COLLINS, R. 2002. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet,* 360**,** 1903-13.

LEWINGTON, S., CLARKE, R., QIZILBASH, N., PETO, R. & COLLINS, R. 2003. Age-specific relevance of usual blood pressure to vascular mortality. *The Lancet,* 361**,** 1391-1392.

LEWIS, N., GELINAS, J. C., AINSLIE, P. N., SMIRL, J. D., AGAR, G., MELZER, B., ROLF, J. D. & EVES, N. D. 2019. Cerebrovascular function in patients with chronic obstructive pulmonary disease: the impact of exercise training. *American Journal of Physiology-Heart and Circulatory Physiology,* 316**,** H380-H391.

LI, P., LIU, Z., WAN, K., WANG, K., ZHENG, C. & HUANG, J. 2023. Effects of regular aerobic exercise on vascular function in overweight or obese older adults: A systematic review and meta-analysis. *Journal of Exercise Science & Fitness*.

LIPSITZ, L. A., MUKAI, S., HAMNER, J., GAGNON, M. & BABIKIAN, V. 2000. Dynamic regulation of middle cerebral artery blood flow velocity in aging and hypertension. *Stroke,* 31**,** 1897-1903.

LIU, J., TSENG, B. Y., KHAN, M. A., TARUMI, T., HILL, C., MIRSHAMS, N., HODICS, T. M., HYNAN, L. S. & ZHANG, R. 2016. Individual variability of cerebral autoregulation, posterior cerebral circulation and white matter hyperintensity. *The Journal of physiology,* 594**,** 3141-3155.

LIU, X., CZOSNYKA, M., DONNELLY, J., BUDOHOSKI, K. P., VARSOS, G. V., NASR, N., BRADY, K. M., REINHARD, M., HUTCHINSON, P. J. & SMIELEWSKI, P. 2015. Comparison of frequency and time domain methods of assessment of cerebral autoregulation in traumatic brain injury. *Journal of Cerebral Blood Flow & Metabolism,* 35**,** 248-256.

LONGHITANO, Y., IANNUZZI, F., BONATTI, G., ZANZA, C., MESSINA, A., GODOY, D., DABROWSKI, W., XIUYUN, L., CZOSNYKA, M. & PELOSI, P. 2021. Cerebral autoregulation in non-brain injured patients: a systematic review. *Frontiers in Neurology,* 12**,** 732176.

LUAN, X., TIAN, X., ZHANG, H., HUANG, R., LI, N., CHEN, P. & WANG, R. 2019. Exercise as a prescription for patients with various diseases. *Journal of sport and health science,* 8**,** 422-441.

LUCAS, S. J., COTTER, J. D., BRASSARD, P. & BAILEY, D. M. 2015. High-intensity interval exercise and cerebrovascular health: curiosity, cause, and consequence. *Journal of Cerebral Blood Flow & Metabolism,* 35**,** 902-911.

LUCAS, S. J., TZENG, Y. C., GALVIN, S. D., THOMAS, K. N., OGOH, S. & AINSLIE, P. N. 2010. Influence of changes in blood pressure on cerebral perfusion and oxygenation. *Hypertension,* 55**,** 698-705.

MACINTOSH, B. J., CRANE, D. E., SAGE, M. D., RAJAB, A. S., DONAHUE, M. J., MCILROY, W. E. & MIDDLETON, L. E. 2014. Impact of a single bout of aerobic exercise on regional brain perfusion and activation responses in healthy young adults. *PloS one,* 9**,** e85163.

MACLEOD, S., TERADA, T., CHAHAL, B. & BOULE, N. 2013. Exercise lowers postprandial glucose but not fasting glucose in type 2 diabetes: a meta-analysis of studies using continuous glucose monitoring. *Diabetes/metabolism research and reviews,* 29**,** 593-603.

MACMAHON, S. 1996. Blood pressure and the prevention of stroke. *Journal of hypertension. Supplement: official journal of the International Society of Hypertension,* 14**,** S39-46.

MAEDA, H., MATSUMOTO, M., HANDA, N., HOUGAKU, H., OGAWA, S., ITOH, T., TSUKAMOTO, Y. & KAMADA, T. 1994. Reactivity of cerebral blood flow to carbon dioxide in hypertensive patients: evaluation by the transcranial Doppler method. *Journal of hypertension,* 12**,** 191-198.

MAGYAR, M. T., VALIKOVICS, A., BERECZKI, D., FICZERE, A., CZURIGA, I. & CSIBA, L. 2001. Transcranial Doppler monitoring in hypertensive patients during physical exercise. *Cerebrovascular Diseases,* 12**,** 186-191.

MAIORANA, A., O'DRISCOLL, G., DEMBO, L., GOODMAN, C., TAYLOR, R. & GREEN, D. 2001. Exercise training, vascular function, and functional capacity in middle-aged subjects. *Medicine and science in sports and exercise,* 33**,** 2022-2028.

MAIORANA, A., O'DRISCOLL, G., TAYLOR, R. & GREEN, D. 2003. Exercise and the nitric oxide vasodilator system. *Sports Medicine,* 33**,** 1013-1035.

MAIORANA, A. J., NAYLOR, L. H., EXTERKATE, A., SWART, A., THIJSSEN, D. H., LAM, K., O'DRISCOLL, G. & GREEN, D. J. 2011. The impact of exercise training on conduit artery wall thickness and remodeling in chronic heart failure patients. *Hypertension,* 57**,** 56-62.

MARK, A. 1996. The sympathetic nervous system in hypertension: a potential long-term regulator of arterial pressure. *Journal of hypertension. Supplement: official journal of the International Society of Hypertension,* 14**,** S159-65.

MARKUS, H. & CULLINANE, M. 2001. Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. *Brain,* 124**,** 457-467.

MARSDEN, K., HAYKOWSKY, M., SMIRL, J., JONES, H., NELSON, M., ALTAMIRANO-DIAZ, L. A., GELINAS, J., TZENG, Y., SMITH, K. & WILLIE, C. 2012. Aging blunts hyperventilation-induced hypocapnia and reduction in cerebral blood flow velocity during maximal exercise. *Age,* 34**,** 725-735.

MARTÍNEZ-REVELLES, S., AVENDAÑO, M. S., GARCÍA-REDONDO, A. B., ALVAREZ, Y., AGUADO, A., PÉREZ-GIRÓN, J. V., GARCÍA-REDONDO, L., ESTEBAN, V., REDONDO, J. M. & ALONSO, M. J. 2013. Reciprocal relationship between reactive oxygen species and cyclooxygenase-2 and vascular dysfunction in hypertension. *Antioxidants & redox signaling,* 18**,** 51-65.

MASSIDDA, M., SCORCU, M. & CALÒ, C. M. 2014. New genetic model for predicting phenotype traits in sports. *International journal of sports physiology and performance,* 9**,** 554-560.

MATSUMOTO, T., OZONO, R., OSHIMA, T., MATSUURA, H., SUEDA, T., KAJIYAMA, G. & KAMBE, M. 2000. Type 2 angiotensin II receptor is downregulated in cardiomyocytes of patients with heart failure. *Cardiovascular research,* 46**,** 73-81.

MATSUZAWA, Y., KWON, T. G., LENNON, R. J., LERMAN, L. O. & LERMAN, A. 2015. Prognostic value of flow-mediated vasodilation in brachial artery and fingertip artery for cardiovascular events: a systematic review and meta-analysis. *Journal of the American Heart Association,* 4**,** e002270.

MCGOWAN, C. L., LEVY, A. S., MILLAR, P. J., GUZMAN, J. C., MORILLO, C. A., MCCARTNEY, N. & MACDONALD, M. J. 2006. Acute vascular responses to isometric handgrip exercise and effects of training in persons medicated for hypertension. *American Journal of Physiology-Heart and Circulatory Physiology,* 291**,** H1797-H1802.

MEISSNER, A. 2016. Hypertension and the brain: a risk factor for more than heart disease. *Cerebrovascular diseases,* 42**,** 255-262.

MENSAH, G. A., ROTH, G. A. & FUSTER, V. 2019. The global burden of cardiovascular diseases and risk factors: 2020 and beyond. American College of Cardiology Foundation Washington, DC.

MEZZANO, S. A., RUIZ-ORTEGA, M. & EGIDO, J. 2001. Angiotensin II and renal fibrosis. *Hypertension,* 38**,** 635-638.

MIKUS, C. R., BOYLE, L. J., BORENGASSER, S. J., OBERLIN, D. J., NAPLES, S. P., FLETCHER, J., MEERS, G. M., RUEBEL, M., LAUGHLIN, M. H. & DELLSPERGER, K. C. 2013. Simvastatin impairs exercise training adaptations. *Journal of the American College of Cardiology,* 62**,** 709-714.

MILLER, A. A., BUDZYN, K. & SOBEY, C. G. 2010. Vascular dysfunction in cerebrovascular disease: mechanisms and therapeutic intervention. *Clinical science,* 119**,** 1-17.

MILLER, G., MAXWELL, J., THOMPSON, A., CABLE, N., LOW, D., GEORGE, K. & JONES, H. 2022. The effects of exercise training in the cold on cerebral blood flow and cerebrovascular function in young healthy individuals. *Autonomic Neuroscience,* 238**,** 102945.

MILLER, V. M. & DUCKLES, S. P. 2008. Vascular actions of estrogens: functional implications. *Pharmacological reviews,* 60**,** 210-241.

MILLS, K. T., STEFANESCU, A. & HE, J. 2020. The global epidemiology of hypertension. *Nature Reviews Nephrology,* 16**,** 223-237.

MINSHALL, R. D., NAKAMURA, F., BECKER, R. P. & RABITO, S. F. 1995. Characterization of bradykinin B2 receptors in adult myocardium and neonatal rat cardiomyocytes. *Circulation research,* 76**,** 773-780.

MITTLEMAN, M. A., MACLURE, M., TOFLER, G. H., SHERWOOD, J. B., GOLDBERG, R. J. & MULLER, J. E. 1993. Triggering of acute myocardial infarction by heavy physical exertion--protection against triggering by regular exertion. *New England Journal of Medicine,* 329**,** 1677-1683.

MIYAI, N., ARITA, M., MORIOKA, I., MIYASHITA, K., NISHIO, I. & TAKEDA, S. 2000. Exercise BP response in subjects with high-normal BP: exaggerated blood pressure response to exercise and risk of future hypertension in subjects with high-normal blood pressure. *Journal of the American College of Cardiology,* 36**,** 1626-1631.

MOCHIN, M. T., UNDERWOOD, K. F., COOPER, B., MCLENITHAN, J. C., PIERCE, A. D., NALVARTE, C., ARBISER, J., KARLSSON, A. I., MOISE, A. R. & MOSKOVITZ, J. 2015. Hyperglycemia and redox status regulate RUNX2 DNA-binding and an angiogenic phenotype in endothelial cells. *Microvascular Research,* 97**,** 55-64.

MOHOLDT, T., WISLØFF, U., NILSEN, T. I. L. & SLØRDAHL, S. A. 2008. Physical activity and mortality in men and women with coronary heart disease: a prospective population-based cohort study in Norway (the HUNT study). *European Journal of Preventive Cardiology,* 15**,** 639-645.

MONCADA, S. & HIGGS, E. 2006. The discovery of nitric oxide and its role in vascular biology. *British journal of pharmacology,* 147**,** S193-S201.

MONCION, K., ALLISON, E. Y., AL-KHAZRAJI, B. K., MACDONALD, M. J., ROIG, M. & TANG, A. 2022. What are the effects of acute exercise and exercise training on cerebrovascular hemodynamics following stroke? A systematic review and meta-analysis. *Journal of Applied Physiology,* 132**,** 1379-1393.

MONS, U., HAHMANN, H. & BRENNER, H. 2014. A reverse J-shaped association of leisure time physical activity with prognosis in patients with stable coronary heart disease: evidence from a large cohort with repeated measurements. *Heart,* 100**,** 1043-1049.

MOORE, S. C., PATEL, A. V., MATTHEWS, C. E., DE GONZALEZ, A. B., PARK, Y., KATKI, H. A., LINET, M. S., WEIDERPASS, E., VISVANATHAN, K. & HELZLSOUER, K. J. 2012. Leisure time physical activity of moderate to vigorous intensity and mortality: a large pooled cohort analysis. *PLoS medicine,* 9**,** e1001335.

MORA, S., COOK, N., BURING, J. E., RIDKER, P. M. & LEE, I.-M. 2007. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation,* 116**,** 2110-2118.

MORAINE, J.-J., LAMOTTE, M., BERRÉ, J., NISET, G., LEDUC, A. & NAEIJEL, R. 1993. Relationship of middle cerebral artery blood flow velocity to intensity during dynamic exercise in normal subjects. *European journal of applied physiology and occupational physiology,* 67**,** 35-38.

MORALEZ, G., ROMERO, S. A., RICKARDS, C. A., RYAN, K. L., CONVERTINO, V. A. & COOKE, W. H. 2012. Effects of dehydration on cerebrovascular control during standing after heavy resistance exercise. *Journal of applied physiology,* 112**,** 1875-1883.

MORIGUCHI, J., ITOH, H., HARADA, S., TAKEDA, K., HATTA, T., NAKATA, T. & SASAKI, S. 2005. Low frequency regular exercise improves flow-mediated dilatation of subjects with mild hypertension. *Hypertension research,* 28**,** 315-321.

MULSER, L. & MOREAU, D. 2023. Effect of acute cardiovascular exercise on cerebral blood flow: A systematic review. *Brain Research***,** 148355.

MULVANY, M. & AALKJAER, C. 1990. Structure and function of small arteries. *Physiological reviews,* 70**,** 921-961.

MUNIR, S., JIANG, B., GUILCHER, A., BRETT, S., REDWOOD, S., MARBER, M. & CHOWIENCZYK, P. 2008. Exercise reduces arterial pressure augmentation through vasodilation of muscular arteries in humans. *American Journal of Physiology-Heart and Circulatory Physiology,* 294**,** H1645-H1650.

MÜNZEL, T., DAIBER, A., ULLRICH, V. & MÜLSCH, A. 2005. Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. *Arteriosclerosis, thrombosis, and vascular biology,* 25**,** 1551-1557.

MUOIO, V., PERSSON, P. & SENDESKI, M. 2014. The neurovascular unit–concept review. *Acta physiologica,* 210**,** 790-798.

MURRAY, E., GUZIK, T. & DELLES, C. 2021. Early hypertension: exploring the vascular phenotype. *Journal of Hypertension,* 39**,** e44-e45.

MURRELL, C., COTTER, J. D., GEORGE, K., SHAVE, R., WILSON, L., THOMAS, K., WILLIAMS, M. J., LOWE, T. & AINSLIE, P. N. 2009. Influence of age on syncope following prolonged exercise: differential responses but similar orthostatic intolerance. *The Journal of Physiology,* 587**,** 5959-5969.

MURRELL, C., WILSON, L., COTTER, J. D., LUCAS, S., OGOH, S., GEORGE, K. & AINSLIE, P. N. 2007. Alterations in autonomic function and cerebral hemodynamics to orthostatic challenge following a mountain marathon. *Journal of applied physiology,* 103**,** 88-96.

MURRELL, C. J., COTTER, J. D., THOMAS, K. N., LUCAS, S. J., WILLIAMS, M. J. & AINSLIE, P. N. 2013. Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: effect of age and 12-week exercise training. *Age,* 35**,** 905-920.

NAYLOR, L. H., DAVIS, E. A., KALIC, R. J., PARAMALINGAM, N., ABRAHAM, M. B., JONES, T. W. & GREEN, D. J. 2016. Exercise training improves vascular function in adolescents with type 2 diabetes. *Physiological reports,* 4**,** e12713.

NEUNTEUFL, T., HEHER, S., KATZENSCHLAGER, R., WÖLFL, G., KOSTNER, K., MAURER, G. & WEIDINGER, F. 2000. Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. *American Journal of Cardiology,* 86**,** 207-210.

NIEBAUER, J. & COOKE, J. P. 1996. Cardiovascular effects of exercise: role of endothelial shear stress. *Journal of the American College of Cardiology,* 28**,** 1652-1660.

NISHIMURA, N., SCHAFFER, C. B., FRIEDMAN, B., LYDEN, P. D. & KLEINFELD, D. 2007. Penetrating arterioles are a bottleneck in the perfusion of neocortex. *Proceedings of the National Academy of Sciences,* 104**,** 365-370.

NUMAN, T., BAIN, A. R., HOILAND, R. L., SMIRL, J. D., LEWIS, N. C. & AINSLIE, P. N. 2014. Static autoregulation in humans: a review and reanalysis. *Medical engineering & physics,* 36**,** 1487-1495.

NUR, E., KIM, Y.-S., TRUIJEN, J., VAN BEERS, E. J., DAVIS, S. C., BRANDJES, D. P., BIEMOND, B. J. & VAN LIESHOUT, J. J. 2009. Cerebrovascular reserve capacity is impaired in patients with sickle cell disease. *Blood, The Journal of the American Society of Hematology,* 114**,** 3473-3478.

OEINCK, M., NEUNHOEFFER, F., BUTTLER, K.-J., MECKEL, S., SCHMIDT, B., CZOSNYKA, M., WEILLER, C. & REINHARD, M. 2013. Dynamic cerebral autoregulation in acute intracerebral hemorrhage. *Stroke,* 44**,** 2722-2728.

OGOH, S. & AINSLIE, P. N. 2009. Cerebral blood flow during exercise: mechanisms of regulation. *Journal of applied physiology,* 107**,** 1370-1380.

OGOH, S., BROTHERS, R. M., BARNES, Q., EUBANK, W. L., HAWKINS, M. N., PURKAYASTHA, S., O-YURVATI, A. & RAVEN, P. B. 2005a. The effect of changes in cardiac output on middle

cerebral artery mean blood velocity at rest and during exercise. *The Journal of physiology,* 569**,** 697-704.

OGOH, S., DALSGAARD, M., SECHER, N. & RAVEN, P. 2007a. Dynamic blood pressure control and middle cerebral artery mean blood velocity variability at rest and during exercise in humans. *Acta physiologica,* 191**,** 3-14.

OGOH, S., DALSGAARD, M. K., YOSHIGA, C. C., DAWSON, E. A., KELLER, D. M., RAVEN, P. B. & SECHER, N. H. 2005b. Dynamic cerebral autoregulation during exhaustive exercise in humans. *American Journal of Physiology-Heart and Circulatory Physiology,* 288**,** H1461-H1467.

OGOH, S., FISHER, J. P., FADEL, P. J. & RAVEN, P. B. 2007b. Increases in central blood volume modulate carotid baroreflex resetting during dynamic exercise in humans. *The Journal of Physiology,* 581**,** 405-418.

OGOH, S., HAYASHI, N., INAGAKI, M., AINSLIE, P. N. & MIYAMOTO, T. 2008. Interaction between the ventilatory and cerebrovascular responses to hypo-and hypercapnia at rest and during exercise. *The Journal of physiology,* 586**,** 4327-4338.

OKAMOTO, T., MASUHARA, M. & IKUTA, K. 2006. Cardiovascular responses induced during high-intensity eccentric and concentric isokinetic muscle contraction in healthy young adults. *Clinical physiology and functional imaging,* 26**,** 39-44.

OKAMOTO, T., MASUHARA, M. & IKUTA, K. 2008. Relationship between plasma endothelin-1 concentration and cardiovascular responses during high-intensity eccentric and concentric exercise. *Clinical physiology and functional imaging,* 28**,** 43-48.

OLIVO, G., NILSSON, J., GARZÓN, B., LEBEDEV, A., WÅHLIN, A., TARASSOVA, O., EKBLOM, M. & LÖVDÉN, M. 2021. Immediate effects of a single session of physical exercise on cognition and cerebral blood flow: A randomized controlled study of older adults. *Neuroimage,* 225**,** 117500.

OPARIL, S., ZAMAN, M. A. & CALHOUN, D. A. 2003. Pathogenesis of hypertension. *Annals of internal medicine,* 139**,** 761-776.

ORGANISATION, W. H. 2023. *Hypertension* [Online]. World Health Organisation Available: https://www.who.int/news-room/fact-sheets/detail/hypertension [Accessed 6 December 2023].

OSBORN, J. W., FINK, G. D., SVED, A. F., TONEY, G. M. & RAIZADA, M. K. 2007. Circulating angiotensin II and dietary salt: converging signals for neurogenic hypertension. *Current hypertension reports,* 9**,** 228-235.

ØSTERGÅRD, T., NYHOLM, B., HANSEN, T. K., RASMUSSEN, L. M., INGERSLEV, J., SØRENSEN, K. E., BØTKER, H. E., SALTIN, B. & SCHMITZ, O. 2006. Endothelial function and biochemical vascular markers in first-degree relatives of type 2 diabetic patients: the effect of exercise training. *Metabolism,* 55**,** 1508-1515.

PADILLA, J., HARRIS, R. A., FLY, A. D., RINK, L. D. & WALLACE, J. P. 2006. The effect of acute exercise on endothelial function following a high-fat meal. *European journal of applied physiology,* 98**,** 256-262.

PAGANO, P. J., CLARK, J. K., CIFUENTES-PAGANO, M. E., CLARK, S. M., CALLIS, G. M. & QUINN, M. T. 1997. Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II. *Proceedings of the National Academy of Sciences,* 94**,** 14483-14488.

PAGE, I. H. 1949. Pathogenesis of arterial hypertension. *Journal of the American Medical Association,* 140**,** 451-458.

PAGE, I. H. 1963. The Nature of Arterial Hypertension: The Eduardo Braun-Menendez Memorial Lecture. *Archives of internal medicine,* 111**,** 103-115.

PANERAI, R., RENNIE, J., KELSALL, A. & EVANS, D. 1998. Frequency-domain analysis of cerebral autoregulation from spontaneous fluctuations in arterial blood pressure. *Medical and Biological Engineering and Computing,* 36**,** 315-322.

PANERAI, R. B. 1998. Assessment of cerebral pressure autoregulation in humans-a review of measurement methods. *Physiological measurement,* 19**,** 305.

PAULSON, O. 1990. Strandgaard S, Edvinsson L. Cerebral autoregulation. *Cerebrovasc Brain Metab Reviews,* 2**,** 161-192.

PAULSON, O., STRANDGAARD, S. & EDVINSSON, L. 1990. Cerebral autoregulation. *Cerebrovascular and brain metabolism reviews,* 2**,** 161-192.

PAVY-LE TRAON, A., COSTES-SALON, M.-C., GALINIER, M., FOURCADE, J. & LARRUE, V. 2002. Dynamics of cerebral blood flow autoregulation in hypertensive patients. *Journal of the neurological sciences,* 195**,** 139-144.

PAYNE, S. 2016. *Cerebral autoregulation: control of blood flow in the brain*, Springer.

PEDRALLI, M. L., MARSCHNER, R. A., KOLLET, D. P., NETO, S. G., EIBEL, B., TANAKA, H. & LEHNEN, A. M. 2020. Different exercise training modalities produce similar endothelial function improvements in individuals with prehypertension or hypertension: A randomized clinical trial. *Scientific reports,* 10**,** 7628.

PERDOMO, S. J., BALZER, J. R., JAKICIC, J. M., KLINE, C. E. & GIBBS, B. B. 2019. Acute effects of aerobic exercise duration on blood pressure, pulse wave velocity and cerebral blood flow velocity in middle-aged adults. *Sport Sciences for Health,* 15**,** 647-658.

PESCATELLO, L. S., MACDONALD, H. V., ASH, G. I., LAMBERTI, L. M., FARQUHAR, W. B., ARENA, R. & JOHNSON, B. T. Assessing the existing professional exercise recommendations for hypertension: a review and recommendations for future research priorities. Mayo Clinic Proceedings, 2015. Elsevier, 801-812.

PESCATELLO, L. S., WU, Y., GAO, S., LIVINGSTON, J., SHEPPARD, B. B. & CHEN, M.-H. 2021. Do the combined blood pressure effects of exercise and antihypertensive medications add up to the sum of their parts? A systematic meta-review. *BMJ Open Sport & Exercise Medicine,* 7**,** e000895.

PETTY, R. G. & PEARSON, J. D. 1989. Endothelium—the axis of vascular health and disease. *Journal of the Royal College of Physicians of London,* 23**,** 92.

PHILLIPS, A. A., CHAN, F. H., ZHENG, M. M. Z., KRASSIOUKOV, A. V. & AINSLIE, P. N. 2016. Neurovascular coupling in humans: physiology, methodological advances and clinical implications. *Journal of Cerebral Blood Flow & Metabolism,* 36**,** 647-664.

PIENIAŻEK, W., DIMITROW, P. P. & JASIŃSKI, T. 2001. Comparison of the effect of perindopril and acebutolol on cerebral hemodynamics in hypertensive patients. *Cardiovascular drugs and therapy,* 15**,** 63-67.

PIEPOLI, M. F., HOES, A. W., AGEWALL, S., ALBUS, C., BROTONS, C., CATAPANO, A. L., COONEY, M.-T., CORRÀ, U. & COSYNS, B. 2016. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *European Journal of Preventive Cardiology,* 23**,** NP1-NP96.

PINCKARD K, B. K., STANFORD KI 2019. Exercise to Improve Cardiovascular Health. *Front Cardiovasc Med,* 4.

POHL, U., HOLTZ, J., BUSSE, R. & BASSENGE, E. 1986. Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension,* 8**,** 37-44.

POLLOCK, M. L., LOWENTHAL, D. T., FOSTER, C., PELS III, A. E., ROD, J., STOIBER, J. & SCHMIDT, D. H. 1991. Acute and chronic responses to exercise in patients treated with beta blockers. *Journal of Cardiopulmonary Rehabilitation and Prevention,* 11**,** 132-144.

PORTEGIES, M. L., DE BRUIJN, R. F., HOFMAN, A., KOUDSTAAL, P. J. & IKRAM, M. A. 2014. Cerebral vasomotor reactivity and risk of mortality: the Rotterdam Study. *Stroke,* 45**,** 42-47.

POZNYAK, A. V., SADYKHOV, N. K., KARTUESOV, A. G., BORISOV, E. E., MELNICHENKO, A. A., GRECHKO, A. V. & OREKHOV, A. N. 2022. Hypertension as a risk factor for atherosclerosis: Cardiovascular risk assessment. *Frontiers in Cardiovascular Medicine,* 9**,** 959285.

PSATY, B. M., LUMLEY, T., FURBERG, C. D., SCHELLENBAUM, G., PAHOR, M., ALDERMAN, M. H. & WEISS, N. S. 2003. Health outcomes associated with various antihypertensive therapies used as first-line agents: a network meta-analysis. *Jama,* 289**,** 2534-2544.

PYKE, K. E. & TSCHAKOVSKY, M. E. 2007. Peak vs. total reactive hyperemia: which determines the magnitude of flow-mediated dilation? *Journal of applied physiology,* 102**,** 1510-1519.

RAS, R. T., STREPPEL, M. T., DRAIJER, R. & ZOCK, P. L. 2013. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. *International journal of cardiology,* 168**,** 344-351.

REINHARD, M., ROTH, M., MULLER, T., CZOSNYKA, M., TIMMER, J. & HETZEL, A. 2003. Cerebral autoregulation in carotid artery occlusive disease assessed from spontaneous blood pressure fluctuations by the correlation coefficient index. *Stroke,* 34**,** 2138-2144.

RIZZONI, D., DE CIUCEIS, C., PORTERI, E., PAIARDI, S., BOARI, G. E., MORTINI, P., CORNALI, C., CENZATO, M., RODELLA, L. F. & BORSANI, E. 2009. Altered structure of small cerebral arteries in patients with essential hypertension. *Journal of hypertension,* 27**,** 838-845.

ROBERTSON, A. D., CRANE, D. E., RAJAB, A. S., SWARDFAGER, W., MARZOLINI, S., SHIRZADI, Z., MIDDLETON, L. E. & MACINTOSH, B. J. 2015. Exercise intensity modulates the change in cerebral blood flow following aerobic exercise in chronic stroke. *Experimental brain research,* 233**,** 2467-2475.

ROBINSON, B. F., EPSTEIN, S. E., BEISER, G. D. & BRAUNWALD, E. 1966. Control of heart rate by the autonomic nervous system: studies in man on the interrelation between baroreceptor mechanisms and exercise. *Circulation Research,* 19**,** 400-411.

ROGNMO, Ø., BJØRNSTAD, T. H., KAHRS, C., TJØNNA, A. E., BYE, A., HARAM, P. M., STØLEN, T., SLØRDAHL, S. A. & WISLØFF, U. 2008. Endothelial function in highly endurance-trained men: effects of acute exercise. *The Journal of Strength & Conditioning Research,* 22**,** 535-542.

ROMERO, S. A. & COOKE, W. H. 2007. Hyperventilation before resistance exercise: cerebral hemodynamics and orthostasis. *Medicine and science in sports and exercise,* 39**,** 1302.

ROSS, R. 1999. Atherosclerosis—an inflammatory disease. *New England journal of medicine,* 340**,** 115-126.

ROTH, G. A., MENSAH, G. A. & FUSTER, V. 2020. The global burden of cardiovascular diseases and risks: a compass for global action. American College of Cardiology Foundation Washington DC.

RUIZ-ORTEGA, M., LORENZO, O., SUZUKI, Y., RUPÉREZ, M. & EGIDO, J. 2001. Proinflammatory actions of angiotensins. *Current opinion in nephrology and hypertension,* 10**,** 321-329.

RULAND, S. & AIYAGARI, V. 2007. Cerebral autoregulation and blood pressure lowering. Am Heart Assoc.

SADOSHIMA, J. 2000. Cytokine actions of angiotensin II. *Circulation research,* 86**,** 1187-1189.
SARKAR, S., GHOSH, S., GHOSH, S. K. & COLLIER, A. 2007. Role of transcranial Doppler ultrasonography in stroke. *Postgraduate Medical Journal,* 83**,** 683-689.
SAVIN, W. M., GORDON, E. P., KAPLAN, S. M., HEWITT, B. F., HARRISON, D. C. & HASKELL, W. L. 1985. Exercise training during long-term beta-blockade treatment in healthy subjects. *The American journal of cardiology,* 55**,** D101-D109.
SAVOIA, C., BURGER, D., NISHIGAKI, N., MONTEZANO, A. & TOUYZ, R. M. 2011. Angiotensin II and the vascular phenotype in hypertension. *Expert reviews in molecular medicine,* 13**,** e11.
SCHEER, A. S., IR DE OLIVEIRA, B., SHAH, A., JACQUES, A., CHASLAND, L. C., GREEN, D. J. & MAIORANA, A. J. 2023. The effects of water-based circuit exercise training on vascular function in people with coronary heart disease. *American Journal of Physiology-Heart and Circulatory Physiology,* 325**,** H1386-H1393.
SCHIFFRIN, E. L. 2006. Effects of aldosterone on the vasculature. *Hypertension,* 47**,** 312-318.
SCHOLTENS, S., SMIDT, N., SWERTZ, M. A., BAKKER, S. J., DOTINGA, A., VONK, J. M., VAN DIJK, F., VAN ZON, S. K., WIJMENGA, C. & WOLFFENBUTTEL, B. H. 2015. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *International journal of epidemiology,* 44**,** 1172-1180.
SCHREUDER, T. H., GREEN, D. J., NYAKAYIRU, J., HOPMAN, M. T. & THIJSSEN, D. H. 2015. Time-course of vascular adaptations during 8 weeks of exercise training in subjects with type 2 diabetes and middle-aged controls. *European journal of applied physiology,* 115**,** 187-196.
SCHULTZ, M. G. & SHARMAN, J. E. 2014. Exercise hypertension. *Pulse,* 1**,** 161-176.
SEALS, D. R., JABLONSKI, K. L. & DONATO, A. J. 2011. Aging and vascular endothelial function in humans. *Clinical science,* 120**,** 357-375.
SERRADOR, J. M., PICOT, P. A., RUTT, B. K., SHOEMAKER, J. K. & BONDAR, R. L. 2000. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke,* 31**,** 1672-1678.
SETTAKIS, G., MOLNÁR, C., KERÉNYI, L., KOLLÁR, J., LEGEMATE, D., CSIBA, L. & FÜLESDI, B. 2003a. Acetazolamide as a vasodilatory stimulus in cerebrovascular diseases and in conditions affecting the cerebral vasculature. *European Journal of Neurology,* 10**,** 609-620.
SETTAKIS, G., PÁLL, D., MOLNÁR, C., BERECZKI, D., CSIBA, L. & FÜLESDI, B. 2003b. Cerebrovascular reactivity in hypertensive and healthy adolescents: TCD with vasodilatory challenge. *Journal of Neuroimaging,* 13**,** 106-112.
SICARD, K. M. & DUONG, T. Q. 2005. Effects of hypoxia, hyperoxia, and hypercapnia on baseline and stimulus-evoked BOLD, CBF, and CMRO2 in spontaneously breathing animals. *Neuroimage,* 25**,** 850-858.
SILVA, J. K. T., MENÊSES, A. L., PARMENTER, B. J., RITTI-DIAS, R. M. & FARAH, B. Q. 2021. Effects of resistance training on endothelial function: a systematic review and meta-analysis. *Atherosclerosis,* 333**,** 91-99.
SILVESTRO, A., SCOPACASA, F., OLIVA, G., DE CRISTOFARO, T., IULIANO, L. & BREVETTI, G. 2002. Vitamin C prevents endothelial dysfunction induced by acute exercise in patients with intermittent claudication. *Atherosclerosis,* 165**,** 277-283.
SIMPSON, D. & CLAASSEN, J. 2018. CrossTalk opposing view: dynamic cerebral autoregulation should be quantified using induced (rather than spontaneous) blood pressure fluctuations. *The Journal of physiology,* 596**,** 7.

SINGH, R.-J., CHEN, S., GANESH, A. & HILL, M. D. 2018. Long-term neurological, vascular, and mortality outcomes after stroke. *International Journal of Stroke,* 13**,** 787-796.

SISCOVICK, D. S., WEISS, N. S., FLETCHER, R. H. & LASKY, T. 1984. The incidence of primary cardiac arrest during vigorous exercise. *New England Journal of Medicine,* 311**,** 874-877.

SJÚRÐARSON, T., BEJDER, J., BREENFELDT ANDERSEN, A., BONNE, T., KYHL, K., RÓIN, T., PATURSSON, P., ODDMARSDÓTTIR GREGERSEN, N., SKORADAL, M. B. & SCHLIEMANN, M. 2022. Effect of angiotensin-converting enzyme inhibition on cardiovascular adaptation to exercise training. *Physiological Reports,* 10**,** e15382.

SMITH, E. C., PIZZEY, F. K., ASKEW, C. D., MIELKE, G. I., AINSLIE, P. N., COOMBES, J. S. & BAILEY, T. G. 2021. Effects of cardiorespiratory fitness and exercise training on cerebrovascular blood flow and reactivity: a systematic review with meta-analyses. *American Journal of Physiology-Heart and Circulatory Physiology,* 321**,** H59-H76.

SMITH, K., MACLEOD, D., WILLIE, C., LEWIS, N., HOILAND, R., IKEDA, K., TYMKO, M., DONNELLY, J., DAY, T. & MACLEOD, N. 2014. Influence of high altitude on cerebral blood flow and fuel utilization during exercise and recovery. *The Journal of physiology,* 592**,** 5507-5527.

SMITH, K. J. & AINSLIE, P. N. 2017. Regulation of cerebral blood flow and metabolism during exercise. *Experimental physiology,* 102**,** 1356-1371.

SMITH, K. J., HOILAND, R. L., GROVE, R., MCKIRDY, H., NAYLOR, L., AINSLIE, P. N. & GREEN, D. J. 2019. Matched increases in cerebral artery shear stress, irrespective of stimulus, induce similar changes in extra-cranial arterial diameter in humans. *Journal of Cerebral Blood Flow & Metabolism,* 39**,** 849-858.

SMYTH, A., O'DONNELL, M., LAMELAS, P., TEO, K., RANGARAJAN, S. & YUSUF, S. 2016. Physical activity and anger or emotional upset as triggers of acute myocardial infarction: the INTERHEART study. *Circulation,* 134**,** 1059-1067.

SORENSEN, K., CELERMAJER, D., GEORGAKOPOULOS, D., HATCHER, G., BETTERIDGE, D. & DEANFIELD, J. 1994. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein (a) level. *The Journal of clinical investigation,* 93**,** 50-55.

SORESCU, D., WEISS, D., LASSÈGUE, B., CLEMPUS, R. E., SZÖCS, K., SORESCU, G. P., VALPPU, L., QUINN, M. T., LAMBETH, J. D. & VEGA, J. D. 2002. Superoxide production and expression of nox family proteins in human atherosclerosis. *Circulation,* 105**,** 1429-1435.

SPIEGELBERG, B. D. 2013. G protein coupled-receptor signaling and reversible lysine acetylation. *Journal of Receptors and Signal Transduction,* 33**,** 261-266.

SPIRA, D., WALSTON, J., BUCHMANN, N., NIKOLOV, J., DEMUTH, I., STEINHAGEN-THIESSEN, E., ECKARDT, R. & NORMAN, K. 2016. Angiotensin-converting enzyme inhibitors and parameters of sarcopenia: relation to muscle mass, strength and function: data from the Berlin Aging Study-II (BASE-II). *Drugs & aging,* 33**,** 829-837.

SS MEEL-VAN DEN ABEELEN, A., LAGRO, J., HEA VAN BEEK, A. & AHR CLAASSEN, J. 2014. Impaired cerebral autoregulation and vasomotor reactivity in sporadic Alzheimer's disease. *Current Alzheimer Research,* 11**,** 11-17.

STATISTICS NETHERLANDS. 2020. *Statistics Netherlands Data and Biobank* [Online]. Available: https://www.lifelines.nl/researcher [Accessed 12th January 2024].

STEVENS, E. G. V., EMMETT, E. S., WANG, Y., MCKEVITT, C. J. & WOLFE, C. D. A. 2017. The Burden of Stroke in Europe: Overview of stroke burden and care in each EU and SAFE member country.

STEWART, K. J., SUNG, J., SILBER, H. A., FLEG, J. L., KELEMEN, M. D., TURNER, K. L., BACHER, A. C., DOBROSIELSKI, D. A., DEREGIS, J. R. & SHAPIRO, E. P. 2004. Exaggerated exercise blood pressure is related to impaired endothelial vasodilator function. *American journal of hypertension,* 17**,** 314-320.

STEWART, R. A., HELD, C., HADZIOSMANOVIC, N., ARMSTRONG, P. W., CANNON, C. P., GRANGER, C. B., HAGSTRÖM, E., HOCHMAN, J. S., KOENIG, W. & LONN, E. 2017. Physical activity and mortality in patients with stable coronary heart disease. *Journal of the American College of Cardiology,* 70**,** 1689-1700.

STOLK, R. P., ROSMALEN, J. G., POSTMA, D. S., DE BOER, R. A., NAVIS, G., SLAETS, J. P., ORMEL, J. & WOLFFENBUTTEL, B. H. 2008. Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. *European journal of epidemiology,* 23**,** 67-74.

STRANDGAARD, S. & PAULSON, O. 1995. Cerebral blood flow in untreated and treated hypertension. *The Netherlands journal of medicine,* 47**,** 180-184.

SU, J. B., BARBE, F., CROZATIER, B., CAMPBELL, D. J. & HITTINGER, L. 1999. Increased bradykinin levels accompany the hemodynamic response to acute inhibition of angiotensin-converting enzyme in dogs with heart failure. *Journal of cardiovascular pharmacology,* 34**,** 700-710.

SUDHIR, K., CHOU, T. M., HUTCHISON, S. J. & CHATTERJEE, K. 1996. Coronary vasodilation induced by angiotensin-converting enzyme inhibition in vivo: differential contribution of nitric oxide and bradykinin in conductance and resistance arteries. *Circulation,* 93**,** 1734-1739.

SUMUKADAS, D., BAND, M., MILLER, S., CVORO, V., WITHAM, M., STRUTHERS, A., MCCONNACHIE, A., LLOYD, S. M. & MCMURDO, M. 2014. Do ACE inhibitors improve the response to exercise training in functionally impaired older adults? A randomized controlled trial. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences,* 69**,** 736-743.

SUMUKADAS, D., WITHAM, M. D., STRUTHERS, A. D. & MCMURDO, M. E. 2007. Effect of perindopril on physical function in elderly people with functional impairment: a randomized controlled trial. *Cmaj,* 177**,** 867-874.

SUN, Y., ZHANG, J., LU, L., CHEN, S. S., QUINN, M. T. & WEBER, K. T. 2002. Aldosterone-induced inflammation in the rat heart: role of oxidative stress. *The American journal of pathology,* 161**,** 1773-1781.

TABET, F., SCHIFFRIN, E. L., CALLERA, G. E., HE, Y., YAO, G., OSTMAN, A., KAPPERT, K., TONKS, N. K. & TOUYZ, R. M. 2008. Redox-sensitive signaling by angiotensin II involves oxidative inactivation and blunted phosphorylation of protein tyrosine phosphatase SHP-2 in vascular smooth muscle cells from SHR. *Circulation research,* 103**,** 149-158.

TADDEI, S., VIRDIS, A., GHIADONI, L., SUDANO, I. & SALVETTI, A. 2001. Endothelial dysfunction in hypertension. *Journal of cardiovascular pharmacology,* 38**,** S11-S14.

TADDEI, S., VIRDIS, A., MATTEI, P., GHIADONI, L., GENNARI, A., FASOLO, C. B., SUDANO, I. & SALVETTI, A. 1995. Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation,* 91**,** 1981-1987.

TAJIRI, N., ZHANG, H., LEE, J.-Y. & BORLONGAN, C. 2019. A brief physical activity protects against ischemic stroke. *Brain Circulation,* 5**,** 112.

TAKAC, I., SCHRÖDER, K. & BRANDES, R. P. 2012. The Nox family of NADPH oxidases: friend or foe of the vascular system? *Current hypertension reports,* 14**,** 70-78.

TAKASE, B., UEHATA, A., AKIMA, T., NAGAI, T., NISHIOKA, T., HAMABE, A., SATOMURA, K., OHSUZU, F. & KURITA, A. 1998. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. *American Journal of Cardiology,* 82**,** 1535-1539.

TAKEDA, S., SATO, N., TAKEUCHI, D., KURINAMI, H., SHINOHARA, M., NIISATO, K., KANO, M., OGIHARA, T., RAKUGI, H. & MORISHITA, R. 2009. Angiotensin receptor blocker prevented β-amyloid-induced cognitive impairment associated with recovery of neurovascular coupling. *Hypertension,* 54**,** 1345-1352.

TAN, C. O. 2012. Defining the characteristic relationship between arterial pressure and cerebral flow. *Journal of applied physiology,* 113**,** 1194-1200.

TANNE, D., FREIMARK, D., POREH, A., MERZELIAK, O., BRUCK, B., SCHWAMMENTHAL, Y., SCHWAMMENTHAL, E., MOTRO, M. & ADLER, Y. 2005. Cognitive functions in severe congestive heart failure before and after an exercise training program. *International journal of cardiology,* 103**,** 145-149.

TAYLOR, R. S., UNAL, B., CRITCHLEY, J. A. & CAPEWELL, S. 2006. Mortality reductions in patients receiving exercise-based cardiac rehabilitation: how much can be attributed to cardiovascular risk factor improvements? *European Journal of Preventive Cardiology,* 13**,** 369-374.

THIJSSEN, D., DE GROOT, P., SMITS, P. & HOPMAN, M. 2007. Vascular adaptations to 8-week cycling training in older men. *Acta physiologica,* 190**,** 221-228.

THIJSSEN, D. H., BLACK, M. A., PYKE, K. E., PADILLA, J., ATKINSON, G., HARRIS, R. A., PARKER, B., WIDLANSKY, M. E., TSCHAKOVSKY, M. E. & GREEN, D. J. 2011. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *American Journal of Physiology-Heart and Circulatory Physiology,* 300**,** H2-H12.

THIJSSEN, D. H., BRUNO, R. M., VAN MIL, A. C., HOLDER, S. M., FAITA, F., GREYLING, A., ZOCK, P. L., TADDEI, S., DEANFIELD, J. E. & LUSCHER, T. 2019. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *European heart journal,* 40**,** 2534-2547.

THIJSSEN, D. H., CABLE, N. T. & GREEN, D. J. 2012. Impact of exercise training on arterial wall thickness in humans. *Clinical science,* 122**,** 311-322.

THIJSSEN, D. H., DE GROOT, P., KOOIJMAN, M., SMITS, P. & HOPMAN, M. T. 2006. Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. *American Journal of Physiology-Heart and Circulatory Physiology,* 291**,** H3122-H3129.

THIJSSEN, D. H., REDINGTON, A., GEORGE, K. P., HOPMAN, M. T. & JONES, H. 2018. Association of exercise preconditioning with immediate cardioprotection: a review. *JAMA cardiology,* 3**,** 169-176.

THOMAS, B. P., YEZHUVATH, U. S., TSENG, B. Y., LIU, P., LEVINE, B. D., ZHANG, R. & LU, H. 2013. Life-long aerobic exercise preserved baseline cerebral blood flow but reduced vascular reactivity to CO2. *Journal of Magnetic Resonance Imaging,* 38**,** 1177-1183.

TIECKS, F. P., LAM, A. M., AASLID, R. & NEWELL, D. W. 1995. Comparison of static and dynamic cerebral autoregulation measurements. *Stroke,* 26**,** 1014-1019.

TINKEN, T. M., THIJSSEN, D. H., BLACK, M. A., CABLE, N. T. & GREEN, D. J. 2008. Time course of change in vasodilator function and capacity in response to exercise training in humans. *The Journal of physiology,* 586**,** 5003-5012.

TINKEN, T. M., THIJSSEN, D. H., HOPKINS, N., BLACK, M. A., DAWSON, E. A., MINSON, C. T., NEWCOMER, S. C., LAUGHLIN, M. H., CABLE, N. T. & GREEN, D. J. 2009. Impact of shear rate modulation on vascular function in humans. *Hypertension,* 54**,** 278-285.

TINKEN, T. M., THIJSSEN, D. H., HOPKINS, N., DAWSON, E. A., CABLE, N. T. & GREEN, D. J. 2010. Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension,* 55**,** 312-318.

TOUYZ, R. M., RIOS, F. J., ALVES-LOPES, R., NEVES, K. B., CAMARGO, L. L. & MONTEZANO, A. C. 2020. Oxidative stress: a unifying paradigm in hypertension. *Canadian journal of cardiology,* 36**,** 659-670.

TRYFONOS, A., COCKS, M., RASOUL, D., MILLS, J., GREEN, D. J. & DAWSON, E. A. 2020. Impact of catheterization on shear-mediated arterial dilation in healthy young men. *European Journal of Applied Physiology,* 120**,** 2525-2532.

TYLDUM, G. A., SCHJERVE, I. E., TJØNNA, A. E., KIRKEBY-GARSTAD, I., STØLEN, T. O., RICHARDSON, R. S. & WISLØFF, U. 2009. Endothelial dysfunction induced by post-prandial lipemia: complete protection afforded by high-intensity aerobic interval exercise. *Journal of the American College of Cardiology,* 53**,** 200-206.

TZENG, Y.-C. & AINSLIE, P. N. 2014. Blood pressure regulation IX: cerebral autoregulation under blood pressure challenges. *European journal of applied physiology,* 114**,** 545-559.

USHIO-FUKAI, M. 2009. Vascular signaling through G protein coupled receptors-new concepts. *Current opinion in nephrology and hypertension,* 18**,** 153.

VALENZUELA RUIZ, P. L., CARRERA BASTOS, P., GONZÁLEZ GÁLVEZ, B., RUIZ HURTADO, G., ORDOVÁS MUÑOZ, J. M., RUILOPE URIOSTE, L. M. & LUCÍA MULAS, A. 2020. Lifestyle interventions for the prevention and treatment of hypertension.

VAN BEEK, A. H., CLAASSEN, J. A., RIKKERT, M. G. O. & JANSEN, R. W. 2008. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *Journal of Cerebral Blood Flow & Metabolism,* 28**,** 1071-1085.

VENKETASUBRAMANIAN, N., YOON, B. W., PANDIAN, J. & NAVARRO, J. C. 2017. Stroke epidemiology in south, east, and south-east asia: A review. *Journal of stroke,* 19**,** 286.

VERBREE, J., BRONZWAER, A.-S. G., GHARIQ, E., VERSLUIS, M. J., DAEMEN, M. J., VAN BUCHEM, M. A., DAHAN, A., VAN LIESHOUT, J. J. & VAN OSCH, M. J. 2014. Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. *Journal of applied physiology,* 117**,** 1084-1089.

VITA, J. A. & KEANEY JR, J. F. 2002. Endothelial function: a barometer for cardiovascular risk? : Am Heart Assoc.

VLACHOPOULOS, C., O'ROURKE, M. & NICHOLS, W. W. 2011. *McDonald's blood flow in arteries: theoretical, experimental and clinical principles*, CRC press.

VUCKOVIC, K. M., PIANO, M. R. & PHILLIPS, S. A. 2013. Effects of exercise interventions on peripheral vascular endothelial vasoreactivity in patients with heart failure with reduced ejection fraction. *Heart, Lung and Circulation,* 22**,** 328-340.

WALSH, J. H., BILSBOROUGH, W., MAIORANA, A., BEST, M., O'DRISCOLL, G. J., TAYLOR, R. R. & GREEN, D. J. 2003. Exercise training improves conduit vessel function in patients with coronary artery disease. *Journal of Applied Physiology,* 95**,** 20-25.

WANG, Y., LI, M., DONG, F., ZHANG, J. & ZHANG, F. 2015. Physical exercise-induced protection on ischemic cardiovascular and cerebrovascular diseases. *International journal of clinical and experimental medicine,* 8**,** 19859.

WANNAMETHEE, G. & SHAPER, A. G. 1992. Physical activity and stroke in British middle aged men. *BMJ,* 304**,** 597-601.

WANNAMETHEE, S. G., SHAPER, A. G. & WALKER, M. 2000. Physical activity and mortality in older men with diagnosed coronary heart disease. *Circulation,* 102**,** 1358-1363.

WEI, E. P., KONTOS, H. A. & PATTERSON JR, J. L. 1980. Dependence of pial arteriolar response to hypercapnia on vessel size. *American Journal of Physiology-Heart and Circulatory Physiology,* 238**,** H697-H702.

WENDEL-VOS, G. W., SCHUIT, A. J., SARIS, W. H. & KROMHOUT, D. 2003. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *Journal of clinical epidemiology,* 56**,** 1163-1169.

WESTHOFF, T., FRANKE, N., SCHMIDT, S., VALLBRACHT-ISRANG, K., ZIDEK, W., DIMEO, F. & VAN DER GIET, M. 2007. Beta-blockers do not impair the cardiovascular benefits of endurance training in hypertensives. *Journal of human hypertension,* 21**,** 486-493.

WESTON, K. S., WISLØFF, U. & COOMBES, J. S. 2014. High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *British journal of sports medicine,* 48**,** 1227-1234.

WESTON, M. E., KOEP, J. L., LESTER, A. B., BARKER, A. R. & BOND, B. 2022. The acute effect of exercise intensity on peripheral and cerebral vascular function in healthy adults. *Journal of Applied Physiology,* 133**,** 461-470.

WHANG, W., MANSON, J. E., HU, F. B., CHAE, C. U., REXRODE, K. M., WILLETT, W. C., STAMPFER, M. J. & ALBERT, C. M. 2006. Physical exertion, exercise, and sudden cardiac death in women. *Jama,* 295**,** 1399-1403.

WHELTON, P. K., CAREY, R. M., ARONOW, W. S., CASEY, D. E., COLLINS, K. J., DENNISON HIMMELFARB, C., DEPALMA, S. M., GIDDING, S., JAMERSON, K. A. & JONES, D. W. 2018. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Journal of the American College of Cardiology,* 71**,** e127-e248.

WHITE, P. C. 1994. Disorders of aldosterone biosynthesis and action. *New England Journal of Medicine,* 331**,** 250-258.

WILLIAMS, P. T. & THOMPSON, P. D. Increased cardiovascular disease mortality associated with excessive exercise in heart attack survivors. Mayo Clinic Proceedings, 2014. Elsevier, 1187-1194.

WILLIAMSON, J. W., MCCOLL, R. & MATHEWS, D. 2004. Changes in regional cerebral blood flow distribution during postexercise hypotension in humans. *Journal of applied physiology,* 96**,** 719-724.

WILLIAMSON, J. W., QUERRY, R., MCCOLL, R. & MATHEWS, D. 2009. Are decreases in insular regional cerebral blood flow sustained during postexercise hypotension? *Medicine & Science in Sports & Exercise,* 41**,** 574-580.

WILLICH, S. N., LEWIS, M., LOWEL, H., ARNTZ, H.-R., SCHUBERT, F. & SCHRODER, R. 1993. Physical exertion as a trigger of acute myocardial infarction. *New England Journal of Medicine,* 329**,** 1684-1690.

WILLIE, C., COLINO, F., BAILEY, D., TZENG, Y., BINSTED, G., JONES, L., HAYKOWSKY, M., BELLAPART, J., OGOH, S. & SMITH, K. 2011. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *Journal of neuroscience methods,* 196**,** 221-237.

WILLIE, C., MACLEOD, D., SHAW, A., SMITH, K., TZENG, Y.-C., EVES, N., IKEDA, K., GRAHAM, J., LEWIS, N. & DAY, T. 2012. Regional brain blood flow in man during acute changes in arterial blood gases. *The Journal of physiology,* 590**,** 3261-3275.

WILLIE, C. & SMITH, K. 2011. Fuelling the exercising brain: a regulatory quagmire for lactate metabolism. *The Journal of physiology,* 589**,** 779.

WILLIE, C. K., AINSLIE, P. N., TAYLOR, C. E., EVES, N. D. & TZENG, Y.-C. 2013. Maintained cerebrovascular function during post-exercise hypotension. *European journal of applied physiology,* 113**,** 1597-1604.

WILLIE, C. K., TZENG, Y. C., FISHER, J. A. & AINSLIE, P. N. 2014. Integrative regulation of human brain blood flow. *The Journal of physiology,* 592**,** 841-859.

WILMORE, J. H., EWY, G. A., FREUND, B. J., HARTZELL, A. A., JILKA, S. M., JOYNER, M. J., TODD, C. A., KINZER, S. M. & PEPIN, E. B. 1985. Cardiorespiratory alterations consequent to endurance exercise training during chronic beta-adrenergic blockade with atenolol and propranolol. *The American journal of cardiology,* 55**,** D142-D148.

WILSON, J. R. & FERRARO, N. 1985. Effect of the renin-angiotensin system on limb circulation and metabolism during exercise in patients with heart failure. *Journal of the American College of Cardiology,* 6**,** 556-563.

WONG, W. T., TIAN, X. Y. & HUANG, Y. 2013. Endothelial dysfunction in diabetes and hypertension: cross talk in RAS, BMP4, and ROS-dependent COX-2–derived prostanoids. *Journal of cardiovascular pharmacology,* 61**,** 204-214.

WOODMAN, R., PLAYFORD, D., WATTS, G., CHEETHAM, C., REED, C., TAYLOR, R., PUDDEY, I., BEILIN, L., BURKE, V. & MORI, T. 2001. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *Journal of applied physiology,* 91**,** 929-937.

WORLD HEALTH ORGANISATION. 2021. *Cardiovascular Diseases* [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) [Accessed 15th January 2024].

WRIGHT, J. M., MUSINI, V. M. & GILL, R. 2018. First-line drugs for hypertension. *Cochrane Database of systematic reviews*.

YAO, F., LIU, Y., LIU, D., WU, S., LIN, H., FAN, R. & LI, C. 2014. Sex differences between vascular endothelial function and carotid intima-media thickness by Framingham Risk Score. *Journal of Ultrasound in Medicine,* 33**,** 281-286.

YU, J. G., ZHOU, R. R. & CAI, G. J. 2011. From hypertension to stroke: mechanisms and potential prevention strategies. *CNS neuroscience & therapeutics,* 17**,** 577-584.

ZHANG, R., ZUCKERMAN, J. H., GILLER, C. A. & LEVINE, B. D. 1998. Transfer function analysis of dynamic cerebral autoregulation in humans. *American Journal of Physiology-Heart and Circulatory Physiology,* 274**,** H233-H241.

ZHANG, W., HUANG, Q., ZENG, Z., WU, J., ZHANG, Y. & CHEN, Z. 2017. Sirt1 inhibits oxidative stress in vascular endothelial cells. *Oxidative Medicine and Cellular Longevity,* 2017.

ZHANG, Y., ZHANG, Y.-J., ZHANG, H.-W., YE, W.-B. & KORIVI, M. 2021. Low-to-moderate-intensity resistance exercise is more effective than high-intensity at improving endothelial function in adults: a systematic review and meta-analysis. *International Journal of Environmental Research and Public Health,* 18**,** 6723.

ZHOU, B., CARRILLO-LARCO, R. M., DANAEI, G., RILEY, L. M., PACIOREK, C. J., STEVENS, G. A., GREGG, E. W., BENNETT, J. E., SOLOMON, B., SINGLETON, R. K., SOPHIEA, M. K., IURILLI, M. L., LHOSTE, V. P., COWAN, M. J., SAVIN, S., WOODWARD, M., BALANOVA, Y., CIFKOVA, R., DAMASCENO, A., ELLIOTT, P., FARZADFAR, F., HE, J., IKEDA, N.,

KENGNE, A. P., KHANG, Y.-H., KIM, H. C., LAXMAIAH, A., LIN, H.-H., MARGOZZINI MAIRA, P., MIRANDA, J. J., NEUHAUSER, H., SUNDSTRÖM, J., VARGHESE, C., WIDYAHENING, I. S., ZDROJEWSKI, T., ABARCA-GÓMEZ, L., ABDEEN, Z. A., ABDUL RAHIM, H. F., ABU-RMEILEH, N. M., ACOSTA-CAZARES, B., ADAMS, R. J., AEKPLAKORN, W., AFSANA, K., AFZAL, S., AGDEPPA, I. A., AGHAZADEH-ATTARI, J., AGUILAR-SALINAS, C. A., AGYEMANG, C., AHMAD, N. A., AHMADI, A., AHMADI, N., AHMADI, N., AHMADIZAR, F., AHMED, S. H., AHRENS, W., AJLOUNI, K., AL-RADDADI, R., ALAROUJ, M., ALBUHAIRAN, F., ALDHUKAIR, S., ALI, M. M., ALKANDARI, A., ALKERWI, A. A., ALLIN, K., ALY, E., AMARAPURKAR, D. N., AMOUGOU, N., AMOUYEL, P., ANDERSEN, L. B., ANDERSSEN, S. A., ANJANA, R. M., ANSARI-MOGHADDAM, A., ANSONG, D., AOUNALLAH-SKHIRI, H., ARAÚJO, J., ARIANSEN, I., ARIS, T., ARKU, R. E., ARLAPPA, N., ARYAL, K. K., ASPELUND, T., ASSAH, F. K., ASSUNÇÃO, M. C. F., AUVINEN, J., AVDIĆOVÁ, M., AZEVEDO, A., AZIMI-NEZHAD, M., AZIZI, F., AZMIN, M., BABU, B. V., BAHIJRI, S., BALAKRISHNA, N., BAMOSHMOOSH, M., BANACH, M., BANADINOVIĆ, M., BANDOSZ, P., BANEGAS, J. R., BARAN, J., BARBAGALLO, C. M., BARCELÓ, A., et al. 2021. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. *The Lancet,* 398**,** 957-980.

ZHU, W., ZENG, J., YIN, J., ZHANG, F., WU, H., YAN, S. & WANG, S. 2010. Both flow-mediated vasodilation procedures and acute exercise improve endothelial function in obese young men. *European journal of applied physiology,* 108**,** 727-732.

ZI, M., CARMICHAEL, N. & LYE, M. 2003. The effect of quinapril on functional status of elderly patients with diastolic heart failure. *Cardiovascular drugs and therapy,* 17**,** 133-139.