

# **DETERMINANTS OF VASCULAR HEALTH IN YOUNG PEOPLE**

**NICOLA DOMINIQUE HOPKINS**

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

**April 2010**

# **LIVERPOOL JOHN MOORES UNIVERSITY**

## **Candidate's declaration form**

Name of candidate: Nicola Hopkins

School: School of Sport and Exercise Sciences

Degree for which thesis is submitted: Doctor of Philosophy (PhD)

**1. Statement of related studies undertaken in connection with the programme of research**

I have undertaken a programme of related studies which developed my research skills, provided a greater understanding of the research process and helped improve my competence in undertaking of a research project.

**2. Concurrent registration for two or more academic awards**

I declare that while registered as a candidate for the University's research degree, I have not been a registered candidate or enrolled student for another award of the LJMU or other academic or professional institution

**3. Material submitted for another award**

I declare that the following material contained in the thesis formed part of a submission for the award of Master of Science (Sports Physiology), Liverpool John Moores University. A small proportion of data from study 2 was used within the Dissertation for the MSc programme. In the current study we measured flow mediated dilation, body composition, fitness, physical activity and sedentary behaviour in 145 children. In the MSc flow mediated dilation, fitness and body composition were measured in 70 children.

Signed..... Date .....  
(Candidate)



## Abstract

In recent years the prevalence of obesity, physical inactivity, sedentary behaviour and low cardiorespiratory fitness in Western countries has increased rapidly. These variables are independently associated with cardiovascular disease risk and/or mortality. Atherosclerosis begins in childhood and endothelial dysfunction is its earliest detectable manifestation. Endothelial dys/function can be quantified using flow mediated dilation (FMD).

The primary aim of this thesis was to investigate childhood associations between endothelial function and a range of modifiable and non-modifiable CV risk factors. We sought to provide novel information regarding relationships between physical activity, sedentary behaviour, body composition, cardiorespiratory fitness and the heritability of endothelial function. In addition, we sought to explore the issue of the scaling of vascular outcomes for body size and composition.

Study one (Chapter 4) addressed the question of scaling, by evaluating the efficacy of scaling vascular dimensions and FMD for different body composition variables using allometric and ratio procedures, with the aim of producing size independent vascular indices. Our data indicate that, if between-group comparisons of baseline brachial artery diameter are to be undertaken, appropriate, allometric scaling for measures of lean or total mass may need to be adopted. The application of such scaling to FMD data is debatable as the associations between FMD and body composition variables were very weak. We therefore concluded that the adoption of scaling FMD for body composition variables cannot be advocated until further research has been undertaken.

Studies 2 and 4 (Chapters 5 and 7) assessed cross-sectional relationships between FMD and modifiable CV risk factors in young people. We examined associations between FMD and objective measurements of body composition, cardiorespiratory fitness, physical activity levels and sedentary behaviour. We observed a weak association between percentage body fat and FMD and no further relationships across cohort. However, depressed endothelial function was significantly related to, and predicted by high intensity physical activity. The relationship between FMD and high intensity PA was further reinforced by the findings from studies 3 and 4 (Chapters 6 and 7), which aimed to address the issue of seasonal variation in FMD and determine its predictors. The studies demonstrated that seasonal decline in vascular function was associated with, and predicted by, a change in high intensity PA but no other variables. These findings demonstrate, for the first time, that high-intensity PA may be an important determinant of vascular dys/function in children. The ramifications of these findings are that interventions aimed at improving vascular health in children may need to be refocused to bring about a progressive increase in physical activity, specifically high intensity physical activity, rather than reducing obesity or sedentary time *per se*.

Finally, studies 5 and 6 (Chapters 8 and 9) utilised a classic twin study design to explore the role of genetics in the modulation of FMD. Through the comparison of intra-twin pair differences in mono- and di-zygotic twins, we were able to provide information relating to the genetic influence on FMD and calculate a heritability estimate. We concluded that, although a one-off measurement of endothelial function is under some level of genetic control, environmental factors may have a larger influence in the determination of FMD in young people (study 5).

In study 6, twins undertook 8 weeks of aerobic exercise training. In this study, intra-twin differences in the adaptation of FMD were compared to changes in other variables. The results highlighted a greater similarity between MZ twins than DZ twins in the change in FMD, suggesting that exercise-induced improvements in FMD may be highly genetically determined.

Taken together, the findings of this thesis infer that, whilst a genetic predisposition to endothelial dysfunction may exist, interventions that aim to increase high intensity physical activity have the potential to enhance vascular health in young people at risk of endothelial impairment and future development of atherosclerotic diseases.



## **Acknowledgements**

I would first of all like to thank Professor Danny Green, for all his encouragement, guidance and (sometimes over-) enthusiasm over the last 3 years, I am extremely grateful. He have been an inspiration, albeit from the other side of the world! I would like to thank my supervisor Professor Gareth Stratton for his support, advice and for managing to fit our thought provoking meetings into his hectic schedule. Thanks to Professor Tim Cable for his contribution and advice. And finally thank you to my surrogate supervisor, Professor Keith George, for helping me out in Danny's absence, and for dragging me through allometric scaling.

I would like to thank Liverpool City Council Lifestyles Gyms and all the staff for their help implementing the exercise training programme, without their help the intervention would not have been possible. Thanks to all the twins and their families for agreeing to participate in the study.

Thanks to all my friends and family. A super huge thanks to my mum and dad, for their encouragement, support and love over the years (and all the ££!!). Thanks to my brothers for always keeping me grounded, whether I want to be or not. And finally thanks to Lee, my lovely assistant, for all his love, advice and for keeping me laughing. Great.

### **Declaration**

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

### **Publications and communications directly and indirectly associated with this thesis.**

#### ***Publications directly based on the work contained in this thesis***

1. **Hopkins, N.D.** G. Stratton, T.M. Tinken, N. McWhannell, N.D. Ridgers, L.E.F. Graves, K. George, N.T. Cable and D.J. Green. Relationships between measures of fitness, physical activity, body composition and vascular function in children. *Atherosclerosis*. 204: 244-249, 2009.
2. **Hopkins ND**, Green DJ, Tinken TM, Sutton L, McWhannell N, Thijssen DHJ, Cable NT, Stratton G, George K. Does conduit artery diameter vary according to the anthropometric characteristics of children or men? *American Journal of Physiology*. 297, 2182-2187, 2009.
3. **Hopkins ND**, Green DJ, Tinken TM, Sutton L, McWhannell N, Thijssen DHJ, Cable NT, Stratton G, George K. Does brachial artery flow-mediated dilation scale to anthropometric characteristics? *Accepted at the European Journal of Applied Physiology 2010*
4. **Hopkins, N.D.** G. Stratton, T.M. Tinken, N. Ridgers, L.E.F. Graves, N.T. Cable and D.J. Green. Seasonal reduction in physical activity and flow mediated dilation in children. *Under 2<sup>nd</sup> review at Medicine and Science in Sports and Exercise*.
5. **Hopkins, N.D.** G. Stratton, T.M. Tinken, J. Maia, L.E.F. Graves, N.T. Cable and D.J. Green. Heritability of arterial function, fitness and physical activity in youth: A study of mono- and di-zygotic twins. *Under 2<sup>nd</sup> review at Journal of Pediatrics*.

***Submitted articles directly based on the work contained in this thesis***

1. **Hopkins, N.D.** G. Stratton, T.N Cable and D.J. Green. Impact of exercise training on body composition in youth: A study of mono- and di-zygotic twins. *Submitted to the Journal of Applied Physiology, March 2010.*
2. **Hopkins, N.D.** G. Stratton, T.M. Tinken, J. Maia, L.E.F. Graves, N.T. Cable and D.J. Green. Impact of exercise training on endothelial function, and fitness in young people: A study of mono- and di-zygotic twins. *Submitted to Arteriosclerosis, Thrombosis and Vascular Biology, March 2010.*
3. **Hopkins, N.D.** G. Stratton, T.M. Tinken, N. Ridgers, L.E.F. Graves, N.T. Cable and D.J. Green. Lack of relationship between sedentary behaviour and vascular health in children. *Submitted to European Journal of Cardiovascular Prevention and Rehabilitation*

***Other publications completed by the candidate during PhD tenure***

1. Thijssen, D.H.J., L.M. Bullens, M.M. van Bommel, E.A. Dawson, **N. Hopkins**, T.M. Tinken, M.A. Black, M.T.E. Hopman, N.T. Cable, and D.J. Green. Does arterial shear explain the magnitude of flow-mediated dilation? A comparison between young and older humans. *Am J Physiol Heart Circ Physiol.* 296: H57-H64, 2009.
2. Tinken, T.M., D.H.J. Thijssen, **N.D. Hopkins**, M.A. Black, E.A. Dawson, C.T. Minson, S.C. Newcomer, M.H. Laughlin, N.T. Cable, and D.J. Green. Impact of shear rate modulation on vascular function in humans. *Hypertension* 54(2): 278-285.



3. Tinken, T.M., D.H.J. Thijssen, **N.D. Hopkins**, E.A. Dawson, N.T. Cable, and D.J. Green. Manipulation of shear stress during exercise training modifies artery function and remodelling in humans. *Submitted to Circulation*. 2009
4. Thijssen, D.H.J., M.M. van Bommel, L.M. Bullens, E.A. Dawson, **N.D. Hopkins**, T.M. Tinken, M.A. Black, M.T.E. Hopman, N.T. Cable, and D.J. Green. The impact of baseline diameter on flow-mediated dilation differs in young and older humans. *Am J Physiol Heart Circ Physiol* 295: H1594-H1598, 2008.
5. Dawson, E.A, G.P. Whyte, M.M. Black, H Jones, **N.D. Hopkins**, D Oxborough, D Gaze, R.E. Shave, M Wilson, K.P. George, D.J. Green. Changes in vascular and cardiac function after prolonged strenuous exercise in humans. *J Appl Physiol* 105 (5): 1562-1568, 2008.
6. Atkinson, G, A.M. Batterham, M.A. Black, N.T. Cable, **N.D. Hopkins**, E.A. Dawson, D.H.J. Thijssen, H Jones, T.M. Tinken, and D.J. Green. Is the ratio of flow-mediated dilation (FMD) and shear rate a statistically-sound approach to normalization? *The Journal of Applied Physiology* 107: 1893-1899, 2009.
7. T.M. Tinken; D.H.J. Thijssen; **N.D. Hopkins**; E.A. Dawson; N.T. Cable; D.J. Green. Shear Stress Mediates Endothelial Adaptations to Exercise Training in Humans. *Hypertension* 55: 312-318, 2010.

### ***Oral communications***

1. European College of Sports Science, ECSS, Portugal, JULY 2008; **Hopkins, N.D.** G. Stratton, T.M. Tinken, N. McWhannell, N.D. Ridgers, L.E.F. Graves, K. George, N.T. Cable and D.J. Green. Relationships between measures of fitness, physical activity, body composition and vascular function in children.
2. European College of Sports Science, ECSS, Norway, June 2009; **Hopkins, N.D.** G. Stratton, T.M. Tinken, N. Ridgers, L.E.F. Graves, N.T. Cable and D.J. Green. High Intensity Physical Activity Correlates with Improvement in Vascular Function in 9-11 Year Olds.
3. PWP, France, September 2009; **Hopkins, N.D.** G. Stratton, T.M. Tinken, N. Ridgers, L.E.F. Graves, N.T. Cable and D.J. Green. Association Between High Intensity Physical Activity and Vascular Function in 9-11 Year Olds. ***Prize received for oral presentation.***
4. American College of Sport Medicine (ACSM) Annual Conference, Seattle, USA, 2009; Tinken, T.M., D.H.J. Thijssen, **N.D. Hopkins**, M.A. Black, E.A. Dawson, C.T. Minson, S.C. Newcomer, M.H. Laughlin, N.T. Cable, and D.J. Green. Impact of shear rate modulation on vascular function in humans. Presented at
5. European College of Sports Science (ECSS), Norway, 2009; Atkinson, G., A.M. Batterham, N.T. Cable, **N.D. Hopkins**, E.A. Dawson, D.H.J. Thijssen, H. Jones, T.M. Tinken, D.J. Green. Is the ratio of flow-mediated dilation (FMD) and shear rate a statistically-sound approach to normalisation?

### ***Poster communications***

1. Faculty of Health Research Day, LJMU May 2009; **Hopkins, N.D.** G. Stratton, T.M. Tinken, N. McWhannell, N.D. Ridgers, L.E.F. Graves, K. George, N.T. Cable and D.J. Green. Relationships between measures of fitness, physical activity, body composition and vascular function in children.
2. Postgraduate Research Day, LJMU May 2009; **Hopkins, N.D.** G. Stratton, T.M. Tinken, N. McWhannell, N.D. Ridgers, L.E.F. Graves, K. George, N.T. Cable and D.J. Green. Relationships between measures of fitness, physical activity, body composition and vascular function in children
3. ASCM, Seattle, USA, May 2009; **Hopkins ND**, Thijssen DHJ, Tinken TM, Sutton, L, McWhannell N, George K, Cable NT, Stratton G, Green DJ. Anthropometric correlates of vascular function in children.
4. European College of Sports Science (ECSS), Norway, 2009; Tinken, T.M., D.H.J. Thijssen, **N.D. Hopkins**, M.A. Black, E.A. Dawson, C.T. Minson, S.C. Newcomer, M.H. Laughlin, N.T. Cable, and D.J. Green (2009). Impact of shear rate modulation on vascular function in humans.



## List of Contents

|   |           |
|---|-----------|
| 1. Chapter 1  |           |
| <i>Introduction .....</i>   | <b>1</b>  |
| 2. Chapter  |           |
| <i>Literature review.....</i>   | <b>10</b> |
| 2.1. Atherosclerosis in young adults and children                                   | 11        |
| 2.2. Cardiovascular disease risk factors in childhood                               | 13        |
| 2.2.1. <i>Rising obesity levels</i>   | 14        |
| 2.2.2. <i>Physical inactivity</i>   | 16        |
| 2.2.3. <i>Sedentary behaviour</i>   | 18        |
| 2.2.4. <i>Reduced cardiorespiratory fitness levels</i>                              | 21        |
| 2.2.5. <i>Summary</i>   | 23        |
| 2.3. Early manifestations of atherosclerosis – Endothelial Dysfunction              | 23        |
| 2.3.1. <i>Measuring endothelial function in vivo – Flow mediated dilation (FMD)</i> | 25        |
| 2.3.2. <i>Prognostic relevance of FMD</i>   | 29        |
| 2.3.3. <i>Summary</i>   | 32        |
| 2.4. What factors affect FMD?   | 32        |
| 2.4.1. <i>Aging</i>   | 33        |
| 2.4.2. <i>Traditional cardiovascular risk factors</i>                               | 36        |
| 2.4.3. <i>Genetics</i>  | 37        |
| 2.4.4. <i>Sedentary behaviour</i>   | 39        |
| 2.4.5. <i>Exercise and physical activity</i>  | 39        |
| 2.5. Summary and aims of thesis   | 42        |
| 3. Chapter 3  |           |
| .....   | <b>44</b> |
| <i>General Methodology</i>  |           |
| 3.1. Inclusion criteria and testing conditions                                      | 45        |
| 3.2. Methodologies and techniques   | 45        |
| 3.2.1. <i>Assessment of conduit artery function</i>                                 | 45        |
| 3.2.2. <i>Post test analysis of artery diameter</i>                                 | 47        |
| 3.2.3. <i>Assessment of cardiorespiratory fitness</i>                               | 48        |
| 3.2.4. <i>Assessment of physical activity and sedentary behaviour</i>               | 50        |
| 3.2.5. <i>Assessment of body composition</i>  | 52        |

|  |            |
|--|------------|
| <b>4. Chapter 4</b>  |            |
| .....  | <b>55</b>  |
| <i>Scaling brachial artery diameter and function for anthropometric variables</i>                          |            |
| 4.0. Introduction  | 56         |
| 4.1. Methods   | 57         |
| 4.2. Results   | 59         |
| 4.3. Discussion  | 64         |
| 4.4. Conclusion  | 67         |
| <br><b>5. Chapter 5</b>  |            |
| .....  | <b>69</b>  |
| <i>Relationship between measures of body composition, physical activity, fitness and vascular function</i> |            |
| 5.0. Introduction  | 70         |
| 5.1. Methods   | 71         |
| 5.2. Results   | 73         |
| 5.3. Discussion  | 78         |
| 5.4. Conclusion  | 81         |
| <br><b>6. Chapter 6</b>  |            |
| .....  | <b>84</b>  |
| <i>Associations between seasonal decline in vascular function and physical activity</i>                    |            |
| 6.0. Introduction  | 85         |
| 6.1. Methods   | 85         |
| 6.2. Results   | 88         |
| 6.3. Discussion  | 93         |
| 6.4. Conclusion  | 97         |
| <br><b>7. Chapter 7</b>  |            |
| .....  | <b>99</b>  |
| <i>Vascular function and sedentary behaviour</i>   |            |
| 7.0. Introduction  | 100        |
| 7.1. Methods   | 101        |
| 7.2. Results   | 102        |
| 7.3. Discussion  | 107        |
| 7.4. Conclusion  | 109        |
| <br><b>8. Chapter 8</b>  |            |
| .....  | <b>111</b> |
| <i>Heredity of vascular function, physical activity, fitness and body composition</i>                      |            |
| 8.0. Introduction  | 112        |
| 8.1. Methods   | 112        |
| 8.2. Results   | 114        |
| 8.3. Discussion  | 117        |
| 8.4. Conclusion  | 121        |

9. Chapter 9

.....123

*Heredity of exercise induced changes in vascular function, fitness and body composition*

|                   |     |
|-------------------|-----|
| 9.0. Introduction | 124 |
| 9.1. Methods      | 125 |
| 9.2. Results      | 127 |
| 9.3. Discussion   | 130 |
| 9.4. Conclusion   | 134 |

10. Chapter 10

.....135

*Synthesis of findings*

|  |     |
|--|-----|
| 10.0. Aims and objectives  | 136 |
| 10.1. General discussion   | 139 |
| 10.1.1. <i>Scaling issues</i>  | 139 |
| 10.1.2. <i>Physical activity, fitness, body composition, sedentary behaviour and FMD in children</i> | 141 |
| 10.1.3. <i>Heritability of FMD</i>   | 145 |
| 10.2. Limitations and future directions  | 148 |
| 10.3. Conclusion   | 149 |

11. References

.....151



## **List of Figures**

|   |           |
|---|-----------|
| <b>Figure 2.1</b> Leading causes of death worldwide   | <b>11</b> |
| <b>Figure 2.2</b> Percentages of subjects with early lesions, or advanced lesions, plotted for successive 3-year age groups     | <b>13</b> |
| <b>Figure 2.3</b> Childhood obesity trends in Liverpool school children   | <b>15</b> |
| <b>Figure 2.4</b> Percentage reduction in CVD events associated with physical activity that is explained by risk factors        | <b>18</b> |
| <b>Figure 2.5</b> CVD mortality rates per 1000 person-years according to television (TV) viewing time (h/d)                     | <b>20</b> |
| <b>Figure 2.6</b> Nitric oxide mediated endothelium vasodilation  | <b>24</b> |
| <b>Figure 2.7</b> Longitudinal, B-mode images of lumen-arterial wall interface and continuous Doppler velocity.                 | <b>24</b> |
| <b>Figure 2.8</b> Flow mediated dilation technique using high resolution Doppler ultrasound.                                    | <b>26</b> |
| <b>Figure 2.9</b> Outer femoral arterial diameter of a dog demonstrating the vasomotor responses to different stimuli           | <b>27</b> |
| <b>Figure 2.10</b> NO contribution to FMD using proximal and distal cuff  | <b>29</b> |
| <b>Figure 2.11</b> Relation between flow-mediated dilation in a coronary artery and flow-mediated dilation in a brachial artery | <b>30</b> |
| <b>Figure 2.12</b> Time course of functional (FMD) and structural (CADC) arterial adaptation to exercise training               | <b>41</b> |
| <b>Figure 3.1</b> Terason, t3000, Teratech used to assess FMD.  | <b>46</b> |
| <b>Figure 3.2</b> User interface from FMD analysis software, showing calculation of baseline and peak diameters                 | <b>48</b> |

|  |            |
|--|------------|
| <b>Figure 3.3</b> A child undertaking $\text{VO}_{2\text{peak}}$ test (left pane). Hip mounted accelerometer worn during the $\text{VO}_{2\text{peak}}$ test, to measure habitual physical activity (right pane)                             | <b>49</b>  |
| <b>Figure 3.4</b> Example of physical activity output generated from the Mahuffe analysis software   | <b>52</b>  |
| <b>Figure 3.5</b> Assessment of body composition using dual-energy x-ray absorptiometry  | <b>53</b>  |
| <b>Figure 4.1.</b> Relationship between body mass and (a) baseline brachial artery diameter scaled using the simple ratio method for body mass and (b) baseline brachial artery diameter scaled by allometric scaling for body mass in boys. | <b>63</b>  |
| <b>Figure 7.1</b> Baseline relationship between FMD and sedentary behaviour  | <b>106</b> |
| <b>Figure 7.2</b> Relationship between change in sedentary time and change in FMD  | <b>106</b> |
| <b>Figure 8.1.</b> Relationship between FMD in each pair of MZ and DZ twins  | <b>117</b> |
| <b>Figure 9.1</b> Twins undertaking the exercise training intervention   | <b>126</b> |
| <b>Figure 9.2</b> Relationship between change in FMD in MZ and DZ twins  | <b>129</b> |

## **List of Tables**

|   |            |
|---|------------|
| <b>Table 2.1.</b> Summary of findings examining the prognostic value of FMD in high risk individuals                                | <b>34</b>  |
| <b>Table 4.1.</b> Body composition and vascular measures  | <b>60</b>  |
| <b>Table 4.2</b> Correlates for baseline artery diameter and FMD  | <b>61</b>  |
| <b>Table 4.3.</b> Correlations ( $y/x:x$ or $y/x^b:x$ ) used to check the size-independence of vascular outcomes                    | <b>62</b>  |
| <b>Table 5.1</b> Descriptive characteristic)  | <b>74</b>  |
| <b>Table 5.2</b> Percentage $\dot{V} O_2$ peak at treadmill speeds for boys and girls   | <b>75</b>  |
| <b>Table 5.3</b> Comparison of means across FMD and FMD/SR tertiles   | <b>77</b>  |
| <b>Table 6.1</b> Descriptive characteristics, collected in June and November  | <b>60</b>  |
| <b>Table 7.1</b> Descriptive characteristics  | <b>104</b> |
| <b>Table 7.2</b> Spearman's correlations between baseline SB and vascular outcomes and change in SB and change in vascular outcomes | <b>105</b> |
| <b>Table 8.1</b> Descriptive characteristics for MZ and DZ twins  | <b>115</b> |
| <b>Table 8.2.</b> Intraclass correlation coefficients   | <b>116</b> |
| <b>Table 9.1.</b> Pre and post exercise data in mono- and di-zygotic twins  | <b>128</b> |
| <b>Table 9.2.</b> Intraclass correlation coefficients performed on change scores for each twin subset                               | <b>129</b> |



## **List of abbreviations**

|                  |                                  |
|------------------|----------------------------------|
| FMD              | flow mediated dilation           |
| SR               | shear rate                       |
| NO               | nitric oxide                     |
| CAD              | coronary artery disease          |
| CHD              | coronary heart disease           |
| CVD              | cardiovascular disease           |
| VF               | vascular function                |
| ACh              | acetylcholine                    |
| SD               | standard deviation               |
| HR               | heart rate                       |
| BP               | blood pressure                   |
| PA               | physical activity                |
| PA <sub>4</sub>  | minutes spent at 4km             |
| PA <sub>6</sub>  | minutes spent at 6 km            |
| PA <sub>8</sub>  | minutes spent at 8km             |
| PA <sub>10</sub> | minutes spent at 10km            |
| DXA              | dual-energy X-ray absorptiometry |
| %lean            | percentage total lean mass       |
| %fat             | percentage total fat mass        |
| BM,              | body mass                        |
| LM               | lean body mass                   |
| FM               | body fat mass                    |
| AM               | whole arm mass                   |
| ALM              | arm lean mass                    |
| AFM              | arm fat mass                     |
| FAM              | Forearm mass                     |
| FLM,             | forearm lean mass                |
| FFM              | forearm fat mass                 |
| TPHV             | time to peak height velocity     |
| MZ               | monozygotic                      |
| DZ               | di-zygotic                       |
| H <sup>2</sup>   | heritability                     |

# Chapter 1

## Introduction

## **1 Introduction**

### **Cardiovascular risk in young people**

In recent decades cardiovascular disease mortality has increased rapidly and CVD is now the leading cause of death in high and middle income societies (WHO 2004). Perhaps more concerning, however, is the rate at which CVD mortality and morbidity is increasing in developing countries such as China and Japan. The rapidly increasing prevalence of CVD and its co-morbidities has been the stimulus for experts to make some bleak predictions for the future. Hossain *et al.* (Hossain et al. 2007) have concluded that there is set to be a global explosion of type 2 diabetes worldwide with a 150% increase in some Asian countries by 2030. Furthermore Olshansky *et al.* (Olshansky et al. 2005) have suggested that unless obesity is dealt with at a population level, life expectancy will actually decline for the first time in decades.

Manifestations of CVD usually occur during mid-to-late life, however overt indications of CVD are preceded by a long preclinical phase, which has its origins in childhood. Sary *et al.* (Sary 1989) examined the aortas and coronary arteries of 1160 subjects who died between full term birth and 29 yrs. They reported that, by the age of 12-14 yrs, 65% of children already presented with a substantial accumulation of foam cells accompanied by lipid droplets and extracellular lipids. Isolated macrophage foam cells were found in 45% of infants aged <8 months. Numerous studies have since reported that the prodromal stages of atherosclerotic disease occur during fetal development (Napoli et al. 1997; Palinski and Napoli 1999). Although prenatal events play a role in the development of atherosclerosis, disease progression is also partially determined by a range of post-natal modifiable environmental risk factors. For the purposes of this thesis we will focus on relationships between obesity, cardiorespiratory fitness, physical

activity, sedentary behaviour and the early stages of atherosclerosis during childhood and adolescence.

It is estimated that globally 155 million children that are overweight/obese, of which around one-seventh are aged <5 years old (Deckelbaum and Williams 2001; Hossain et al. 2007). The presence of obesity during childhood has ramifications for adult health. Previous studies have illustrated that both adiposity level (Srinivasan et al. 1996) and BMI (Janssen et al. 2005) during childhood are predictors of obesity, metabolic conditions and coronary heart disease (CHD) in adulthood. Childhood obesity is associated with co morbidities such as hypertension, dyslipidaemia, glucose intolerance and insulin resistance, all of which have been identified as independent risk factors for atherosclerotic disease. Presence of these risk factors during childhood leads to increased risk for adult cardiovascular disease (Khan et al. 2003). Indeed Mossberg et al. reported that childhood obesity is associated with increased cardiovascular mortality regardless of adult weight (Mossberg 1989).

Participation in regular physical activity is greater in children than adults, however; in the UK one-third of children aged between 2-11 years do not achieve the recommended target of 60 minutes of at least moderate physical activity/day, and ~17% fail to achieve even 30 minutes of moderate physical activity per day (Sproston and Primatesta 2003). Limited physical activity in youth predisposes to developing an inactive lifestyle later in life (Malina 1996; Telama et al. 1997). Low physical activity early in life is also associated with a less favourable CV risk factor profile (Pate et al. 1995). A dose response relationship is evident between PA and CHD (Berlin and Colditz 1990; Francis 1996; Whaley and Blair 1995) and physical inactivity is associated with many known CHD risk factors including increased blood pressure and decreased high density



lipoprotein-cholesterol (HDL-C). Furthermore, PA appears to modulate CHD incidence independent of CV risk factor modification (Green et al. 2008).

There are currently no definitive guidelines on the acceptable daily amount of sedentary behaviour (SB), however recent research suggests that 30% of children in the UK are watching television to excess (>4hours per day) (Samdal et al. 2007). Recent evidence in adults suggests that each 1 hour increment of TV viewing per day was associated with an 18% increase in CV mortality risk, and those who watched >4 hours per day had an 80% increase in the risk of CVD mortality compared to those watching <2 hours per day. In children, SBs have been associated with obesity, insulin resistance and blood pressure (Ekelund et al. 2007), however there is no evidence directly linking SB to markers of CVD in youth.

With reduced PA and SB, levels of fitness in young people have declined concurrently. Tomkinson *et al* (Tomkinson and Olds 2007) conducted a meta-analysis examining data from ~25.5 million 6- to 19-year olds between 1958 and 2003. A decline of 0.36% per annum in maximal aerobic field running tests was observed. Stratton *et al.* (Stratton et al. 2007) suggest that children's fitness levels are declining consistently across all fitness categories regardless of BMI status. Poor cardiorespiratory fitness in childhood is related to increased body fatness (Eisenmann et al. 2005), metabolic syndrome (Brage et al. 2004) and numerous CV risk factors (Farrell et al. 1998). Furthermore, in adults, low cardiorespiratory fitness level is associated with premature all cause and CVD mortality (Lee et al. 1999).

## **The importance of the vascular endothelium**

The vascular endothelium is collectively recognised as a large endocrine organ responsible for the maintenance of vascular tone, regulation of platelet aggregation, coagulation and fibrinolysis through the secretion of numerous substances. Critically, a normally functioning endothelium mediates anti-atherogenic properties that protect against vasoconstriction, smooth muscle cell growth and inflammatory responses (Davignon and Ganz 2004). When endothelial dysfunction is present, the endothelium may adopt a phenotype that facilitates inflammation, thrombosis, vasoconstriction and atherosclerotic lesion formation. Dysfunction of the endothelium is a sentinel event in the progression of atherosclerotic disease. It precedes the clinical manifestations of CVD and is present in young children who have CVD risk factors (Celermajer et al. 1992).

Nitric oxide bioactivity is used as a surrogate marker of endothelial dys/function and can be measured non-invasively using flow mediated dilation (FMD) (Celermajer et al. 1992). This method involves direct imaging of large conduit arteries and assessment of arterial diameter. Numerous studies have demonstrated a close relationship between peripheral endothelial function and coronary artery function (Anderson et al. 1995; Takase et al. 2005; Takase et al. 1998), leading researchers to conclude that FMD in brachial arteries can be used as a surrogate measure for coronary artery endothelial function. In addition FMD is strongly and independently related to cardiovascular risk and mortality in patients with CVD (Gocke et al. 2002b; Wang et al. 2009), peripheral vascular disease (PVD) (Brevetti et al. 2003; Gocke et al. 2003), CAD (Chan et al. 2003) and chronic heart failure (Meyer et al. 2005). In healthy older adults FMD provided additional prognostic information to traditional CV risk factors (Yeboah et al. 2007).



Evidence suggests that endothelial dysfunction is an integral mechanism in the early stages of atherosclerotic disease, as the loss of the protective effect of the endothelium and NO facilitates atherogenesis. However endothelial dysfunction is reversible and numerous interventions that modify CV risk factors, or reduce CV morbidity and mortality, concomitantly improve endothelial function (Maiorana et al. 2003). These risk factors include aging, all traditionally accepted CV risk factors (Celermajer et al. 1992) and genetic factors (Benjamin et al. 2004).

The presence of traditional cardiovascular risk factors during childhood is related to impairment of FMD. Hypercholesterolemia, obesity and type 2 diabetes mellitus (Aggoun et al. 2005) and smoking are associated with endothelial dysfunction (Celermajer et al. 1992). Although the relative contribution of each of these risk factors is unknown, all have been independently associated with impaired FMD. The precise cause of the endothelial dysfunction is often difficult to identify as children may present with more than one of these risk factors, however numerous mechanisms have been implicated in the development of dysfunction, these include increased inflammation, oxidative stress and altered glucose metabolism (Raitakari et al. 2004).

The role of genetics in the determination of endothelial health is poorly defined. Little research has been undertaken to assess the relative genetic and environmental contributions to measures of vascular function. In adults, there is large variation in the heritability estimates that have been calculated for FMD, which range from 0.14 to 0.39 (Benjamin et al. 2004; Jartti et al. 2002; Zhao et al. 2007). These data imply that FMD may be weakly-to-moderately influenced by genetics. However, no study has sought to examine the impact of interventions such as exercise training on the heritability of

changes in FMD. Furthermore, there are currently no data that examine the genetic contribution to FMD during childhood or adolescence.

The beneficial effect of exercise training on endothelial function in clinical populations of adults is relatively clear (Green et al. 2003). Less evidence of the beneficial effect of exercise is available in paediatric populations who are at high future CV risk. Although numerous studies have confirmed that obesity induced endothelial dysfunction can be normalised through supervised exercise training in both children and adolescents (Meyer et al. 2006; Watts et al. 2004a; Watts et al. 2004b; Woo et al. 2004), there are limited data examining the role of exercise training in other CV risk populations of this age group. Additionally, studies undertaken in healthy paediatric populations indicate that daily leisure time PA could be an important mediator of endothelial health (Abbott et al. 2002; Pahkala et al. 2008). The relationships between FMD, SB and fitness are yet to be explored in children.

## **Summary**

In summary, attenuation of FMD is evident in subjects, including children, with CV risk factors such as smoking, obesity, diabetes and hyperlipidemia. Endothelial dysfunction can be normalised by interventions, including exercise training, that modify CV risk factors. Yet, there are large gaps in the paediatric literature pertaining to the interaction of factors such as genetics, PA, SB and fitness level with FMD.

The general aim of this thesis is to provide evidence in these areas, where knowledge is lacking, whilst the specific aims are:

1. To address the issue of scaling vascular outcomes for body size/composition components in children



2. To explore the associations between FMD and body composition, cardiorespiratory fitness and physical activity using a cross section of healthy children.
3. To determine whether FMD is affected by seasonal variability, and assess the associations with seasonal changes in body composition and physical activity in healthy children.
4. To investigate the basal association between FMD and sedentary behaviour, and address the question of whether change in sedentary behaviour is related to change in endothelial function in healthy children.
5. Using a classic twin study design, assess the heritability of FMD in healthy children.
6. To determine the heritability of the change in FMD in monozygotic and dizygotic twins in response to 8 weeks of high intensity, gym based exercise training.

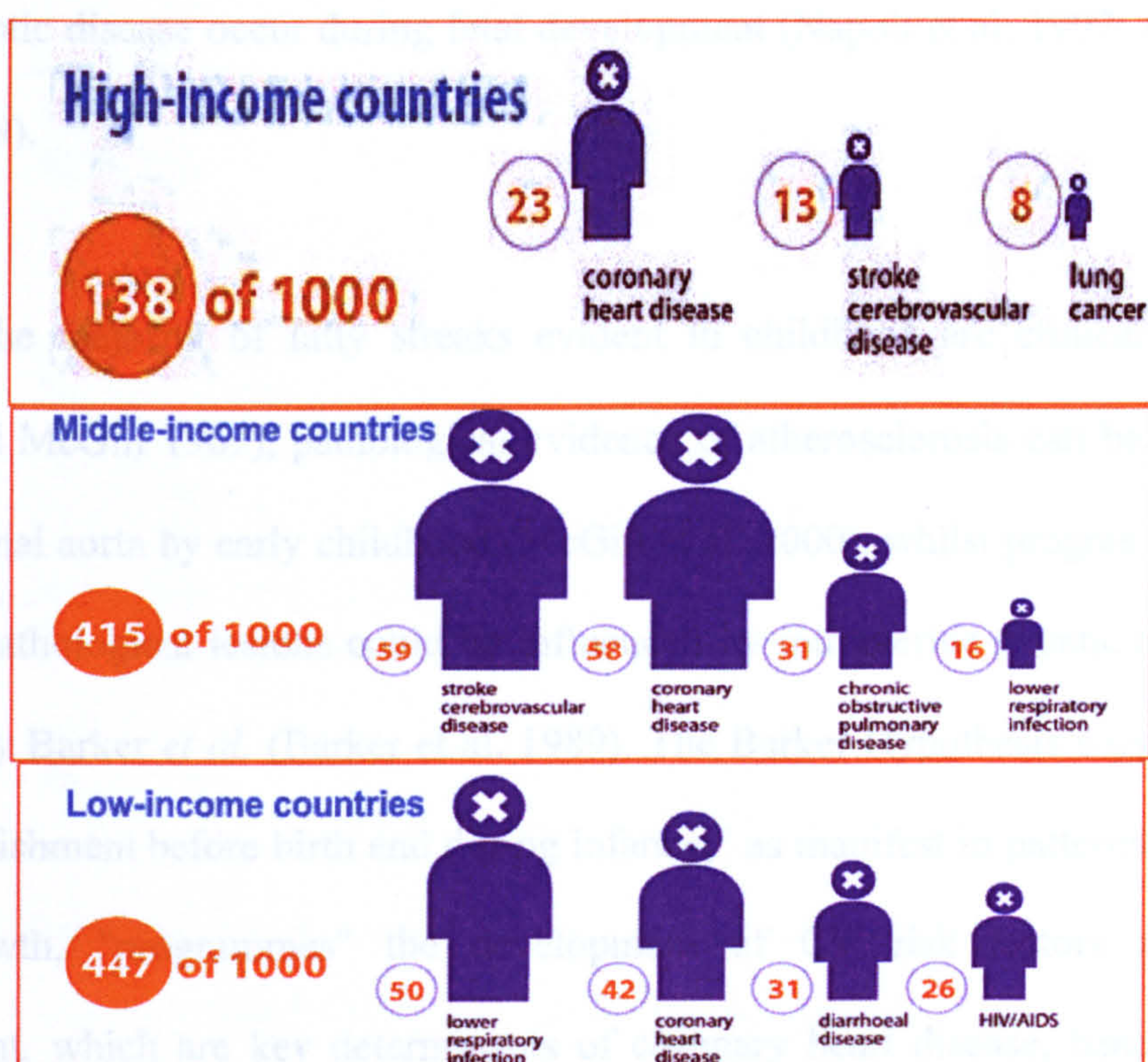
# Chapter 2

## Literature Review

## **2 Literature review**

In recent years cardiovascular diseases (CVD), including coronary heart disease (CHD), cerebrovascular disease and stroke, have become the leading causes of death in high and middle income societies (WHO 2004, figure 2.1), whilst CVD mortality rates in developing countries are rising rapidly. Worldwide, 18 million people die from CVD per annum (Hossain et al. 2007). Atherosclerosis is the progressive disease that precedes overt CVD. It is characterized by vascular inflammation and infiltration of lipids, cholesterol, calcium and cellular debris into the sub-intima of the vessel wall, resulting in plaque formation, vascular remodelling, acute and chronic luminal obstruction, blood flow abnormalities and, ultimately, diminished oxygen supply to target organs (Stary et al. 1995). Although clinically detectable manifestations of CVD, such as angina, stroke and myocardial infarction become apparent around the 4<sup>th</sup> decade of life or later, the atherosclerotic disease process has a long preclinical stage which originates in childhood (Celermajer et al. 1992).





**Figure 2.1** Leading causes of death worldwide (World Health Organisation, 2004)

## 2.1 Atherosclerosis in young adults and children

Evidence of atherosclerosis in young adults and children has been provided by autopsy studies. The earliest of these investigations examined the arteries of American soldiers killed in Korea (Enos et al. 1953). Some evidence of coronary disease was demonstrated in 77% of this cohort of 300 men, mean age 22.1 yrs. The average age of a sample of 20 soldiers who presented with >50% luminal narrowing of a coronary artery was 22.6yrs. Sary *et al.* (Sary 1989) were able to add to these findings, in a study examining 1160 subjects who died between full term birth and 29 yrs. They reported that, by the age of 12-14 yrs, 65% of children already presented with a substantial accumulation of foam cells accompanied by lipid droplets and extracellular lipids. A further 8% of children had progressed beyond these early stage plaques and had advanced to the pre-atheroma or atheroma (figure 2.2). Isolated macrophage foam cells were found in 45% of infants aged <8 months. Numerous studies have since reported that the prodromal stages of



atherosclerotic disease occur during fetal development (Napoli et al. 1997; Palinski and Napoli 1999).

Although the majority of fatty streaks evident in childhood are clinically harmless (Strong and McGill 1969), pathological evidence of atherosclerosis can be apparent in the abdominal aorta by early childhood (McGill et al. 2000), whilst progression to more advanced pathological lesions could be influenced by intrauterine events, as originally proposed by Barker *et al.* (Barker et al. 1989). The Barker Hypothesis suggests that "a baby's nourishment before birth and during infancy," as manifest in patterns of fetal and infant growth, "programmes" the development of CV risk factors and arterial development, which are key determinants of coronary heart disease, hence low birth weight could itself be a CV risk factor.

Recently, Skilton *et al.* (Skilton et al. 2005) compared the maximal aortic intima-medial thickness (IMT) of 25 neonates with intrauterine growth restriction to 25 normal-high weight neonates. Both birth weight ( $r=-0.32$ ,  $P=0.03$ ) and study group ( $r=0.33$ ,  $P=0.02$ ) were associated with increased IMT. The difference between groups for IMT, adjusted for birth weight, remained significant independent of maternal age and smoking status. The authors concluded that aortic wall thickening, an early marker of atherosclerosis in children, is increased in neonates with intrauterine growth restriction compared with babies with normal birth weight and that prenatal events could predispose to CV risk in later life.

**Figure 2.2** Percentages of subjects with early lesions, or advanced lesions, plotted for successive 3-year age groups (Stary 1989).

The studies described above indicate that prior to the overt detection of CVD, which usually occurs in the fourth decade of life or later, clinically relevant changes are evident in the vasculature. These preclinical markers precede and accelerate the development of atherosclerotic plaque and can be present in young children and even new born babies exposed to CV risk factors.

## **2.2 Cardiovascular disease risk factors in childhood**

Although prenatal events play a role in the development of atherosclerosis, disease progression is also determined by a range of post-natal modifiable environmental risk factors. Such risk factors include obesity, hypertension, insulin resistance/diabetes, hypercholesterolemia, smoking, physical inactivity and low cardiorespiratory fitness. For the purposes of this thesis we will focus on relationships between obesity, cardiorespiratory fitness, physical activity, sedentary behaviour and the early stages of atherosclerosis during childhood and adolescence.

### 2.2.1. Rising obesity levels

Obesity has achieved epidemic proportions worldwide (Xavier and Sunyer 2002). It is estimated that 1.1 billion people are overweight, whilst 312 million people are obese (Hossain et al. 2007). In developing countries obesity has tripled over the last 20 years as westernized lifestyles have increasingly been adopted. By 2025 it is estimated that more than 40% of the population will be obese (Kopelman 2000) and ~420 million people worldwide will be glucose intolerant (Hossain et al. 2007), as obesity causes an explosion of diseases and co morbidities including type 2 diabetes.

Even more concerning is the rate at which the prevalence of childhood and adolescent obesity is increasing. Hossain *et al.* (Hossain et al. 2007) have reported that 155 million children worldwide are overweight or obese, a figure which includes approximately 22 million children aged <5 years (Deckelbaum and Williams 2001). In Europe it has been reported that the prevalence of childhood overweight is rising at a rate of ~1% per year (Lobstein and Frelut 2003). The most recent data for national obesity rates in the UK are from the Health Survey for England 2003 (Sproston and Primatesta 2003). In 2-10 year olds, 16% of boys and 12 % of girls were over the 95<sup>th</sup> percentile of BMI. These figures rose in 11-14 year olds to 24% of boys and 26% of girls. The Sportslink project, an ongoing study which monitors the health and fitness of Liverpool school children and provides annual data on obesity prevalence in the north west of England, suggests that in 2007/2008 around one-third of children aged 9-10 were categorised overweight or obese (Boddy et al. 2010) (figure 2.3).

**Figure 2.3** Childhood obesity trends in Liverpool school children (Boddy et al. 2010), data is adjusted for fitness level and indices of multiple deprivation. Obesity is defined using Cole *et al.* (Cole et al. 2000).

Previous studies have illustrated that both adiposity level (Srinivasan et al. 1996) and BMI (Janssen et al. 2005) during childhood are predictors of obesity, metabolic conditions and coronary artery disease (CAD) in adulthood. Childhood obesity is associated with co-morbidities such as hypertension, dyslipidaemia, glucose intolerance and insulin resistance, all of which have been identified as independent risk factors for atherosclerotic disease. Data from the Bogalusa Heart Study showed that ~60% of overweight 5-10 year olds presented with 1 CVD risk factor. Within the same cohort, ~20% of overweight children possessed 2 risk factors (Freedman et al. 2008). Presence of these risk factors during childhood leads to increased risk for adult cardiovascular disease (Khan et al. 2003). Indeed Mossberg et al. reported that childhood obesity is associated with increased cardiovascular mortality regardless of adult weight (Mossberg 1989). The current trends for obesity have led experts to forecast that, unless obesity is reduced at a population level, the steady rise in life expectancy observed for over a



century may come to an end, with future generations living shorter and less healthy lives than enjoyed by their parents (Olshansky et al. 2005).

### **2.2.2. Physical (in)activity**

The current epidemic of overweight and obesity has raised the important question of aetiology. Fundamentally, obesity must be explained by increased energy intake (Rennie et al. 2005) and/or decreased daily energy expenditure (Dollman et al. 2005). Some evidence suggests that, in adults, there has been a decrease in energy intake (Prentice and Jebb 1995) in recent decades, whilst data in children show no secular increase in either energy or fat intake (Schlicker et al. 1994; Troiano et al. 2000). Nonetheless, in theory, there is an important role for sedentary lifestyle and decreased physical activity as major contributors to obesity. Current guidelines recommend that adults undertake 30 mins·d<sup>-1</sup> of moderate to vigorous physical activity in order to maintain health and limit the morbidities associated with low physical activity levels (Sproston and Primatesta 2003). However, figures from the Department of Health show that around two-thirds of men and three-quarters of women in the UK do not currently meet physical activity guidelines. Participation in regular physical activity is greater in children than adults, however in the UK one-third of children aged between 2-11 that do not achieve the recommended target of 60 minutes of at least moderate physical activity/day, and ~17% fail to achieve even 30 minutes of moderate physical activity per day (Sproston and Primatesta 2003). Limited physical activity in youth predisposes to developing a sedentary lifestyle later in life (Malina 1996; Telama et al. 1997), as childhood physical activity patterns track into adolescence (Kristensen et al. 2008) and adulthood (Telama 2009; Telama et al. 2005). Low physical activity early in life is also associated with a less favourable CV risk factor profile (Pate et al. 1995).

Major prospective epidemiological studies indicate that there is a dose-response relationship between overall physical activity and CHD (Berlin and Colditz 1990; Francis 1996; Whaley and Blair 1995). Physical inactivity is associated with many known CHD risk factors including increased blood pressure and decreased high density lipoprotein-cholesterol (HDL-C) and attention has been drawn to the role of risk factor modification through physical activity as a possible mediating mechanism for CVD reduction. Physical activity tends to change individual risk factors modestly, around the order of 5% for blood lipids (Leon and Sanchez 2001), 3-5 mmHg for blood pressure (Fagard 2001) and 1% for haemoglobin A1c (Thompson et al. 2001). Nonetheless, increased physical activity can result in a 30% decrease in the risk of CVD events (Thompson et al. 2003). Numerous studies have demonstrated that the association between physical activity and CVD is independent of other CV risk factors. A recent analysis of 27000 subjects (Mora et al. 2007) reported that differences in risk factors explained ~60% of the cardiovascular risk reduction associated with exercise (figure 2.4). This data infers that ~40% of the risk reduction associated with exercise cannot be explained by effects of physical activity on established risk factors, highlighting the role of a physically active lifestyle in the prevention of CVD, independent of risk factor modification. It has also been suggested that the 'risk factor gap' could be partially explained by the improvement in vascular endothelial function, which occurs as a direct result of repeated exercise bouts. That is, physical activity exposes the vasculature to increased bouts of repetitive shear stress which transduce functional and structural adaptations that may decrease atherosclerotic risk (Green et al. 2008).

**Figure 2.4** Percentage reduction in CVD events associated with physical activity that is explained by risk factors (Mora et al. 2007).

### **2.2.3. Sedentary behaviour**

Whilst physical inactivity is an increasingly common term, a more appropriate label for this concept may be *sedentary behaviour* (SB), as this term encompasses the fact that a diverse range of behaviours can be considered "inactive". However, sedentary behaviour is not synonymous with inactivity and researchers prefer to define SB as behaviours where sitting or lying are the dominant postures and energy expenditure is <1.5 METs (Pate et al. 2008). Sedentary behaviour typically involves both work/school time behaviours and leisure time pursuits and encompasses activities such as screen time, active transport and sitting to talk, read or listen to music (Pate et al. 2008). Although there are currently no definitive guidelines limiting daily time spent in sedentary behaviour, it has been suggested that engaging in >4 hours of television viewing per day is 'excessive' (Marshall and Welk 2008). Taking television (TV) viewing time as an example, and using 4 hours/day as a guideline, ~30% of young

people in the UK watch television in excess (Samdal et al. 2007). In addition, Brodersen *et al.* (Brodersen et al. 2007) found that hours of screen based media use in children increased by ~2.5 hours per week over a 5 year period.

As SB increases in prevalence, evidence is beginning to emerge linking sedentary behaviours with all cause and CVD mortality. Recently, Dunstan *et al.* (Dunstan et al. 2010) examined the relationships between TV viewing time and all cause and CVD mortality in 8800 adults. They reported that increased television viewing was associated with both all cause and CVD mortality (figure 2.5). Each 1 hour increment of TV viewing per day was associated with an 18% increase in CV mortality, and those who watched >4 hours per day had an 80% increase in the risk of CVD mortality compared to those watching <2 hours per day. Furthermore, a dose response relationship has been reported between daily sitting time and CVD mortality (Katzmarzyk et al. 2009). Katzmarzyk et al. (Katzmarzyk et al. 2009) evaluated the association between daily sitting time and CVD mortality in 17 000 adults aged between 18-90 years old. The researchers concluded that those who reported that they engaged in sitting time 'almost all of the day' were at 54% increased risk of CVD mortality, and that this relationship was independent of leisure time activity.



**Figure 2.5** CVD mortality rates per 1000 person-years according to television (TV) viewing time (h/d). (Dunstan et al. 2010)

Much of the research into SB in young people has been focused on its relationship with obesity (Ekelund et al. 2007; Shimai et al. 1993). Children who engage in >2 hours/day of screen based media were 1.5 times more likely to be overweight than those children who engaged in <2 hour/day. The risk of overweight associated with increased screen based media was greatly exacerbated when subjects were insufficiently active (odds ratio ~3.7) (Laurson et al. 2008). Additionally, Ekelund *et al.* (Ekelund et al. 2007) recently documented weak associations between SB, systolic and diastolic blood pressure and clustered metabolic risk in children. The same group have also shown that time spent watching television was related to insulin resistance. Taken together the studies detailed above provide plausible indirect evidence to suggest that sedentary behaviour may have a role in the development and progression of CVD, however research is needed to further explore this hypothesis.

Some researchers have suggested that the negative health impact of increased sedentary behaviour may result as SB displaces time spent performing physical activity (Marshall

et al. 2004). However, explorations of this hypothesis have demonstrated that moderate–vigorous PA (Dunstan et al. 2004; Healy et al. 2008), which has been consistently associated with reduced mortality, is only weakly correlated with TV viewing time. This data implies that there is some unique and distinct impact of sedentary behaviour on health outcomes, apart from the lack of PA. Although researchers concede that it is possible that TV viewing time significantly displaces light-intensity physical activity (Dunstan et al. 2010), which has been shown to be beneficially associated with markers of cardiometabolic risk, including 2-hour post challenge blood glucose (Healy et al. 2007).

#### **2.2.4. Reduced cardiorespiratory fitness**

There has been a secular decline in the fitness levels of young people in recent decades (Huotari et al. 2009; Stratton et al. 2007; Tomkinson et al. 2003). Tomkinson and Olds, (Tomkinson and Olds 2007) conducted a meta-analysis examining trends in aerobic performance over 45 years across 33 countries. The study examined data from ~25.5 million 6- to 19-year olds between 1958 and 2003 and provides the most comprehensive global trend in paediatric fitness levels to date. A decline of 0.36% per annum in maximal aerobic field running tests was observed, this figure was consistent regardless of age, gender and geographical group. Following a peak in fitness levels during the 1970's, the rate at which fitness has declined has increased in magnitude with each passing decade. Further evidence illustrating the decline in children's fitness is provided by Dollman *et al.* (Dollman et al. 1999) who demonstrated that between 1985-1997, although there was no difference in fitness levels of children in the fittest quartile, the least fit quartile of children were markedly less fit in 1997 than 1985. This implies that the decline in fitness is not homogenous across fitness categories.

However, some studies, including those undertaken in Liverpool, suggest that children's fitness levels are declining consistently across fitness categories and regardless of BMI status (Stratton et al. 2007). Stratton *et al.* (Stratton et al. 2007) suggested that there has been a 23% decline in the fitness levels of yr 6 school children between 1998-2004. The Liverpool data also suggest that the proportion of children considered 'unfit' increased by 36% in girls and 50% in boys over the same period.

The implications of low fitness levels in the paediatric population are far reaching, as poor cardiorespiratory fitness is related to increased body fatness (Eisenmann et al. 2005), metabolic syndrome (Brage et al. 2004) and numerous CV risk factors (Farrell et al. 1998). In adults, low cardiorespiratory fitness level is associated with premature mortality. Lee *et al.* (Lee et al. 1999), using a database of 21925 men, found a direct relationship between cardiorespiratory fitness and all cause and cardiovascular disease mortality; the fitter men exhibiting a decreased risk of mortality. They also observed that cardiorespiratory levels in men influence the negative health effects of obesity; that is, those men who are lean and unfit have a greater risk of cardiovascular disease mortality than those who were obese but possessed high fitness levels. These researchers have concluded that moderate fitness protects against the influence of other predictors of CVD mortality. A key implication of these studies from the Aerobics Centre Longitudinal Database, and more recent studies undertaken in women (Wessel et al. 2004), is that fitness may be a more important cardiovascular risk factor than overweight or obesity, and that the latter may owe their predictive capacity to the fact that they are surrogate markers for inactivity. Farrell *et al.* (Farrell et al. 2002) concluded that as cardiorespiratory fitness is itself an independent predictor of all cause



mortality and that failure to measure this variable will confound any relationship between body mass and mortality.

### **2.2.5. Summary**

Obesity, physical activity, sedentary behaviour and fitness levels are independently associated with CVD risk and/or mortality. The relative importance of each has been the subject of heated academic debate over recent years, with attention largely focused on the 'fitness vs. fatness' debate. A similar and evolving debate relates to the relative importance of encouraging physical activity versus the avoidance of sedentary behaviours. Although partitioning out the relative contribution of fitness, fatness, physical activity and sedentary behaviour to CVD risk may aid aetiological understanding and help inform prevention and treatment strategies, such debate may be perceived as somewhat "academic" when one considers that physical activity is an effective strategy to modulate fitness, fatness and sedentary behaviour. Consequently, it has been suggested that physical activity promotion should be a foundation of clinical therapy and public health policy, whether the aim is to promote health or weight control (Blair and Church 2004).

### **2.3 Early manifestations of atherosclerosis – Endothelial dysfunction**

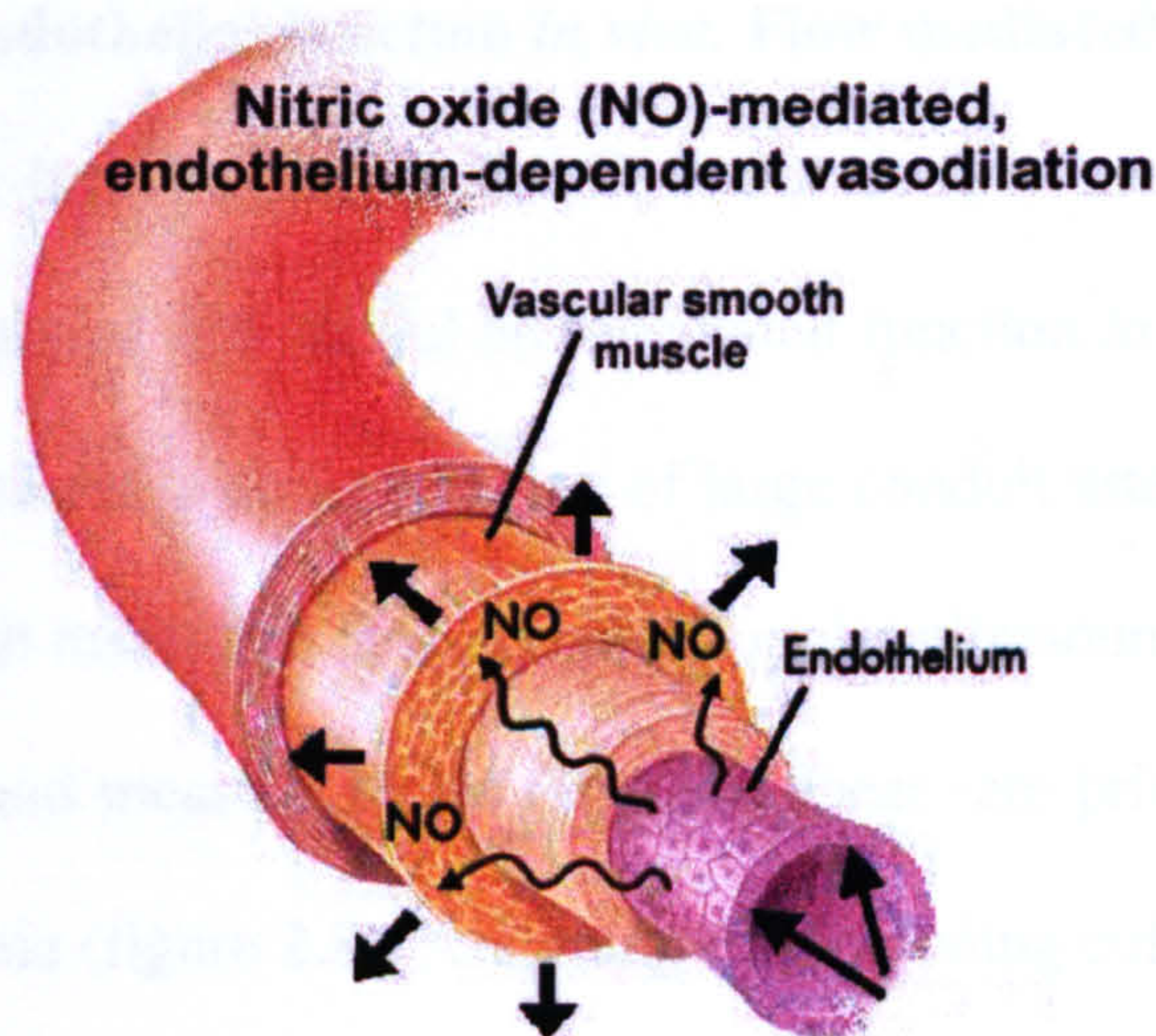
Originally considered a passive layer of inert cells lining the vascular wall, the endothelium is now collectively recognised as a large endocrine organ responsible for the maintenance of vascular tone, regulation of platelet aggregation, coagulation, and fibrinolysis through the secretion of numerous substances. Critically, a normally functioning endothelium mediates anti-atherogenic properties that protect against vasoconstriction, smooth muscle cell growth and inflammatory responses (Davignon and Ganz 2004). When endothelial dysfunction is present, the endothelium may adopt a



phenotype that facilitates inflammation, thrombosis, vasoconstriction and atherosclerotic lesion formation.

Endothelial dysfunction is a sentinel event in the progression of atherosclerotic disease. It precedes the clinical manifestations of CVD and is present in young children who have CVD risk factors (Celermajer et al. 1992). The health of the vascular endothelium represent a barometer for cardiovascular risk (Vita and Keaney 2002) and the vascular endothelium has become a therapeutic target for risk reduction.

Of the vasoactive substances secreted by the endothelium, nitric oxide (NO) has been the most extensively studied. Nitric oxide is a labile, lipid soluble gas synthesized in endothelial cells from the amino-acid L-arginine through the action of endothelial nitric oxide synthase (eNOS) (Palmer et al. 1988). It rapidly diffuses into the vascular smooth muscle of the tunica media where it binds to the enzyme guanylate cyclase (Ignarro et al. 1986), resulting in an increase in cyclic guanosine monophosphate, which induces smooth muscle relaxation and vascular dilation (Furchgott and Jothianandan 1991).



**Figure 2.6** Nitric oxide mediated endothelium vasodilation



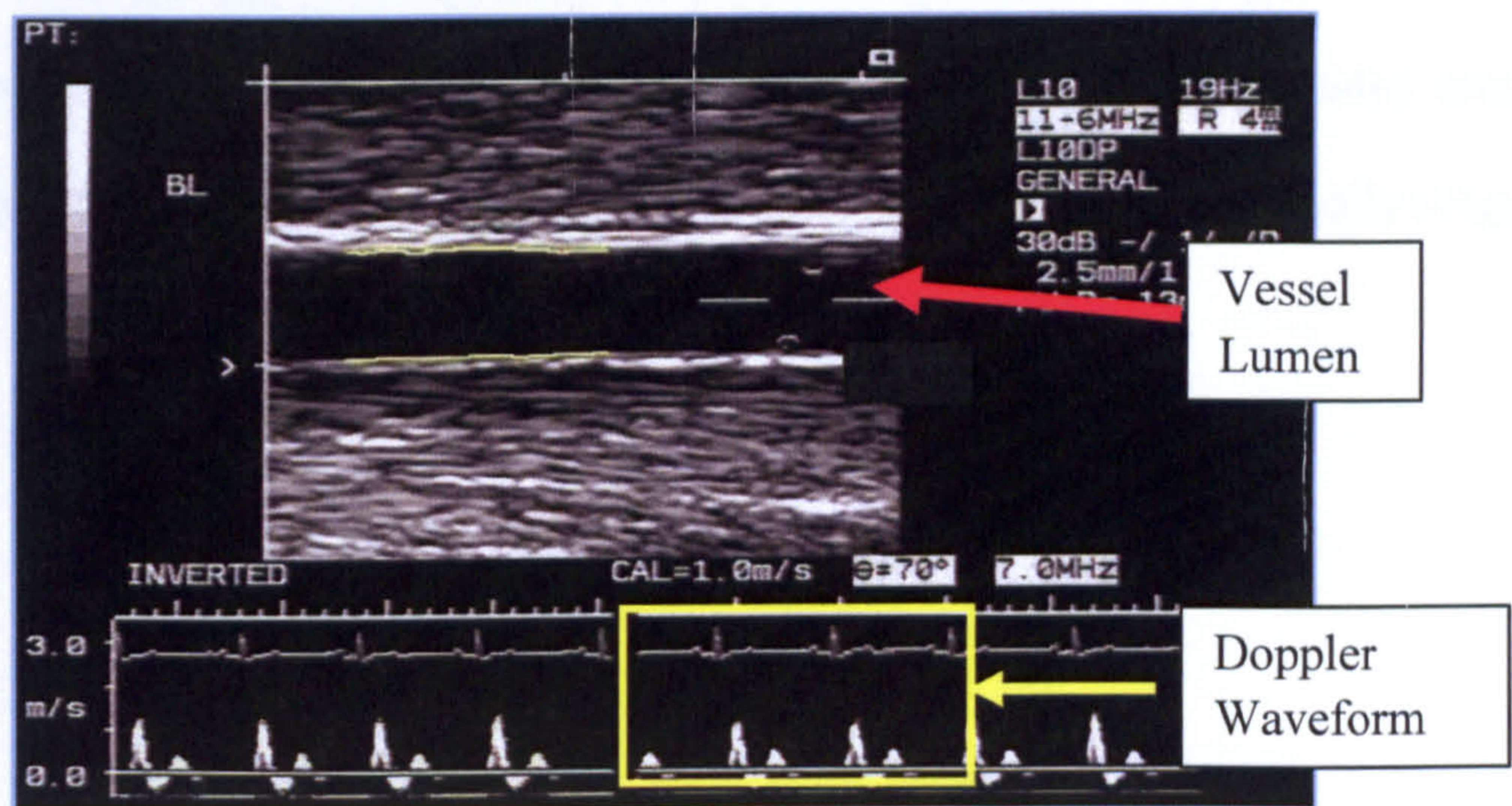
Nitric oxide is continuously secreted at rest, but the endothelium can be stimulated to release increased levels of NO, in turn, causing acute arterial dilation. Such a response can be brought about pharmacologically, using receptor agonists such as acetylcholine (ACh) and also physiologically via increased flow and consequent shear stress along the vessel wall (figure 2.6). Acute release of NO causes vasodilatation in an attempt to normalise shear during periods of increased flow, induced by exercise or ischaemia (Hutcheson and Griffith 1991). Although the signalling cascade linking mechanical stimulation to the release of NO is not yet fully understood, a number of mechanisms are thought to be involved. Endothelial potassium channel activation (Oleson et al. 1988), calcium influx in endothelial cells (Dull and Davies 1991), release of bradykinin (Hecker et al. 1993) and phosphorylation of serine residue (Groves et al. 1995) are all mechanisms proposed to cause an increase in NO bioavailability in response to increased flow and arterial wall shear stress. A central feature of endothelial dysfunction is increased oxidant degradation of NO, resulting in reduced bioavailability of NO (Harrison 1996). Nitric oxide bioactivity is therefore used as a surrogate marker of endothelial dys/function.

### **2.3.1 Measuring endothelial function *in vivo*. Flow mediated Dilation**

In recent years a non-invasive approach, referred to as flow mediated dilation (FMD), has been adopted to assess endothelial NO-mediated function *in vivo* (Celermajer et al. 1992). This method involves direct imaging of large conduit arteries and assessment of arterial diameter. High resolution B-mode and Doppler ultrasound (Figure 2.7) are used to visualise arteries and measure blood flow and shear rate prior to, and following, a period of limb ischemia (figure 2.8). Assuming the occluding cuff is placed distal to the imaged artery (Doshi et al. 2001) and the period of ischaemia is 5 minutes in duration (Mullen et al. 2001), the vasomotor response of the vessel is largely NO mediated



(Joannides et al. 1995; Kooijman et al. 2008). The response can therefore be quantified as an index of endothelial function.



**Figure 2.7** Longitudinal, B-mode images of lumen-arterial wall interface and continuous Doppler velocity.



**Figure 2.8** Flow mediated dilation technique using high resolution Doppler ultrasound.

Arterial flow mediated dilation was first described in 1933 (Schretzenmayr 1933). However, the importance of the endothelium in mediating vasodilator responses to flow



was not recognised until Pohl *et al.* (Pohl et al. 1986) measured the change in canine femoral artery diameter. In this experiment, one femoral artery had a healthy intact endothelium, whilst the endothelium of the contra-lateral leg were denuded via balloon angioplasty. The investigation demonstrated attenuated FMD in the denuded artery, whereas the artery with the intact endothelium responded by dilating normally (Figure 2.9).

**Figure 2.9** Outer femoral arterial diameter of a dog demonstrating the vasomotor responses to different stimuli before (control) and after intimal denudation (Pohl et al. 1986).

Sinoway *et al.* (Sinoway et al. 1989) were the first group to demonstrate FMD *in vivo* in humans using vascular ultrasound. Following this, Celermajer *et al.* (Celermajer et al. 1992) were able to demonstrate that impaired FMD was apparent in populations with CV risk factors. This seminal study investigated the FMD response in subjects aged between 8-67 years, including 50, non smoking, normotensive, non-diabetic controls, 20



adults who had smoked 20 or more cigarettes per day for at least 5 years, 10 children with familial hypercholesterolaemia and 20 subjects aged 54-67 years with established coronary artery disease (CAD). They concluded that flow mediated dilation was depressed in asymptomatic groups who possessed CV risk factors, including young children. This, in turn, suggested that physiologically important changes to the vascular endothelium may occur as early as the first decade of life. These findings have since been confirmed, as all established CV risk factors impair FMD and treatments for risk factors ameliorate this effect. Impaired FMD is now recognized as an early event and a surrogate marker for cardiovascular disease.

Confirmation that the FMD response was almost exclusively mediated by NO was provided by Joannides *et al.* (Joannides et al. 1995). When the radial artery was imaged following a 4 minute period of occlusion distal to the imaged site, these investigators reported a significant reduction in vasodilation following the infusion of the NO blocker  $N^G$ -monomethyl-L-arginine (L-NMMA). These findings have been corroborated in the brachial artery following 5 minutes of ischaemia with the occlusion cuff placed distal to the imaged site (Doshi et al. 2001; Mullen et al. 2001) and more recently in the femoral artery (Kooijman et al. 2008).

Correct placement of the occluding cuff during the FMD technique is essential in order to obtain a NO mediated vasodilator response. Doshi *et al.* (Doshi et al. 2001) showed that NO blockade almost completely eradicated the vasodilator response to FMD when the cuff was positioned distally, however when the cuff was placed proximal to the imaged site there was no effect on the response (Fig 2.10). The placement of the cuff therefore has implications as to whether or not the vasodilator response is NO-dependent or -independent. The duration of the hyperaemic stimulus is also important to

the integrity of the FMD technique. Durations of ischaemia which are greater than 5 minutes produce larger vasodilator responses, but these are relatively unaffected by NO blockade. Nonetheless, NO blockade following an occlusion period of 5 minutes almost completely abolishes the FMD response (Mullen et al. 2001). It is clear that in order to produce a vasomotor response that is reflective of NO bioavailability, FMD must adhere to stringent guidelines, with any deviation in protocol potentially increasing the contribution of alternative vasodilator pathways (Green 2005).

**Figure 2.10** NO contribution to FMD using proximal and distal cuff position (Doshi et al. 2001).

### **2.3.2. Prognostic Relevance of FMD**

A number of studies have examined whether peripheral endothelial function parallels coronary artery function (Anderson et al. 1995; Takase et al. 2005; Takase et al. 1998). The earliest study was conducted in 50 patients undergoing catheterization for evaluation of coronary artery disease (CAD) (Anderson et al. 1995). Coronary endothelial function was assessed using serial intracoronary infusion of the endothelium-dependent agonist acetylcholine. Peripheral vascular function was assessed

in the brachial artery following reactive hyperaemia. This study demonstrated that patients with evidence of CAD exhibited diminished endothelial function in the brachial artery and the authors also suggested that there was a 'close' relationship between brachial and coronary artery function ( $r=0.36$ ,  $p=0.01$ ). The relatively weak correlation found by Anderson *et al.* (Anderson et al. 1995) can perhaps be explained by the use of different stimuli to produce the vasodilator response. Takase *et al.* (Takase et al. 1998) addressed this limitation and were able to induce endothelium-dependent vasodilation in both the coronary and brachial arteries brought about by increases in blood flow and shear stress. They observed a much stronger correlation between coronary and brachial artery function ( $r=0.79$ ,  $p<0.001$ ) (Figure 2.11), concluding that FMD in brachial arteries can be used as a surrogate measure for coronary artery endothelial function.

**Figure 2.11** Relation between flow-mediated dilation in a coronary artery stimulated by 20 mg of ATP and flow-mediated dilation in a brachial artery stimulated by hyperaemia (Takase et al. 1998).

A mounting body of evidence has emerged linking impaired FMD to adverse CV end points in high risk populations, highlighting the prognostic value of FMD. FMD is strongly and independently related to cardiovascular risk and mortality in patients with



CVD (Gocke et al. 2002b; Wang et al. 2009), peripheral vascular disease (PVD) (Brevetti et al. 2003; Gocke et al. 2003), CAD (Chan et al. 2003) and chronic heart failure (Meyer et al. 2005). Table 2.1 provides a brief summary of findings of studies which have examined FMD and its prognostic value in high risk patients. These studies indicate that impaired FMD in high risk patient groups is associated with a greater risk of cardiac events. Furthermore, FMD is a strong independent predictor of events which exceeds the prognostic capacity of compound risk scores such as the Framingham equation.

Less data exists regarding the capacity of FMD to predict CV endpoints in asymptomatic patients. A study in 2007 examined the prognostic value of FMD in a cohort of healthy older adults (72-98 years old) (Yeboah et al. 2007). Event free survival rates were significantly higher in those subjects with intact FMD, and FMD was a significant predictor of CV events, however the inclusion of FMD added only ~1% further predictive accuracy to current traditional CV risk factors. Similarly, FMD was found to be a significant predictor of CV events in older adults (mean age 66 yrs) (Shimbo et al. 2007), however, its predictive value was not independent of traditional CV risk factors. Further to these findings in older adults, FMD also provides additional prognostic information than is provided by Framingham risk scores when classifying patients into risk categories (Yeboah et al. 2009). Yeboah *et al.* (Yeboah et al. 2007) have attempted to contextualise the findings of these studies, suggesting that as age is an independent CVD risk factor and endothelial dysfunction is an early process in the pathogenesis of atherosclerosis, measurement of FMD in older cohorts might be less useful in older populations whose brachial FMD is greatly diminished and who are likely to have advanced atherosclerosis.

### **2.3.3. Summary**

As atherosclerosis is a disease which is systemic in its development, researchers are provided with an opportunity to gain insight into the health of the coronary arteries using non invasive and direct measurement of conduit artery health via flow mediated dilation. Impaired FMD is a significant predictor of adverse CV events, moreover, improvements in FMD result in a more favourable prognosis. These findings suggest that FMD is an invaluable tool for assessing those at highest risk of future CV disease

### **2.4 What factors affect FMD in children?**

The mean FMD in healthy children appears to be between 8-11% (Fernhall and Agiovlasitis 2008), however the range of normal values is extremely large, with the 95% CI reportedly between 4-18% (Tounian et al. 2001; Woo et al. 2004). It is unclear if the large range of 'normal' values is due to biological variation or measurement differences between studies. The measurement of FMD is technically demanding, requiring specific training, and FMD is also sensitive to numerous transient factors that may influence endothelial function acutely such as circadian rhythm, viral illness and food intake prior to measurement (Celermajer 2008). Due in part to these difficulties, there has been a lack of technical consistency in FMD protocols across different laboratories. However there have been recent attempts to standardize the procedure (Corretti et al. 2002; Thijssen et al. In press) in an effort to allow comparison of data across different studies in the future.

Evidence suggests that endothelial dysfunction is an integral mechanism in the early stages of atherosclerotic disease, as the loss of the protective effect of the endothelium and NO facilitates atherogenesis. However endothelial dysfunction is reversible and numerous interventions that modify CV risk factors, or reduce CV morbidity and



mortality, concomitantly improve endothelial function (Maiorana et al. 2003). These risk factors include all traditionally accepted CV risk factors (Celermajer et al. 1992) as well as genetic factors (Benjamin et al. 2004)

#### **2.4.1 Aging**

Healthy aging is associated with an attenuation of the FMD response (Celermajer et al. 1994). Gerhard *et al.* (Gerhard et al. 1996) observed a significant decrease in endothelium-dependent dilation measured using brachial artery infusions of methacholine chloride (0.03 to 10.0 µg/min) with each decade of age from the thirties. Age was found to be a strong univariate and multivariate predictor of endothelium-dependent vasodilation. Evidence indicates that a reduction in NO availability is responsible for the age related decline in endothelial function and it has been suggested that increased oxidative stress may contribute to the reduction in NO bioactivity (Eskurza et al. 2006), coupled with a reduction in NO production thought to be brought about by a reduction in the availability of NO substrates (Eskurza et al. 2005). Endothelial function measured by FMD does not appear to change throughout childhood in healthy, non obese children (Fernhall and Agiovlasitis 2008), however endothelial function has not yet been investigated longitudinally during childhood and adolescence and little is therefore known about the pattern of FMD responses throughout early life course.



Table 2.1. Summary of findings examining the prognostic value of FMD in high risk individuals

| Author  | Subjects  | Events  | Principle finding   |
|---|---|---|---|
| Gocke <i>et al.</i> (Gocke et al. 2002a)        | 187 patients undergoing vascular surgery  | 45 CV events (death, MI, stroke, ischaemic ventricular fibrillation, myocardial necrosis) | <ul style="list-style-type: none"><li>• Impaired FMD predicted events (odds ratio 9.0, 95% CI 1.2 to 68; P=0.03)</li></ul>  |
| Gocke <i>et al.</i> (Gocke et al. 2003)         | 199 patients with peripheral vascular disease   | 35 CV events (death, MI, stroke, unstable angina)   | <ul style="list-style-type: none"><li>• FMD is a strong independent predictor in PVD</li><li>• Risk 9* higher when FMD &lt;8.1% (odds ratio 9.5; 95% CI 2.3 to 40).</li><li>• FMD predicts adverse events</li></ul> |
| Chan <i>et al.</i> (Chan et al. 2003)           | 152 CAD patients  | 22 events over 34 month follow up   | <ul style="list-style-type: none"><li>• FMD predicts adverse events</li></ul>   |
| Meyer <i>et al.</i> (Meyer et al. 2005)         | 75 CHF patients   | Clinical deterioration or death   | <ul style="list-style-type: none"><li>• FMD strongest multivariate predictor</li></ul>  |
| Neunteufl <i>et al.</i> (Neunteufl et al. 2000) | 73 patients with angina pectoris  | 83 events over 5 yrs  | <ul style="list-style-type: none"><li>• FMD independently predictive of cardiac events</li></ul>  |
| Wang <i>et al.</i> (Wang et al. 2009)           | 101 patients with acute myocardial infarction post percutaneous coronary intervention | 29 events over 12 months  | <ul style="list-style-type: none"><li>• FMD was related to events (HR: 0.705, 95% CI: 0.573-0.868, P=0.0010)</li></ul>  |

| <i>Karatzis et al.</i><br>(Karatzis et al. 2006) | 98 male survivors of acute coronary syndromes                                   | Cardiac events occurred in 20 patients   | <ul style="list-style-type: none"> <li>FMD predicted events (hazard ratio 3.0)</li> <li>FMD strongest independent predictor in this group</li> </ul>       |
|--|---|--|--|
| <i>Kitta et al.</i> (Kitta et al. 2005)          | 141 percutaneous coronary intervention patients. Bare metal stents              | In stent restenosis in 46 patients   | <ul style="list-style-type: none"> <li>Impaired follow up FMD independently associated with in stent restenosis</li> </ul>                                 |
| <i>Frick et al.</i> (Frick et al. 2005)          | 398 patients admitted for chest pain  | 44 events over 39 months follow up   | <ul style="list-style-type: none"> <li>FMD did not predict events</li> <li>IMT and CAD were the only predictors of events</li> </ul>                       |
| <i>Kitta et al.</i> (Kitta et al. 2009)          | 251 CAD patients with impaired FMD, receiving CV risk factor reduction therapy  | 27 (26%) events in those with persistently impaired FMD<br>15 (10%) events in those with improved FMD (p=0.01) | <ul style="list-style-type: none"> <li>Persistently impaired FMD was the strongest multivariate predictor (HR 2.9)</li> </ul>                              |
| <i>Fathi et al.</i> (Fathi et al. 2004)          | 444 patients at high risk of CV events  | 70 events over 24 months   | <ul style="list-style-type: none"> <li>Lowest FMD tertile (&lt;2%) had significantly more events</li> <li>FMD did not predict CV events</li> </ul>         |
| <i>Brevetti et al.</i><br>(Brevetti et al. 2003) | 131 peripheral vascular disease patients  | 39 events over 23 months   | <ul style="list-style-type: none"> <li>FMD was an independent predictor of events (RR, 4.8; 95% CI, 1.1 to 23.3; P&lt;0.05)</li> </ul>                     |
| <i>Modena et al.</i><br>(Modena et al. 2002)     | 400 post menopausal hypertensive women receiving 6 months BP lowering treatment | 99 events over 67 months   | <ul style="list-style-type: none"> <li>No improvement in FMD predicted risk of non fatal cardiac events (incident rate ratio, 7.3, p&lt;0.0001)</li> </ul> |



#### 2.4.2 Traditional cardiovascular risk factors

Endothelial dysfunction is observed in children with hypercholesterolemia, obesity and type 2 diabetes mellitus (Aggoun et al. 2005) and in adolescents who smoke (Celermajer et al. 1992). Celermajer *et al.* (Celermajer et al. 1993) demonstrated that, even in asymptomatic young adults (aged 15-57), cigarette smoking was associated with dose-related impairment of FMD. Celermajer *et al.* (Celermajer et al. 1996) also demonstrated the effects of passive smoking in healthy young adults, again reporting a dose response relationship between passive smoking and impaired FMD. There was no difference reported in the magnitude of FMD impairment between active and passive smokers.

Type 2 diabetes (T2D) and insulin resistance have been persistently linked with endothelial dysfunction (Avogaro et al. 1997; Hogikyan et al. 1998). Hyperglycaemia is thought to be critical in the development of endothelial dysfunction in T2D. Studies in healthy subjects have demonstrated that transient hyperglycemia induced by an oral glucose load acutely impairs endothelium-dependent vasodilation (Title et al. 2000). Furthermore, impaired endothelium-dependent flow-mediated dilation is closely linked to 3-hour postprandial blood glucose (Shige et al. 1999).

Significantly reduced FMD has been observed in obese children and adolescents when compared to lean, age matched controls (Watts et al. 2004a; Watts et al. 2004b; Woo et al. 2004). Although obesity *per se* is known to be detrimental to vascular health, it appears that body fat distribution may also play an important role in the determination of FMD. In adults, Arcaro *et al.* (Arcaro et al. 1999) found that visceral abdominal fat predicted the degree of endothelial dysfunction, independent of body weight. Obese



individuals often have related co-morbidities, making it difficult to define the precise mechanisms responsible for endothelial function. It has been suggested that the cause is multifactorial, with factors such as increased inflammation, oxidative stress and altered glucose metabolism implicated (Raitakari et al. 2004). Interventions that reduce body fat through caloric restriction result in improvement in endothelial function in both adults (Raitakari et al. 2004) and children (Woo et al. 2004). Similarly, exercise training improves FMD in obese children and adults, independent of changes in BMI (Watts et al. 2004a; Watts et al. 2004b; Woo et al. 2004). The mechanisms thought to mediate these endothelial improvement include reduction in plasma glucose concentration (Bell et al. 2007) associated with weight loss, which may attenuate the impaired FMD associated with obesity related hyperglycemia (Title et al. 2000).

### 2.4.3 Genetics

Genetic variation is likely to be an important determinant of the development of atherosclerosis and its clinical sequelae. Research has primarily focused on the genetic influence on late cardiac endpoints (Cambien et al. 1992). Less attention has been paid to the genetic determination of preclinical markers of atherosclerosis, such as endothelial function, and currently there are no data describing the role of genes in the determination of vascular function in paediatric populations.

The sparse literature pertaining to the role of genes in adult endothelial health has generated inconsistent results. Benjamin *et al.* (Benjamin et al. 2004) examined FMD in a cross section of ~2900 older adults from the Framingham Heart Study. Heritability was estimated at 0.14 using variance component methods. This modest heritability estimate implies that genes account for a relatively small amount of the variability in FMD, whilst environmental factors may exert a larger influence. To investigate the

heritability of FMD in greater detail, twin study designs have recently been employed. The classic twin study allows the partitioning of genetic and environmental influences on a trait, such as FMD, by comparing the intra-twin differences within groups of mono- (MZ) and di-zygotic (DZ) twins. As MZ twins are genetically identical and DZ twins share, on average, 50% of their genes, the ICC coefficient between MZ intra-twin differences should theoretically be 1 and the correlation between DZ twins 0.5 (half of the MZ value) if genetic factors are solely responsible for variation in a certain phenotype (Maia et al. 2002). If correlation coefficients remain in the same ratio (2:1), but are lower than these values, then both genes and also non-shared environmental factors are responsible for the variance. If the 2:1 ratio is not evident, then genes, non-shared environmental factors and shared environmental factors may all be contributing to the variation.

Jartii *et al.* (Jartti et al. 2002) examined the differences in FMD in twin pairs who were discordant for migration between Finland and Sweden, where CHD mortality is lower. Results demonstrated that in genetically identical MZ twin pairs aged between 42-69 years, the twin that had migrated to Sweden had superior endothelial function than their Finnish sibling. This finding suggests environmental factors exert an influence on FMD. The study yielded a heritability estimate of 0.24, indicating a stronger genetic component to FMD than previously suggested by Benjamin *et al.* (Benjamin et al. 2004). The final study to investigate genetic influences on FMD reported the strongest genetic contribution to FMD to date. Zhao *et al.* (Zhao et al. 2007) measured FMD in 94 middle aged male twin pairs. They produced a heritability estimate of 0.39, highlighting the possible role of heredity in the aetiology of atherosclerotic disease.

In summary, there are few studies that have attempted to determine the genetic contribution to FMD. In addition, these studies have been undertaken in adults and the results lack a cohesive outcome. Finally, no studies undertaken using twin sets have examined the impact of interventions such as exercise training on the heritability of changes in outcome measures. Further research in this area is needed to investigate the heredity of FMD in children.

#### **2.4.4 Sedentary behaviour**

The study of sedentary behaviour and its association with health outcomes is in its infancy. While evidence is beginning to emerge that links SB with CVD mortality, there are currently no data examining the relationship between SB and markers of future CVD risk such as FMD. Although SB has been linked with adiposity and metabolic profile in children (Ekelund et al. 2007). Given that FMD and SB are both associated with obesity and metabolic abnormalities, there is a clear rationale for research to be undertaken which provides insight into the relationship between these variables.

#### **2.4.5 Exercise and physical activity**

The beneficial effect of exercise training on endothelial function in clinical populations of adults is relatively clear (Green et al. 2003). Less evidence of the beneficial effect of exercise is available in paediatric populations who are at high future CV risk. Although numerous studies have confirmed that obesity induced endothelial function can be normalised through exercise training in both children and adolescents (Meyer et al. 2006; Watts et al. 2004a; Watts et al. 2004b; Woo et al. 2004), there are limited data examining the role of exercise training in other CV risk populations of this age group.



Intervention studies that aim to enhance already normal endothelial function in healthy populations have produced mixed results. Clarkson *et al.* (Clarkson *et al.* 1999) demonstrated an improvement in FMD following a 10 week programme of daily aerobic and anaerobic exercise training in military recruits. Conversely, in a randomised cross-over study of combined aerobic and resistance exercise in healthy middle-aged men, training did not significantly affect endothelium-dependent function (Maiorana *et al.* 2001). One possible explanation for these apparently disparate findings in healthy subjects relates to the time course of change in artery function and structure. Tinken *et al.* (Tinken *et al.* 2008) recently demonstrated that adaptations in arterial function occur rapidly in response to short term exercise training, however following the completion of 8 weeks of exercise training, endothelial function had returned to basal levels. The findings suggest that exercise-induced functional changes in conduit arteries precede structural adaptations. Moreover, the initial adaptation in function is attenuated as arterial remodelling occurs (figure 2.12). These data raise the possibility that previous studies of healthy adults have missed adaptations in endothelial function because they occur earlier than the time at which repeated assessments are derived.

**Figure 2.12** Time course of functional (FMD) and structural (CADC) arterial adaptation to exercise training (Tinken et al. 2008)

The studies described above have been undertaken using laboratory based and supervised exercise training interventions. At present there are few data examining the effect of physical activity on endothelial function in children. In a study of 47 5-10 year old children, habitual physical activity was the most influential variable on FMD when multivariate analysis was undertaken (Abbott et al. 2002). Pahkala *et al.* (Pahkala et al. 2008) measured FMD and physical activity in ~480 children and observed that, whilst there was a relationship between FMD and PA in boys, no such relationship was evident in girls, possibly due to the relatively lower PA levels evident in the cohort of girls. These studies indicate that daily leisure time PA could be an important mediator of endothelial health in children, and that the modification of endothelial function observed when exercise training is undertaken could be a reflection of an increase in PA .



## **2.5 Summary and research aim**

Overt CVD is preceded by a long preclinical stage of atherogenesis which has its origins in childhood. The earliest detectable manifestation of atherosclerotic disease is endothelial dysfunction, which appears to be a sentinel event in the development of CVD. The health of the endothelium can be measured using ultrasonic assessment of FMD. A strong correlation has been reported between brachial artery FMD and coronary artery function, and moreover, impaired FMD is an independent predictor of adverse cardiac events. Attenuation of FMD is evident in subjects, including children, with CV risk factors such as smoking, obesity, diabetes and hyperlipidemia. However, endothelial dysfunction can be normalised by interventions, including exercise training, that modify CV risk factors. Yet there are large gaps in the paediatric literature pertaining to the interaction of factors such as genetics, PA, SB and fitness level with FMD. The aim of this thesis is to provide evidence in these areas, where knowledge is lacking. We aim:

1. To address the issue of scaling vascular outcomes for body size/composition components in children
2. Explore the associations between FMD and body composition, cardiorespiratory fitness and physical activity using a cross section of healthy children.
3. Determine whether FMD is affected by seasonal variability, and assess the associations with seasonal changes in body composition and physical activity in healthy children.
4. Investigate the basal association between FMD and sedentary behaviour, and address the question of whether change in sedentary behaviour is related to change in endothelial function in healthy children.

## **2.5 Summary and research aim**

Overt CVD is preceded by a long preclinical stage of atherogenesis which has its origins in childhood. The earliest detectable manifestation of atherosclerotic disease is endothelial dysfunction, which appears to be a sentinel event in the development of CVD. The health of the endothelium can be measured using ultrasonic assessment of FMD. A strong correlation has been reported between brachial artery FMD and coronary artery function, and moreover, impaired FMD is an independent predictor of adverse cardiac events. Attenuation of FMD is evident in subjects, including children, with CV risk factors such as smoking, obesity, diabetes and hyperlipidemia. However, endothelial dysfunction can be normalised by interventions, including exercise training, that modify CV risk factors. Yet there are large gaps in the paediatric literature pertaining to the interaction of factors such as genetics, PA, SB and fitness level with FMD. The aim of this thesis is to provide evidence in these areas, where knowledge is lacking. We aim:

1. To address the issue of scaling vascular outcomes for body size/composition components in children
2. Explore the associations between FMD and body composition, cardiorespiratory fitness and physical activity using a cross section of healthy children.
3. Determine whether FMD is affected by seasonal variability, and assess the associations with seasonal changes in body composition and physical activity in healthy children.
4. Investigate the basal association between FMD and sedentary behaviour, and address the question of whether change in sedentary behaviour is related to change in endothelial function in healthy children.



5. Using a classic twin study design to assess the heritability of FMD in healthy children.
6. Determine the heritability of change in FMD in monozygotic and dizygotic twins in response to 8 weeks of high intensity, gym based exercise training.

## **Chapter 3**

### **General Methods**



### **3.1. Inclusion criteria and testing conditions**

Subjects recruited to all studies in this thesis were healthy, normotensive and had not been diagnosed with any cardiovascular disease/risk factors, or metabolic conditions. Subjects were not using any medications and did not smoke. All studies were approved by the Liverpool John Moores Ethics Committee and subjects, and their parents where indicated, gave written informed consent or assent.

Vascular testing was performed in a dedicated and temperature controlled (21-23°C) laboratory, which allowed testing to be undertaken quietly and in isolation, minimizing psychological stress.

On arrival at the laboratory, all procedures were fully explained to participants and consent was obtained. All subjects were studied at the same time of day to control for the impact of circadian variation. As physical activity can affect the assessment of artery function, subjects were required to complete these tests prior to any exercise tests that may also have been undertaken. Subjects arrived at the laboratory following a minimum of a 4 hour fast, an 8 hour abstinence from alcohol and caffeine, and having avoided strenuous activity for 24 hours. Prior to conduit artery assessment participants were required to lay supine on a bed.

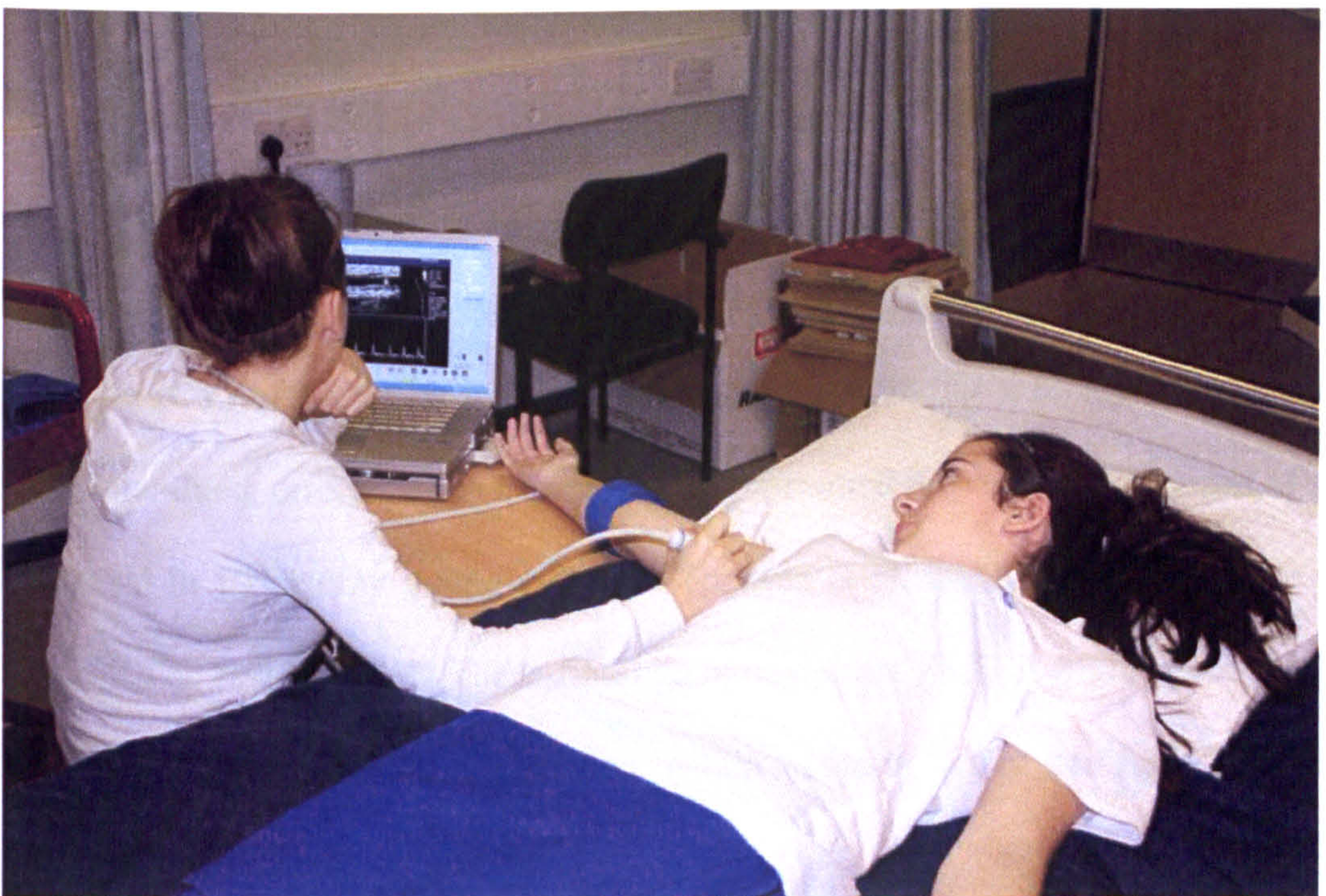
### **3.2 Methodologies and techniques**

#### **3.2.1 Assessment of conduit artery endothelial function**

Flow mediated dilation (FMD) was used to assess endothelium-dependent vasodilatation of the brachial artery. Brachial artery internal diameter was measured using high resolution ultrasonography (Acuson, Aspen, Siemens Corp, Malvern, Penn and Terason, t3000, Teratech,) with a 10-12-MHz probe to visualise the brachial artery



in longitudinal section. B mode images were obtained at a reproducible point in the distal third of the upper arm (figure 3.1). Ultrasonic parameters were set to optimize longitudinal, two-dimensional B mode images of the luminal-arterial wall interface with the focal zone set to the near wall. Once set, these parameters remained fixed and the probe was held in a constant position. Continuous Doppler velocity assessment was obtained using the lowest possible insonation angle (always  $<60^\circ$ ). Nitric oxide (NO)-mediated endothelial function was assessed by measuring the flow mediated dilation (FMD) in response to a 5 minute ischemic stimulus (Mullen et al. 2001), induced by forearm cuff inflation. A one minute baseline measurement was taken prior to cuff inflation. A pneumatic rapid cuff inflator (Hokanson, Bellevue, U.S.A), placed around the forearm distal to the humeral epicondyles, was inflated to 220mmHg for 5 minutes (Corretti et al. 2002) to induce forearm ischemia. Recording of the image ceased on inflation of the cuff and was recommenced 30 seconds prior to deflation. Recording continued for a period of 3 minutes post cuff deflation.



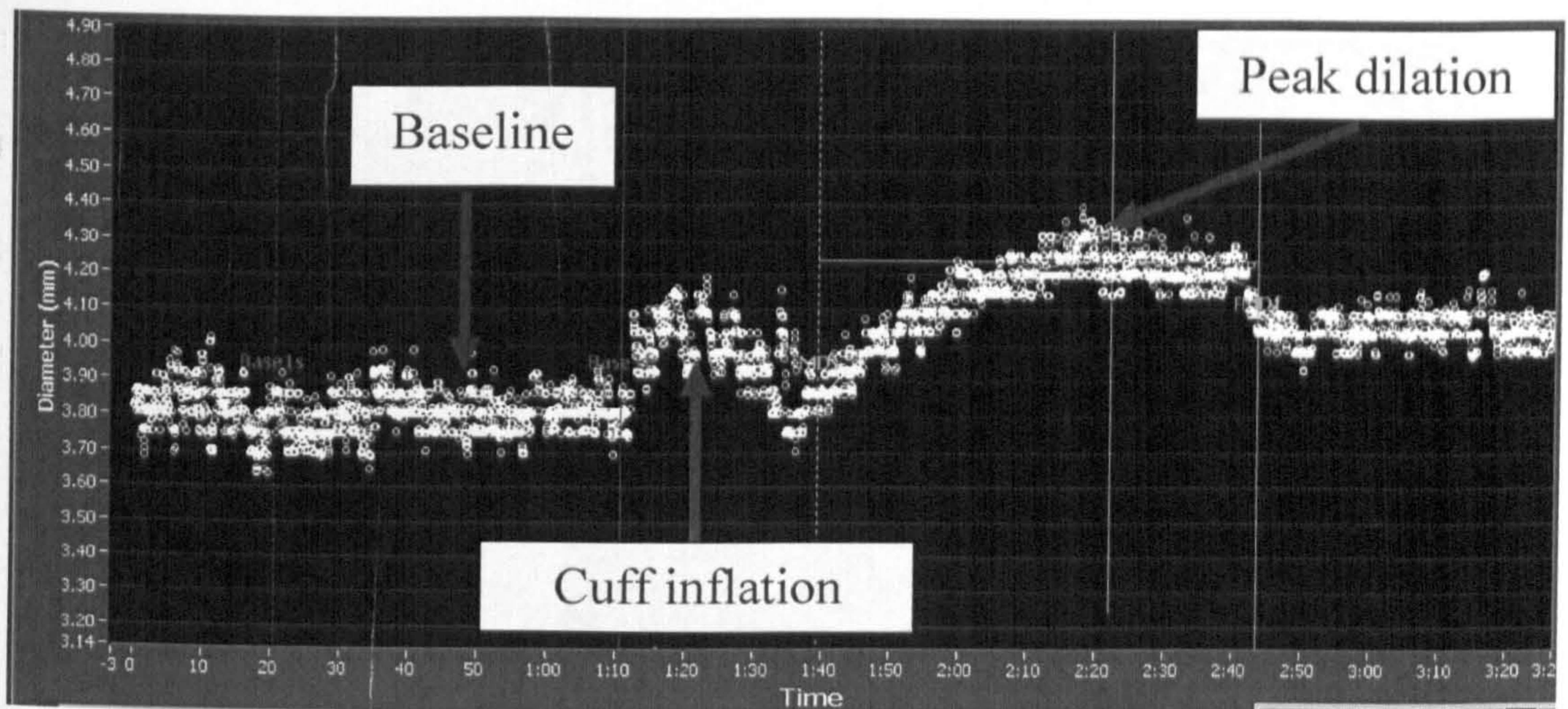
**Figure 3.1** Terason, t3000, Teratech used to assess FMD.



### **3.2.2 Post test analysis of artery diameter**

Post-test analysis of brachial artery diameter was performed with the use of custom designed automated edge-detection and wall-tracking software. B-mode frames are assessed by automated edge-detection software through the use of pixel density and frequency distribution algorithm (Figure 2.7). An optimal region of interest was selected from the first B-mode image based on the quality of the image and discrimination between the intima/lumen. This region typically incorporates around 300 pixel columns, which are then averaged to provide a mean diameter for each frame, with subsequent frames analyzed at a rate of 30Hz. Although the initial selection of the area of interest is operator determined, all proceeding analysis is carried out without investigator bias. Following cuff deflation, peak diameter was automatically detected using an algorithm which identifies the maximum bracket of data subsequent to performance of a moving window smoothing function (Figure 3.2) (Black et al. 2008). We have undertaken comprehensive reproducibility analyses of repeated FMDs using the above techniques. In an early study from our group, using comparable data acquisition and automated observer-independent analysis techniques as applied in the current thesis, repeated FMD of the brachial artery performed at the same time of day, within 1 week in 24 healthy volunteers (Woodman et al. 2001). We found a coefficient of variation (CV) of 14.7%, indicating good reproducibility for a biological parameter. We also examined, on 3 separate occasions, the reproducibility of FMD assessed at a 30 min interval in 10 healthy young subjects (Thijssen et al. 2009). The CVs were 6.7, 10.9 and 9.5%, respectively. These findings indicate that FMD is highly reproducible when repeated measures are undertaken within subjects under carefully controlled experimental conditions.





**Figure 3.2** User interface from FMD analysis software, showing calculation of baseline and peak diameters.

### 3.2.3 Assessment of cardiorespiratory fitness

The gas analyser (Jaeger Oxycon Pro, Warwick, UK) was calibrated before each testing session. Once calibration was complete, participant's age, gender, height and weight were entered into the system. Each subject wore a harness, adjusted for individual height and weight. A face mask was then placed around the nose and mouth and the pneumotach attached onto the face mask. Resting breaths were measured prior to exercise.

A discontinuous treadmill based protocol (figure 3.3) was used to determine peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ) in studies 2 and 3. As the cohort was relatively young, a discontinuous protocol was chosen in an attempt to prevent subjects from ending the test as a result of muscle fatigue. The protocol required subjects to initially walk at 4 and 6  $\text{km}\cdot\text{h}^{-1}$ , then run at 8, 10, 12, and 14  $\text{km}\cdot\text{h}^{-1}$ , to the point of volitional exhaustion. The test consisted of 3 minute stages, followed by a 30 second rest interval.





**Figure 3.3** A child undertaking  $\text{VO}_{2\text{peak}}$  test (left pane). A hip mounted accelerometer worn during the  $\text{VO}_{2\text{peak}}$  test, and used to measure habitual physical activity (right pane).

For studies 5 and 6 there was large variations in both the chronological and biological age of the participants. Consequently,  $\dot{V} \text{O}_{2\text{peak}}$  test speeds were calibrated individually by anchoring treadmill speeds to set Froude numbers. Dynamic similarity theory states that geometrically similar bodies that utilise pendulum like mechanics for movement will have similar gait dynamics if the Froude number is kept constant (Alexander 1989). The theory implies that a human's optimal walking speed will be at a Froude number of 0.25 and the transition from walk to run will occur close to a Froude number of 0.5 regardless of differences in body size (Minetti 2001).

The Froude number is calculated using the equation  $\text{Fr} = v^2/(g \cdot l)$ , where  $v$  is speed of movement ( $\text{m.s}^{-1}$ ),  $g$  is gravity, and  $l$  is a characteristic length (in this case leg length, m). Using this equation it is possible to calculate walking/running speeds at set Froude



numbers to accommodate differences in children's ages and maturation status. As the majority of subjects in studies 5 and 6 were adolescent, it was deemed appropriate to use a continuous protocol. Subjects completed 2 minute stages, beginning with a walking speed equivalent to Fr 0.25. At the completion of the walking speed stage, the speed was increased to the equivalent of Fr 0.5. Subsequent increments were determined by the difference in the speed for stages 1 and 2 ( $\sim 2\text{km}\cdot\text{hr}^{-1}$ ) until the point of volitional exhaustion.

During all  $\text{VO}_{2\text{peak}}$  tests oxygen consumption ( $\dot{V}\text{O}_2$ ) and carbon dioxide production ( $\dot{V}\text{CO}_2$ ) were measured breath-by-breath using an online gas analysis system (Jaeger Oxycon Pro, Warwick, UK). Throughout the test, all participants wore a heart rate monitor (Polar, Kempele, Finland) and a uni-axial accelerometer (GT1M model, Actigraph, Florida, USA). Peak  $\dot{V}\text{O}_2$  was determined as the highest 15-s averaged oxygen uptake achieved during the test when the following criteria were satisfied: a respiratory exchange ratio  $\geq 1.0$ ; HR plateau prior to final stage in protocol if HR had reached  $\geq 195$ . Subjective indicators of maximal effort were also taken into account, these included: unsteady gait; hypernea; facial flushing and sweating.

#### **3.2.4 Assessment of physical activity (PA) and sedentary behaviour (SB)**

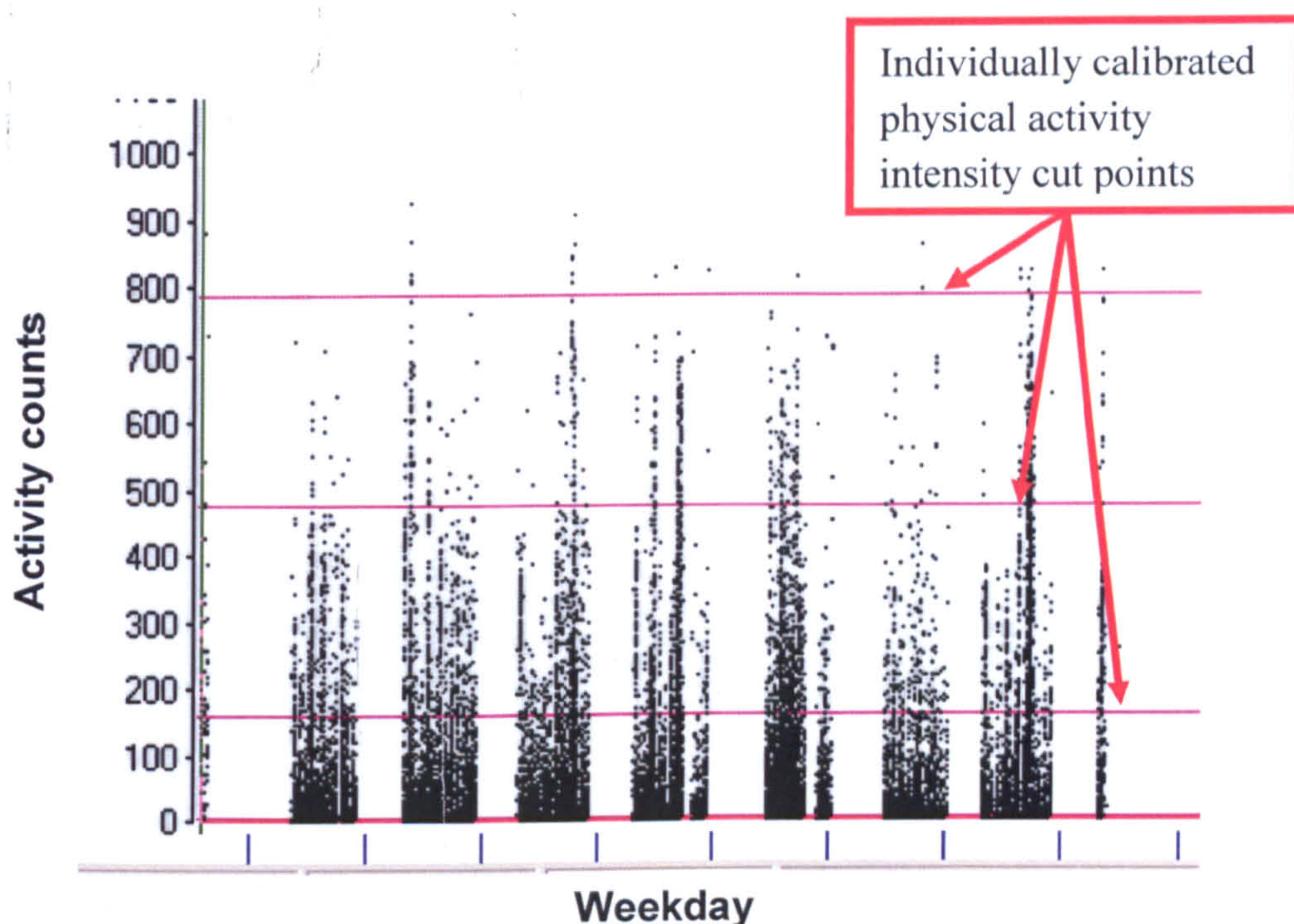
Physical activity was objectively measured using a uni-axial accelerometer (GT1M model, Actigraph, Florida, USA) for seven consecutive days. The accelerometer was programmed to record PA data (raw activity counts and steps) every 5 seconds. On the first day of monitoring, the children underwent a familiarisation period. At the end of the data collection period, all data were downloaded and checked for compliance. Sustained 20 minutes periods of zero counts were deemed to indicate that the accelerometer had been removed, and total missing counts for those periods represented



the duration that the monitors were not worn (Catellier et al. 2005). For inclusion in the analyses, children were required to have produced counts for at least 9 hours a day for at least three days (Mattocks et al. 2008).

In studies 2 and 3 accelerometry data from the peak oxygen uptake test were used to determine individual thresholds for each child at each of the set treadmill speeds (4, 6, 8, 10 km.hr<sup>-1</sup>). Using individual count thresholds and a sedentary threshold of 100 counts per minute, (Treuth et al. 2004) time in minutes per included day spent between 4 and 5.99 km.hr<sup>-1</sup> (PA<sub>4</sub>), 6 and 7.99 km.hr<sup>-1</sup> (PA<sub>6</sub>), 8 and 9.99 km.hr<sup>-1</sup> (PA<sub>8</sub>) and above 10 km.hr<sup>-1</sup> (PA<sub>10</sub>) were determined for each individual. Similarly, for studies 5 and 6, accelerometry data from the peak oxygen uptake test and a sedentary threshold of 100 counts per minute were used to determine the time spent per valid day sedentary, between sedentary and treadmill speed one (PA<sub><Fr0.25</sub>, equivalent to a brisk walk), treadmill speed one and treadmill speed two (PA<sub>Fr0.25-0.5</sub>, equivalent to a light jog) and ≥ treadmill speed three (PA<sub>>Fr0.5</sub>, equivalent to a run) was established (figure 3.4). In addition, the total steps and total PA (average accelerometer counts/minutes of valid recording; CPM) were obtained for each day. The total time spent above each speed for each valid day (total activity divided by number of days) were separated into weekday (Monday-Friday), weekend (Saturday and Sunday) and weighted whole week data, where the weekdays provided 5/7ths of weekly activity and the weekends provided 2/7ths.





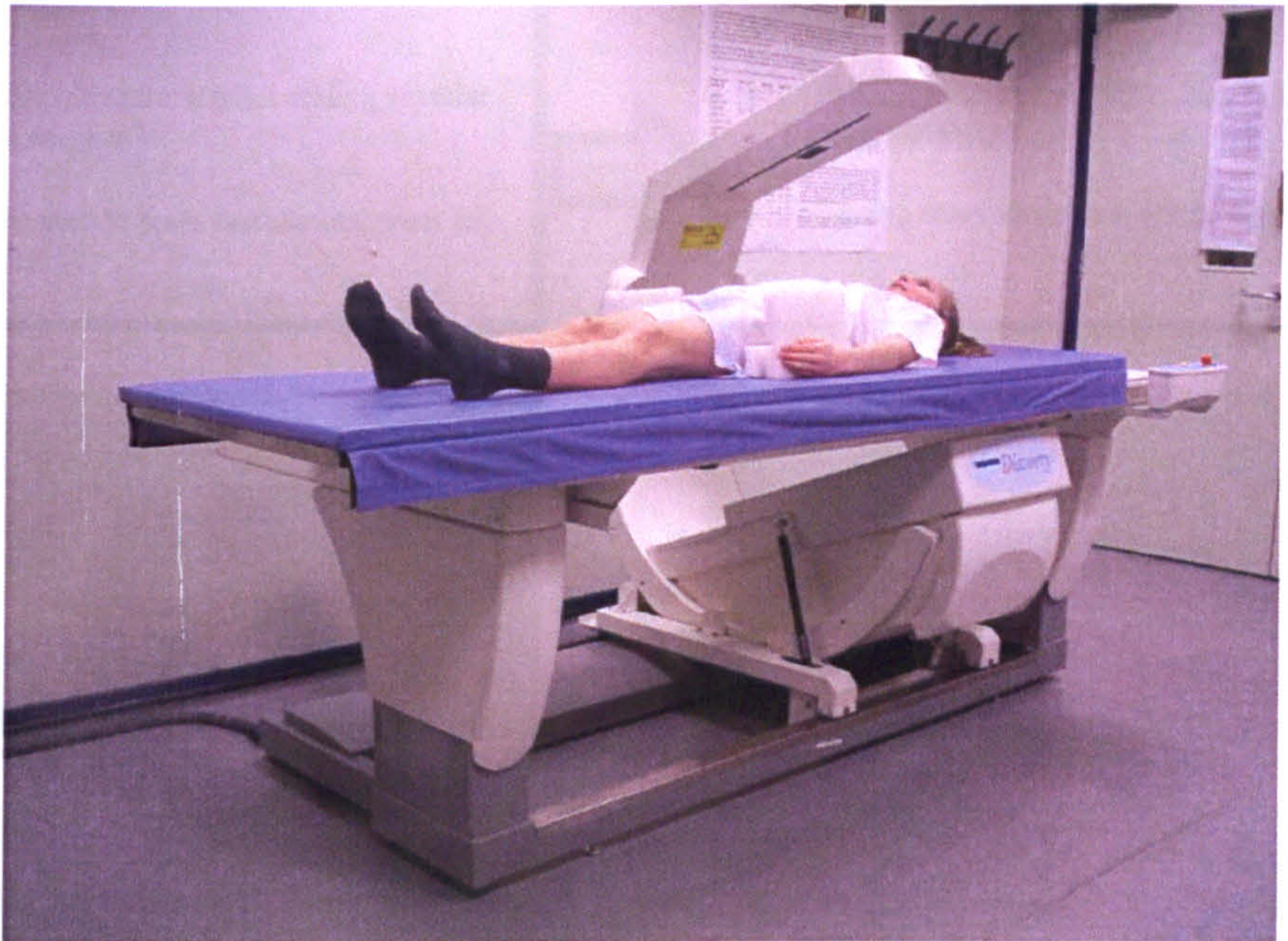
**Figure 3.4** Example of physical activity output generated from the Mahuffe analysis software

### 3.2.5 Assessment of body composition

Body mass and stature were recorded using standard methods. Dual-energy x-ray absorptiometry (DXA) (Hologic QDR Series Discovery A, Bedford, MA) was used to determine whole body, body fat, bone mineral density and mineral free/ fat free residual tissue in each subject. Participants wore shorts and a t-shirt and were required to remove all metal items from their person. A whole body fanbeam scan was taken for each subject according to standardized procedures recommended by the manufacturer (QDR Discovery A, Hologic, MA.). Calibration using the manufacturer's phantom was performed on the day of each scan. Subjects were required to lie supine and remain motionless for the duration of the scan (approximately 3 min) (figure 3.5). Post test analysis was completed using QDR for windows software version 12:4:3. All scans were performed by trained operators. Analysis was carried out by a single technical operator. Values obtained and reported included body fat mass, abdominal fat mass,



body lean mass and percentage body fat mass. Percentage body lean mass was calculated manually from the DXA derived outputs.



**Figure 3.5** Assessment of body composition using dual-energy x-ray absorptiometry (DXA).



# Study aims and outcomes

## Chapter 4

**Rationale** – Cardiac literature implies scaling vascular outcomes may be necessary

**Aim** -Examine the need to scale vascular outcomes for body size variables.

## Outcome

- Possible need to scale artery diameter for lean mass variables
- Scaling artery function is not necessary.

## Chapter 5

**Rationale** – Few studies explore determinants of vascular health in children

## Outcomes

- Weak associations between VF, measures of PA and fat and  $VO_{2peak}$
- High intensity PA was the only significant

## Chapter 6

**Rationale** – No study has assessed whether seasonal variation exists in FMD.

## Outcomes

- FMD and PA significantly decreased between summer and late autumn.
- Change in high intensity PA was the only

## Chapter 7

**Rationale** – Despite an increase in sedentary behaviour, no evidence links SB with VF.

## Outcome

- FMD is not associated with SB
- Seasonal decline in FMD is not related to the increase in SB

## Chapter 8

**Rationale** – In adults heritability of FMD appears to be relatively low. No data exists in children

**Aim** – Explore the genetic contribution to FMD using

## Outcome

- FMD is under moderate genetic control, heritability was estimated at 0.44
- Fat and  $VO_{2peak}$  are highly genetically

## Chapter 9

**Rationale** – The heredity of exercise induced change in FMD is yet to be determined

## Outcome

- Change in FMD, fat and  $VO_{2peak}$  are under a large amount of genetic control,
- Heritability of FMD was estimated at 0.72



## Chapter 4

# **SCALING BRACHIAL ARTERY DIAMETER AND FUNCTION FOR ANTHROPOMETRIC VARIABLES**

**Based on the publication in the American Journal of Physiology  
(Heart and circulation) 2009, entitled “Does conduit artery  
diameter vary according to the anthropometric characteristics of  
children or men?”**

**and the article under review at the European Journal of Applied  
Physiology entitled “Does brachial artery flow-mediated dilation  
scale to anthropometric characteristics?”**

**(Please refer to appendix for articles)**



### 3 Introduction

In sport, exercise and health science research, quantitative assessment of different components of the cardiovascular system is used to inform such practices as pre-participation screening in athletes (Papadakis et al. 2008) and monitoring of cardiovascular disease risk and progression (Deanfield et al. 2007). However, the comparison of these measurements between, and sometimes within, subjects can be confounded by differences in body size and composition. This has led to the adoption of a number of scaling and normalisation procedures to improve the validity of scientific and clinical interpretation (Batterham et al. 1999). From a theoretical perspective the nature of the relationship between cardiovascular structures and body size has been shown to relate across multiple orders of magnitude. West et al. (West et al. 1997) have proposed a general allometric model to describe scaling relations of the mammalian circulatory system. We have chosen to develop empirical data related to body size and brachial artery dimensions within this theoretical landscape.

Measurements of arterial structure and function are commonly undertaken to assess adaptations to acute and chronic exercise in both health and disease (Green et al. 2004a). Despite the widespread appreciation of scaling practices in research investigating cardiac structures, and the consequent adoption of various cardiac indices (Batterham et al. 1999), the relevance of scaling for body size and composition has not previously been addressed in the context of arterial measurement. This is surprising when one considers that adaptation in artery size and function often occur simultaneously with changes in body size and composition (Huonker et al. 2003; Zeppilli et al. 1995). For example, changes in femoral artery diameter, along with muscle mass, occur rapidly in paraplegic athletes, and unilateral differences in artery size exist between the limbs of below knee amputees and single handed elite racquet



sportsmen (Olive et al. 2003). Furthermore excess body fat has a negative impact upon endothelial function and FMD (Arkin et al. 2008; Oflaz et al. 2003), implying measures of body fatness may therefore need to be taken into account when comparing FMD across groups.

If between- or within-subject comparisons of vascular measures are to be performed, two questions arise. Which measures of body size and composition, if any, determine artery size and function, and what form should any scaling process take. Whereas simple ratio scaling procedures (i.e.  $y/x$ ) have been widely adopted to correct for heart size and function (Brown and Thompson 1987; Fleck et al. 1989), recent studies have suggested that the most appropriate body size independent indices may result from allometric scaling approaches ( $y/x^b$ ) (Batterham et al. 1997; Daniels et al. 1995). Allometric scaling allows for non-linear relationships, as opposed to simple ratio scaling that assumes *a priori* a linear relationship that passes through the origin (i.e.  $b$  exponent of 1).

The purpose of this study was to empirically determine the nature of the relationship between arterial diameter and measures of body and limb size and composition in a cross section of children. In addition we sought to compare the utility of applying ratio and allometric scaling processes to these variables.

## **4.1 Methods**

### **4.1.1 Participants**

One hundred and twenty nine pre pubescent children aged 9-10 yrs (75♀, 54♂) were recruited. All subjects were sequentially studied without specific inclusion criteria. Participants were not taking any vasoactive medications, did not smoke and were



moderately active. Ethics approval was obtained from Liverpool John Moores University Ethics Committee. Informed consent was obtained prior to participation in the study.

#### **4.1.2 Experimental design and procedures**

Participants were required to attend a laboratory on one occasion to complete anthropometric and vascular assessments. Brachial artery diameter was measured after a period of at least 20 minutes quiet rest. All measurements were taken in a temperature controlled room and after at least a 4 hour fast and at least 8 hours abstinence from caffeine or alcohol. All subjects avoided strenuous physical activity for 24 hours prior to testing.

For detailed methodology for arterial measurement please see sections 3.2.1 and 3.2.2.

#### **4.1.3 Anthropometric measurements**

In addition to the protocol for assessment of body composition outlined in section 3.2.5, off line analysis using the software inherent to the DXA system provided whole-body and segmental (whole arms and forearms) values for total mass, fat mass and lean mass (body mass-BM, lean body mass-LM, body fat mass-FM, whole arm mass-AM, arm lean mass-ALM, arm fat mass-AFM, Forearm mass-FAM, forearm lean mass-FLM, and forearm fat mass-FFM).

#### **4.1.4 Statistical analysis**

Statistical analyses were performed using SPSS 14.0 (SPSS) software. All data are reported as group means  $\pm$  SD, and statistical significance was assumed at  $p \leq 0.05$ . Initially, Pearson's product-moment correlation coefficient analysis examined the correlations between brachial artery diameter and FMD, and all body composition



variables. Diameter was then scaled via simple ratio and allometric approaches for individual differences in BM, LM, FM, AM, ALM, AFM, FAM, FLM, and FFM. Ratio scaling involved dividing brachial artery diameter ( $y$ ) by the range of body size parameters (e.g. body mass;  $x$ ) and computing an index ( $y/x$ ). Confidence intervals (95%) were generated for each of the correlation coefficients.

Initial multivariate analysis with a gender and a gender by body size index term were carried out. Gender by body size interactions were non-significant, indicating that there were no gender differences in the relation of arterial size to body size variable. Common power function exponents were therefore generated for boys and girls. Univariate allometric scaling was then used to identify a power function ( $b$ ) exponent. This was achieved via log-log transformation of brachial artery and body size data that was then analysed via least squares linear regression. Separate  $b$  ( $\pm$  95% confidence intervals) exponents were calculated for each body size variable.

To assess whether ratio or allometric scaling (or both) could produce size-independent indices of brachial artery diameter, the scaled variable ( $y/x$  or  $y/x^b$ ) was then correlated with the body size index ( $x$ ). If significant correlations were still evident, this illustrates that the influence of size had not been fully removed.

## **4.2 Results**

Table 4.1 presents anthropometric and vascular data for the cohort. Mean ( $\pm$ SD) BMI was 19.30 ( $\pm$  3.26). Significant correlations existed between baseline brachial artery diameter and measures of whole body/segmental mass and lean mass as well as height. No significant correlations were evident between brachial artery diameter and whole body or segmental measures of fat mass (Table 4.2). Significant correlations were also



evident between FMD and measures of whole body fat and segmental fat mass. Correlations between FMD and whole body and segmental measures of total and lean mass were non significant (Table 4. 2).

Significant correlations provide some evidence that body size and brachial artery diameter are associated and, hence, this association with body size should be removed to facilitate inter- and intra-group comparisons.

Data from the Pearson’s correlations ( $y/x:x$  or  $y/x^b:x$ ) were used to check the size-independence of both vascular outcomes (table 4.3). To aid interpretation of the difference between ratio and allometric-scaling, brachial artery dimensions scaled for BM have been plotted in Figure 4.1. For both brachial artery diameter and FMD indices it is clear that simple ratio scaling is not appropriate. In fact, ratio scaling may over-correct for body size, introducing a size bias which would penalise larger sizes. In contrast, allometric scaling produced size-independent indices

**Table 4.1.** Body composition and vascular measures

|                               | Mean $\pm$ SD   |
|-------------------------------|-----------------|
| Height (m)                    | 1.43 $\pm$ 0.07 |
| Whole Body Mass (kg)          | 39.3 $\pm$ 9.5  |
| Whole body lean mass (kg)     | 28.0 $\pm$ 5.0  |
| Whole body fat mass (kg)      | 10.5 $\pm$ 6.0  |
| Whole arm mass (kg)           | 2.1 $\pm$ 0.5   |
| Whole arm lean mass (kg)      | 1.3 $\pm$ 0.3   |
| Whole arm fat mass (kg)       | 0.7 $\pm$ 0.4   |
| Forearm mass (kg)             | 0.9 $\pm$ 0.2   |
| Forearm lean mass (kg)        | 0.6 $\pm$ 0.1   |
| Forearm fat mass (kg)         | 0.2 $\pm$ 0.1   |
| Baseline artery diameter (mm) | 3.1 $\pm$ 0.4   |
| FMD (%)                       | 10.1 $\pm$ 4.5  |



Table 4.2 Correlates for baseline artery diameter and FMD

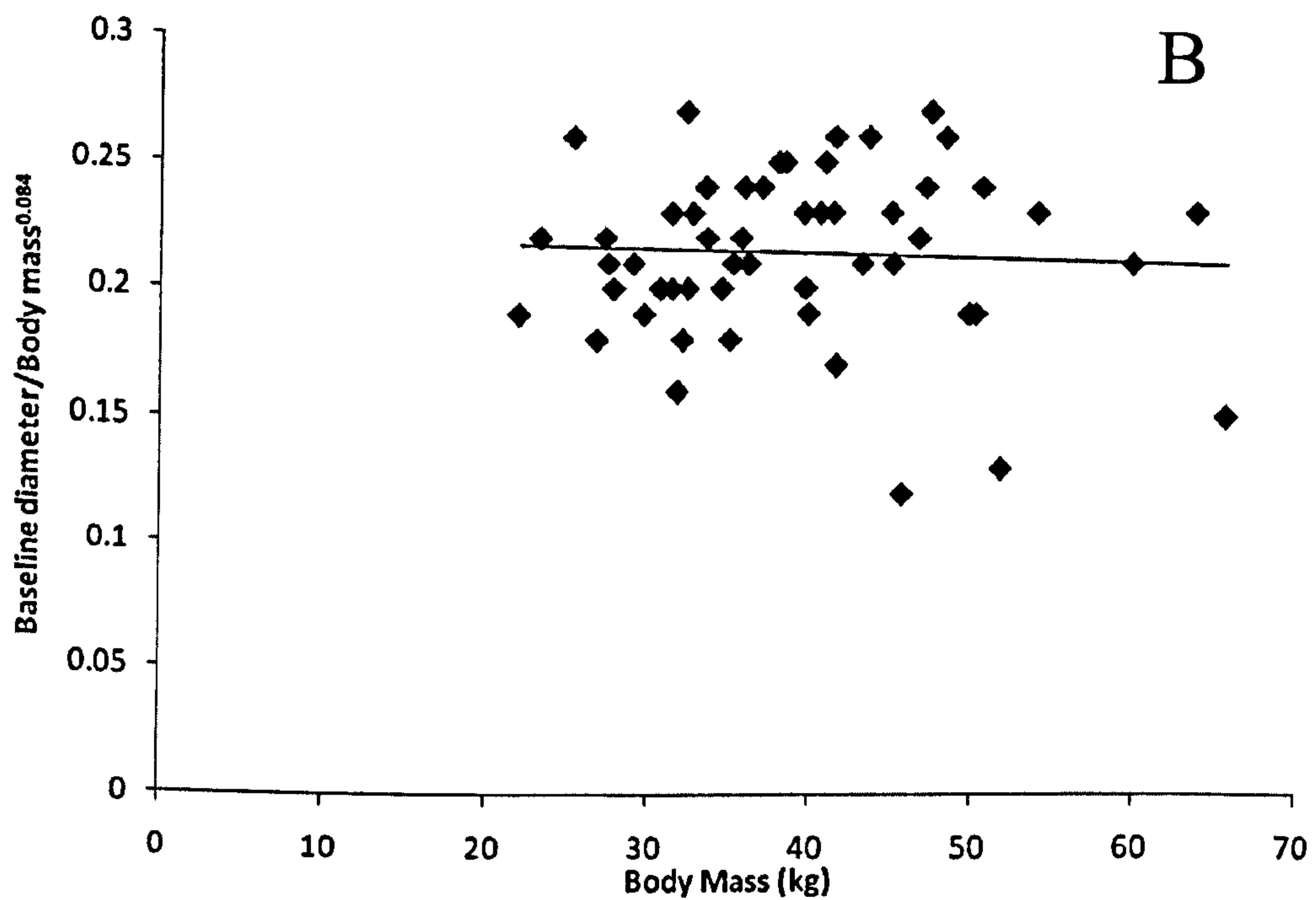
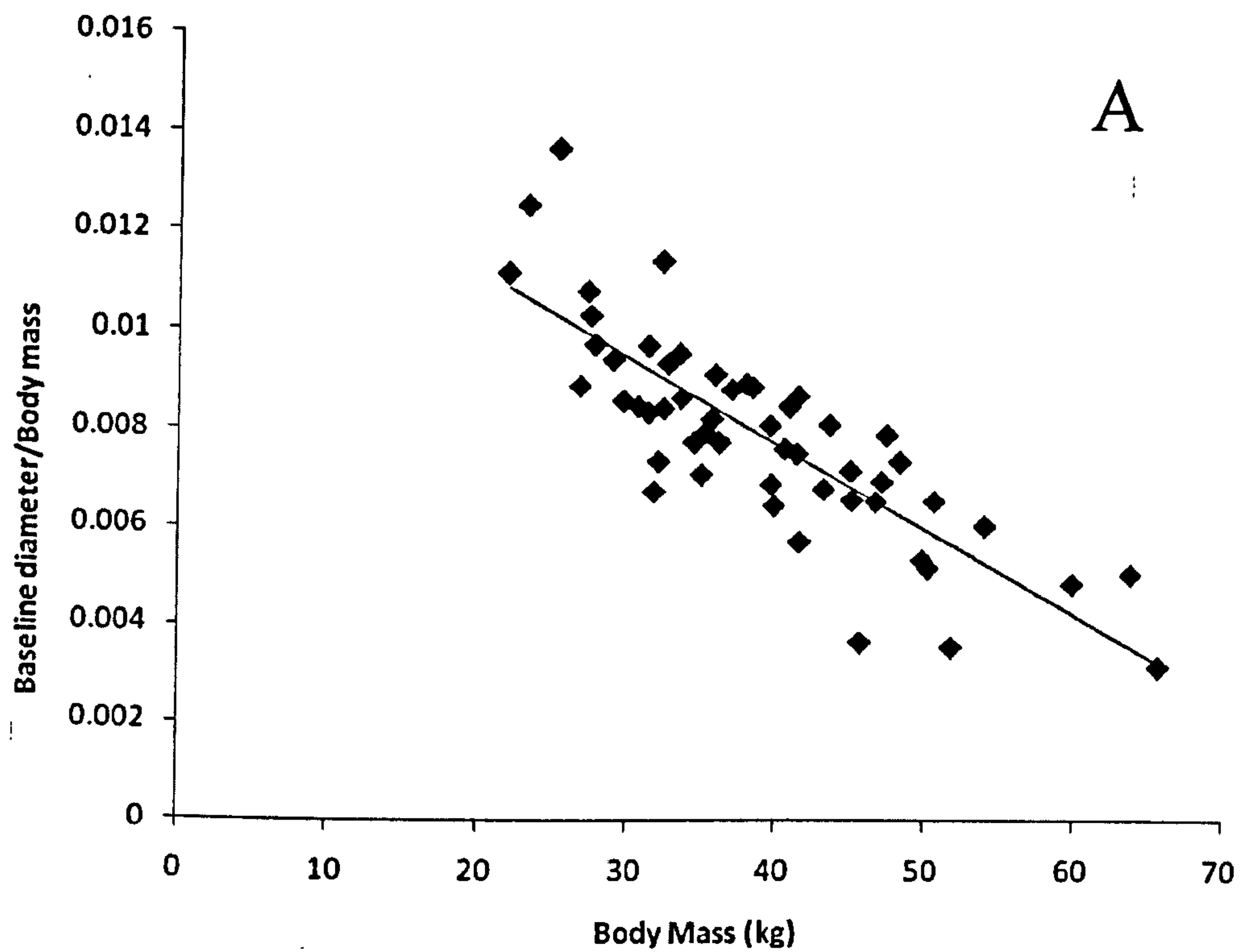
|                      | Baseline Diameter |         |               |         | FMD     |                |
|----------------------|-------------------|---------|---------------|---------|---------|----------------|
|                      | r value           | P value | CI            | r value | P value | CI             |
| Height               | 0.22              | 0.01*   | 0.05 to 0.38  | -0.14   | 0.11    | -0.31 to 0.03  |
| Whole body mass      | 0.21              | <0.01*  | 0.04 to 0.38  | -0.14   | 0.12    | -0.31 to 0.03  |
| Whole body lean mass | 0.28              | <0.01*  | 0.11 to 0.43  | -0.09   | 0.27    | -0.26 to 0.08  |
| Whole body fat mass  | 0.09              | 0.33    | -0.08 to 0.26 | -0.15   | 0.10    | -0.31 to 0.08  |
| Whole arm mass       | 0.23              | 0.01*   | 0.06 to 0.39  | -0.13   | 0.13    | -0.30 to 0.04  |
| Whole arm lean mass  | 0.32              | <0.01*  | 0.16 to 0.47  | -0.01   | 0.88    | -0.31 to 0.03  |
| Whole arm fat mass   | 0.06              | 0.51    | -0.11 to 0.23 | -0.18   | 0.05*   | -0.34 to -0.01 |
| Forearm mass         | 0.24              | <0.01*  | 0.07 to 0.4   | -0.14   | 0.12    | -0.31 to 0.03  |
| Forearm lean mass    | 0.31              | <0.01*  | 0.15 to 0.46  | -0.03   | 0.77    | -0.20 to 0.14  |
| Forearm fat mass     | 0.07              | 0.44    | -0.10 to 0.24 | -0.19   | 0.03*   | -0.35 to -0.02 |



**Table 4.3.** Correlations ( $y/x:x$  or  $y/x^b:x$ ) used to check the size-independence of vascular outcomes

|   | <b>r value</b> | <b>P value</b> | <b>CI</b>      |
|---|----------------|----------------|----------------|
| Baseline diameter/Height:Height               | -0.16          | 0.08           | -0.32 to 0.02  |
| Baseline diameter/Height <sup>b</sup> :Height | 0.15           | 0.01           | -0.03 to 0.31  |
| Baseline diameter/BM:BM                       | -0.79          | <0.01          | -0.85 to -0.72 |
| Baseline diameter/BM <sup>b</sup> :BM         | 0.00           | 0.99           | -0.17 to 0.18  |
| Baseline diameter/BLM:BLM                     | -0.71          | <0.01          | -0.79 to -0.62 |
| Baseline diameter/BLM <sup>b</sup> :BLM       | 0.02           | 0.83           | -0.16 to 0.19  |
| Baseline diameter/AM:AM                       | -0.81          | <0.01          | -0.87 to -0.74 |
| Baseline diameter/AM <sup>b</sup> :AM         | 0.01           | 0.90           | -0.16 to 0.19  |
| Baseline diameter/ALM:ALM                     | -0.74          | <0.01          | -0.82 to -0.66 |
| Baseline diameter/ALM <sup>b</sup> :ALM       | 0.03           | 0.73           | -0.14 to 0.21  |
| Baseline diameter/FAM:FAM                     | -0.77          | <0.01          | -0.84 to -0.70 |
| Baseline diameter/FAM <sup>b</sup> :FAM       | 0.02           | 0.81           | -0.15 to 0.20  |
| Baseline diameter/FLM:FLM                     | -0.71          | <0.01          | -0.79 to -0.61 |
| Baseline diameter/FLM <sup>b</sup> :FLM       | 0.03           | 0.70           | -0.14 to 0.21  |
| FMD/AFM:AFM                                   | -0.63          | <0.01          | -0.72 to -0.51 |
| FMD/AFM <sup>b</sup> :AFM                     | -0.02          | 0.84           | -0.19 to 0.16  |
| FMD/FFM:FFM                                   | -0.62          | <0.01          | -0.71 to -0.50 |
| FMD/FFM <sup>b</sup> :FFM                     | -0.03          | 0.73           | -0.20 to 0.14  |





**Figure 4.1.** Relationship between body mass and (a) baseline brachial artery diameter scaled using the simple ratio method for body mass and (b) baseline brachial artery diameter scaled by allometric scaling for body mass.



### **4.3 Discussion**

To our knowledge this is the first study to empirically explore the issue of body size scaling of conduit artery diameter and function. The findings from the current study illustrate five main points; 1) that whole body and upper limb mass and lean mass data, but not fat mass, correlate significantly with artery diameter, 2) although relationships were modest, measures of segmental adiposity were significantly associated with FMD 3) simple ratio scaling, does not fully remove the influence of any body size variable upon arterial measures, 4) allometric scaling may facilitate body size independent comparisons of arterial diameter and FMD between groups and, 5) as the correlations between FMD and body composition were relatively weak, the rationale for adoption of scaling approaches remains debatable.

Previous studies which have undertaken within- or between-group comparisons to assess the impact of exercise training and inactivity on arterial diameter have not typically attempted to account for differences in body composition (Dinenno et al. 2001; Naylor et al. 2006; Tinken et al. 2008). However, Olive *et al.* (Olive et al. 2003) corrected femoral artery diameter for muscle volume in a between-groups comparison of healthy and spinal cord injured participants, using a simple ratio scaling approach. Scaling in this manner decreased the magnitude of between group differences in femoral artery diameter and maximal hyperaemic blood flow response. Despite a conceptual “step-in-the-right-direction” it is pertinent to emphasize that the empirical relationship between artery diameter (a one-dimensional measure) and muscle volume (a 3-dimensional measure) would be unlikely to meet the criteria for simple ratio scaling, as was the case in the current study. Such data should be revisited and the use of allometric scaling investigated and compared to simple ratio scaling. In another study, de Groot *et*

*al.* (de Groot et al. 2006) assessed the time course of adaptation in leg vascular dimensions within the first 6 weeks following spinal cord injury. The researchers found femoral artery diameter and leg volume decreased simultaneously, and were largely accomplished within 3 weeks. A similar dimensional criticism could be made of this interpretation although formal scaling was not undertaken. These previous studies were not specifically designed to address the question of the importance of scaling procedures, but they infer that some measures of body composition impact upon the magnitude of difference in artery diameter in comparative studies.

There is an established body of evidence in the literature to support the use of scaling procedures when assessing differences in measures of cardiac size and structure (George et al. 1998). It has now become commonplace to use scaling when comparing cardiac variables such as left ventricular mass and cardiac output. This has typically been done using the simple ratio methods. However, George *et al.* (1998) have suggested that the use of such simplistic ratio scaling may not completely remove the influence of body size variables. In contrast, allometric scaling approaches appear to be more effective (George et al. 2001). In keeping with these findings relating to scaling of cardiac variables, our data indicate that allometric scaling is the more effective method of producing body size independent arterial diameter results.

As both total mass and lean mass emerged as influential variables on baseline artery diameters, we suggest that, in 'normal' healthy populations, one is a surrogate marker for the other. The implications of these findings are that valid scaling of artery diameter in normal weight populations does not necessitate the use of DXA derived body composition data, but can be performed adequately using simple measurement of total body mass. Whether or not these findings can be extrapolated to typical patient



populations, in whom adipose tissue constitutes a larger proportion of the body mass, is unknown and requires further investigation. Segmental measures also had an influence on artery diameter which was similar to whole body values. We therefore suggest that investigations comparing artery diameter use allometric scaling to total body mass, although we would prompt individual studies/researchers to generate their own cohort-specific scaling data.

Our findings demonstrate that although FMD was associated with segmental measures of body fat, these relationships were weak, implying that the influence of body fat on FMD in this group of normal, 'healthy' children may be negligible. Consequently, although allometric scaling of FMD for body fat appears to produce size independent indices, allometric scaling may be redundant in this cohort. Yet several studies have demonstrated stronger, significant inverse relationships between body fat and FMD in overweight or obese populations (Raitakari et al. 2004; Sciacqua et al. 2003). The weak associations in the current study could therefore be partially a size effect, as our cohort was relatively lean (Table 4.1). We can only speculate that the relationship between body fat and vascular function might become apparent when over fatness is evident. Future studies should therefore be undertaken to empirically determine whether or not scaling can be used in overweight/obese populations.

There are several limitations to the current study. The group was relatively homogeneous for body composition and thus application of generated allometric exponents to groups such as overweight and obese children, who are most frequently the target of interventions to alter vascular and body composition parameters, is beyond this study. Our study did not include clinical groups (e.g. CV diseases), as such, the utility of the recommended scaling procedures in such populations remains unknown.

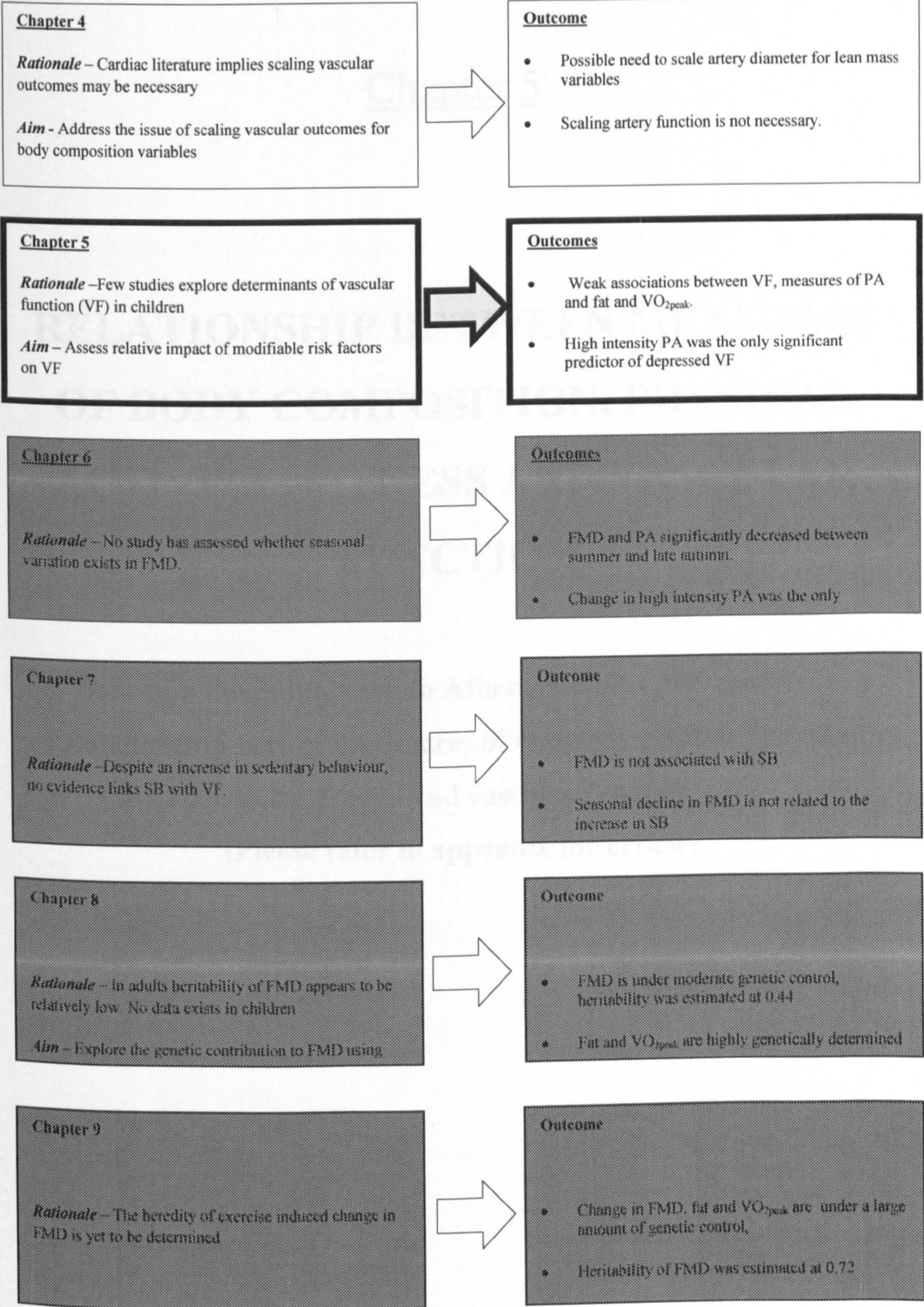
Finally, we measured the brachial artery only, thus we cannot comment on the utility of scaling for body composition variables at other vascular sites.

#### **4.4 Conclusion**

We found significant relationships between brachial artery diameter and measures of whole body and regional mass and lean mass, and between FMD and body fat. Whilst simple ratio scaling did not completely remove the influence of body size variables, allometric scaling produced size-independent indices of arterial diameter in cohorts. However, as the correlations between FMD and body composition were relatively weak, the rationale for adoption of scaling approaches remains debatable. Further studies will be necessary before the widespread adoption of scaling procedures can be advocated.



# Study aims and outcomes





## Chapter 5

# **RELATIONSHIP BETWEEN MEASURES OF BODY COMPOSITION, PHYSICAL ACTIVITY, FITNESS AND VASCULAR FUNCTION**

**Based on the publication in Atherosclerosis 2009, entitled:  
“Relationship between measures of body composition, physical  
activity, fitness and vascular function”  
(Please refer to appendix for article)**



## **5. Introduction**

Excess body fat, physical inactivity and poor cardiorespiratory fitness are increasing in prevalence and have been identified as independent predictors of future CV events and/or mortality (Mossberg 1989; Berlin and Colditz 1990; Lee et al. 1999). However, little is known about the effect of these modifiable risk factors on preclinical markers of future CVD risk in children. The purpose of this study was therefore, to gain insight into the relationships between brachial artery vascular endothelial function and measures of body composition, cardio-respiratory fitness and physical activity (PA) in young people.

Endothelial dysfunction is considered an early and integral manifestation of atherosclerotic disease (Vita and Keaney 2002), which can be evident in the first decade of life (Skilton et al. 2005; Stary 1989) and can be measured using flow mediated dilation (Celermajer et al. 1992). Impaired FMD is present in children and adolescents with CV risk factors such as cigarette smoking, hyperlipidemia, diabetes and hypertension (Celermajer and Ayer 2006) and obesity (Watts et al. 2004b; Woo et al. 2004). Exercise training enhances NO-mediated vasodilator function in obese children (Watts et al. 2004b; Woo et al. 2004) and adolescents (Watts et al. 2004a). Some of the latter effect occurs independently of changes in BMI and body weight (Watts et al. 2005). Taken together, these data raise the possibility that fitness and physical activity may be at least as important, in terms of cardiovascular risk, as overweight or obesity and that the latter may possibly owe their predictive capacity to the fact that they are surrogate markers for inactivity or low fitness (Blair et al. 1996; Wessel et al. 2004). However, no previous studies have attempted to examine the complex relationships between fitness, PA, body composition and vascular endothelial function in children.



## **5.1 Methods**

### **5.1.1 Participants**

145 subjects (59 male, 86 female) aged  $10.30 \pm 0.03$  yr ( $\pm$ SD) were recruited. Subjects were randomly selected from a database of approximately 2000 9-10 year olds participating in the SportsLinX fitness project (Taylor et al. 2004). All subjects were sequentially studied without specific exclusion criteria, and were healthy, normotensive and not suffering from known cardiovascular or metabolic conditions. Ethics approval was obtained from Liverpool John Moores University Ethics Committee. Informed consent was obtained from parents/guardians and children prior to participation in the study.

### **5.1.2 Experimental design and procedures**

Subjects were required to attend a laboratory on one occasion to complete a number of physiological examinations including body composition assessment,  $\dot{V} O_{2\text{peak}}$  testing and assessment of conduit artery function. All subjects were studied at the same time of day to control for the impact of circadian variation. Subjects were informed that they must fast on the morning of testing and avoid any PA. All measurements were taken in a quiet temperature controlled room. Please refer to section 3.2 for detail on the methodologies used in this study.

### **5.1.3 Normalization of FMD for the eliciting shear rate**

In accordance with the most up to date recommendations (Pyke and Tschakovsky 2007), FMD data from this study were normalized according to the shear rate (SR) stimulus which elicits the endothelium-dependent dilation. Correction of FMD for its eliciting stimulus was recently recommended where comparisons are made between subjects who have arteries of different size, since shear rate (and hence the stimulation



to diameter change) is dependent upon artery size. It was suggested that correction, or “normalisation”, of FMD for its eliciting shear stimulus allows for valid comparison of the artery function, independent of size-related differences that may exist in eliciting shear between individuals. Normalisation therefore allows for real differences in arterial function to be distinguished from differences which may result from a different stimulus magnitude. Evidence suggests that the area under the curve of shear rate following cuff deflation is the most appropriate measure of the stimulus to FMD (FMD/SR<sub>AUC</sub>) (Pyke and Tschakovsky 2005; Pyke and Tschakovsky 2007). We therefore chose to use these guidelines to correct the FMD response for the shear rate AUC stimulus until peak diameter.

#### **5.1.4 Statistical analysis**

Statistical analyses were performed using SPSS 14.0 (SPSS) software. Results are expressed as the mean±SD. Data distribution was initially examined for normality using the Shapiro-Wilk test. For results obtained that were below the boundary of normality, which was set at 0.05, non parametric equivalent tests were used. The effect of maturation was assessed by entering TPHV and the dependent variables (DV) into a correlation analysis, where significant relationships were observed maturation was considered to be a confounder. For those dependent variables where maturation was considered a confounder, a forwards linear regression was performed with TPHV entered as the independent variable, if a significant outcome was observed standardised residuals were calculated and used in place of the original data.

Data for boys and girls were compared using unpaired Student's *t*-tests or the Mann Whitney test. Spearman's correlations were performed on the entire data set to evaluate



the strength of relationships between variables. Further to this, stepwise backwards linear regression was carried out to identify predictors of FMD and FMD/SR<sub>AUC</sub>. The whole cohort was then split into tertiles for FMD and FMD/SR<sub>AUC</sub>, and Spearman's correlations and regression analyses were performed within the tertiles.

## **5.2 Results**

No differences between the sexes existed in age, height, body mass, BMI, and systolic and diastolic blood pressures (Table 5.1). In addition, there was no difference between the sex sub-groups in years to peak height velocity.

Compared to boys, girls exhibited significantly lower  $\dot{V} O_{2peak}$  and time spent at both  $<6 \text{ km.hr}^{-1}$  and  $\geq 6 \text{ km.hr}^{-1}$ . Girls also demonstrated significantly higher fat mass and resting HR. Whilst baseline brachial artery diameter was slightly larger in boys, no difference existed between the groups in terms of brachial artery function (FMD or FMD/SR<sub>AUC</sub>) or time to peak diameter. For subsequent analysis FMD data were therefore pooled.

The mean ( $\pm$ SD) values for the children's  $\dot{V} O_{2peak}$  and percentage of  $\dot{V} O_{2peak}$  for each PA intensity are shown in Table 5.2.



**Table 5.1** Descriptive characteristics (mean±SD)

|   |            | <b>Group</b> | <b>Males</b>  | <b>Females</b> | <b>P</b>      |
|---|------------|--------------|---------------|----------------|---------------|
|   | <b>(n)</b> |              | <b>(n=59)</b> | <b>(n=86)</b>  | <b>(MvsF)</b> |
| <b>Age (y)</b>  | 145        | 10.33±0.31   | 10.36±0.30    | 10.32±0.31     | 0.41          |
| <b>Body Mass (kg)</b>   | 145        | 39.5±9.2     | 39.3±1.0      | 39.6±0.9       | 0.89          |
| <b>Height (m)</b>   | 145        | 1.42±0.07    | 1.43±0.07     | 1.42±0.07      | 0.88          |
| <b>TPHV (y)</b>   | 145        | -3.43±0.39   | -3.41±0.38    | -3.44±0.40     | 0.63          |
| <b>BMI</b>  | 145        | 19.3±3.3     | 19.2±3.5      | 19.3±3.1       | 0.63          |
| <b>Fat mass (kg)</b>  | 144        | 11.3±5.4     | 10.6±5.9      | 11.8±5.0       | 0.04          |
| <b>%fat mass (DXA)</b>  | 144        | 27.1±6.9     | 24.5±7.1      | 28.9±6.1       | 0.00          |
| <b>Lean mass (kg)</b>   | 144        | 27.2±5.3     | 28.3±4.9      | 26.7±5.5       | 0.11          |
| <b>%lean mass (DXA)</b>   | 144        | 70.1±8.8     | 73.1±6.6      | 68.0±9.5       | 0.00          |
| <b>Systolic BP (mmHg)</b>   | 145        | 107±10       | 109±11        | 106±9          | 0.09          |
| <b>Diastolic BP(mmHg)</b>   | 145        | 64±5         | 64±6          | 63±4           | 0.21          |
| <b><math>\dot{V} O_2</math> ml.kg<sup>-1</sup>.min<sup>-1</sup></b> | 141        | 45±6         | 47±6          | 43±6           | 0.00          |
| <b>PA&lt;6 km (min)</b>   | 132        | 65.3±26.8    | 72.0±28.6     | 60.9±24.8      | 0.02          |
| <b>PA≥6 km (min)</b>  | 132        | 28.1±18.1    | 36.6±22.4     | 22.5±11.8      | 0.00          |
| <b>FMD (%)</b>  | 129        | 10.1±4.5     | 10.2±4.3      | 10.0±4.6       | 0.75          |
| <b>FMD/AUC (x 10<sup>4</sup>)</b>                                   | 129        | 2.97±1.78    | 3.23±2.07     | 2.78±1.52      | 0.51          |



**Table 5.2** Percentage  $\dot{V} O_{2peak}$  at treadmill speeds for boys and girls (mean $\pm$ SD)

|   | Boys             | Girls          |
|---|------------------|----------------|
| $\dot{V} O_{2peak}$ (ml.kg <sup>-1</sup> .min <sup>-1</sup> ) | 47.1 $\pm$ 5.4** | 42.6 $\pm$ 5.7 |
| % $\dot{V} O_{2peak}$ at 4km.h <sup>-1</sup>                  | 38.3 $\pm$ 4.5*  | 40.6 $\pm$ 5.5 |
| % $\dot{V} O_{2peak}$ at 6km.h <sup>-1</sup>                  | 54.9 $\pm$ 6.2** | 58.5 $\pm$ 6.6 |
| % $\dot{V} O_{2peak}$ at 8km.h <sup>-1</sup>                  | 78.3 $\pm$ 7.0** | 85.2 $\pm$ 6.1 |
| % $\dot{V} O_{2peak}$ at 10km.h <sup>-1</sup>                 | 91.3 $\pm$ 5.5** | 95.3 $\pm$ 4.5 |

Significant t-test sex results, \* boys vs. girls,  $p < 0.05$ ; \*\* boys vs. girls,  $p < 0.01$

**5.2.1 Relationships between FMD and other variables**

Spearman’s correlation coefficient revealed a weak negative correlation between FMD and %fat ( $r=-0.18$ ,  $p=0.04$ ), no further significant correlations were observed for FMD. Significant correlations were, however, found between FMD/SR<sub>AUC</sub> and body composition measurements including percentage fat mass ( $r=-0.23$ ,  $p=0.009$ ) and percentage lean mass ( $r=0.21$ ,  $p=0.02$ ). In addition a significant correlation was observed between TPHV and FMD/SR<sub>AUC</sub> ( $r=-0.24$ ,  $p=0.008$ ).

A significant correlation also existed between FMD/SR<sub>AUC</sub> and  $\dot{V} O_{2peak}$  ( $r=0.25$ ,  $p=0.004$ ). In terms of PA, FMD/SR<sub>AUC</sub> was correlated with time spent at 6 km.hr<sup>-1</sup> ( $r=0.28$ ,  $p=0.014$ ), time spent at 10 km ( $r=0.318$ ,  $p=0.007$ ), time spent above 6km ( $r=0.36$ ,  $p=0.001$ ), CPM ( $r=0.298$ ,  $p=0.008$ ) and number of steps ( $r=0.25$ ,  $p=0.028$ ).

### 5.2.2 Tertile analysis

In addition to the above analysis, the cohort was split into tertiles for FMD and FMD/SR<sub>AUC</sub>, the principal outcomes of interest. The mean $\pm$ SD for each group is shown in table 5.3. Correlation and regression analysis were subsequently carried out within each of these tertiles.

#### *Tertile analysis for FMD*

Spearman's correlation revealed that for the lowest FMD tertile, there was a significant correlation between FMD and PA<sub>8</sub> ( $r=0.38$ ,  $p=0.004$ ) and PA<sub>10</sub> ( $r=0.43$ ,  $p=0.026$ ). No significant correlations existed for variables within the middle or upper FMD tertile.

Regression analysis performed within the lowest FMD tertile revealed that PA<sub>10</sub> predicted FMD accounting for 13% of the variance, with  $p$  values approaching significance ( $R^2=0.13$ ,  $p=0.065$ ). No variables significantly predicted FMD in the middle or highest tertile.

#### *Tertile analysis for FMD/SR<sub>AUC</sub>*

Analysis of lowest tertile for FMD/SR<sub>AUC</sub> resulted in significant correlations between FMD/SR<sub>AUC</sub> and PA<sub>8</sub> ( $r=0.57$ ,  $p=0.003$ ), and PA<sub>10</sub> ( $r=0.48$ ,  $p=0.03$ ). No relationships were evident in the middle and upper tertile.

Regression analysis for FMD/SR lowest tertile revealed 21.2% of the variance in FMD/SR<sub>AUC</sub> could be accounted for by PA<sub>8</sub> ( $R^2=0.212$ ,  $p=0.023$ ). There were no further significant outcomes from the regression



Table 5.3 Comparison of means across FMD and FMD/SR tertiles (mean±SD)

|                                       | FMD % Tertiles |            |           | FMD/SR Tertiles |            |             |
|---------------------------------------|----------------|------------|-----------|-----------------|------------|-------------|
|                                       | 1 (Lower)      | 2 (Middle) | 3 (Upper) | 1 (Lower)       | 2 (Middle) | 3 (Upper)   |
| FMD                                   | 5.4±1.7*       | 9.8± 1.3*  | 15.3±2.4* | 7.00±3.04*      | 9.90±3.81* | 13.00±3.98* |
| FMD/AUC (x 10 <sup>4</sup> )          | 2.0±1.0*       | 2.7±1.0*   | 4.4±2.0*  | 1.4±5.0*        | 2.5±3.0*   | 4.9±1.6*    |
| Fat%                                  | 28.0±6.9       | 28.3± 6.4  | 25.5± 6.8 | 29.5*±6.7       | 26.7± 6.8  | 25.9*±6.3   |
| $\dot{V} O_2$ ml.kg.min <sup>-1</sup> | 44.3±6.1       | 43.2±5.8   | 46.1±5.4  | 42.6*±5.2       | 45.3±6.6   | 45.7*±5.5   |
| PA <sub>10</sub>                      | 5.4±5.1        | 6.6±6.1    | 7.0±5.9   | 4.7*±3.8        | 5.8±5.1    | 8.4*±6.9    |

\* Significant ANOVA results,  $p < 0.05$

### **5.3 Discussion**

To our knowledge this is the first study to examine relationships between FMD, appropriately corrected for its eliciting stimulus (Pyke and Tschakovsky 2005; Pyke and Tschakovsky 2007), and variables associated with increased CV risk. The results of this study in a cohort of 129, 10-11 year old children, indicate that significant correlations exist between high intensity PA and vascular function, particularly for those subjects in the lowest FMD tertile. No such relationships were observed between PA levels and FMD in the upper tertiles for vascular function. Although there were modest global relationships between body fat, lean mass and  $\dot{V} O_{2peak}$ , there were no correlations within tertiles between FMD and measures of either fitness or fatness. These data therefore suggest that interventions focused on increasing the amount of high intensity PA, rather than enhancing cardiorespiratory fitness or reducing body fat *per se*, may be most beneficial in terms of improvement of vascular function in subjects at risk of future cardiovascular disease.

Much recent focus has concentrated on the rapid increase in overweight and obesity levels in children and adolescents. In adults, obesity is associated with the development of insulin resistance, type 2 diabetes and micro- and macro-vascular disease and it is also associated with endothelial dysfunction in both adults (Arcaro et al. 1999) and children (Geilen and Hambrecht 2004). However, some epidemiological evidence suggests that obesity *per se* may be a less powerful predictor of vascular event risk and all cause mortality than physical inactivity (Farrell et al. 2002; Wessel et al. 2004) or low cardiopulmonary fitness (Blair et al. 1996). Due to limitations associated with self-report measures of physical in/activity used in these large cohort studies, and the attractiveness of  $\dot{V} O_{2peak}$  as an optimum measure of functional capacity, a focus on



cardiopulmonary fitness as a predictor of risk has emerged (Blair et al. 1996). However, the use of objective monitors in recent years has enabled the assessment of children's PA levels in free-living situations, and in the present study we adopted a novel and valid approach to quantify the intensity at which PA was undertaken, based on individual cut points related to treadmill velocity. We found that PA measurements were the best predictors of endothelial dysfunction in this unbiased and typical sample of school children aged 10-11 yrs. If these findings are confirmed, then a subtle shift in the focus of prevention, from merely reducing levels of overweight/obesity or increasing fitness, toward increasing PA time and intensity, should be supported as evidence-based. Of course interventions such as appropriately prescribed exercise training, which enhance endothelial function in obese (Watts et al. 2004b; Woo et al. 2004) children, will simultaneously increase PA, fitness and improve body composition (Watts et al. 2005).

Whilst it may be argued that all interventions aimed at decreasing fatness or increasing fitness/PA are laudable, some previous findings suggest that different interventions do, in fact, induce divergent physiological adaptations. After a 6 week intervention program, Woo et al. (Woo et al. 2004) observed increases in FMD in overweight/obese children who were assigned to either dietary modification alone or dietary modification plus exercise training. However, after 1 year, further improvement in FMD occurred only in those subjects in whom exercise was undertaken. Further to these findings, Watts *et al*, established that endothelial dysfunction in obese children and adolescents could be normalised through exercise training independent of dietary modification and in the absence of change in BMI (Watts et al. 2004a; Watts et al. 2004b). The studies of both Woo and Watts induced improvements in vascular health via implementation of relatively high intensity exercise training programmes in obese subjects. Although

neither study directly measured the intensity of PA levels before or after intervention, improvements in FMD due to increases in PA cannot be ruled out and were in fact likely.

One previous study, carried out in 45 children aged 5-10 yrs, related habitual PA and brachial FMD (Abbott et al. 2002). Physical activity, assessed using the doubly labelled water approach, was the most influential variable in terms of correlation with FMD ( $r = 0.39$ ,  $P = 0.007$ ). This study did not assess fitness, DXA body composition or the relative intensity of PA, and did not normalise FMD to the shear stress stimulus, but the results essentially lend support to the findings of the current investigation and to the hypothesis that regular PA exerts a cardio-protective effect through its direct interaction with the vascular endothelium.

Several potential mechanisms exist which might explain the relationships we observed between PA and endothelial function. It is well established that exercise training can enhance vascular endothelial function in both peripheral and coronary arteries (Green et al. 2004b), and Hambrecht and colleagues (Hambrecht et al. 2003) have suggested that exercise training improves endothelial function *in vivo* by up regulating eNOS protein expression and by increased phosphorylation of this enzyme. These findings are consistent with a shear stress stimulus for enhanced NO bioactivity as a result of increased PA. Alternate explanations for the positive relationship between PA and endothelial function may relate to the impact of exercise on oxidative stress and inflammation.

There are several limitations to the current study. The study population was sequentially studied without specific exclusion criteria, hence the investigation did not



specifically target children who were overweight/obese, or of any particular fitness level. Although approximately 1/3<sup>rd</sup> of the children in the study that were classed as overweight, only 15 subjects could be defined as obese. With regard to  $\dot{V} O_{2peak}$  results, the data of 28 subjects were less than 40 ml.kg<sup>-1</sup>.min<sup>-1</sup>, and 28 subjects achieved a  $\dot{V} O_{2peak}$  of over 50 ml.kg<sup>-1</sup>.min<sup>-1</sup>. The study population was therefore typical in terms of both body composition and fitness level. It is possible that different relationships between variables may be present in selected sub-samples, but this study was not powered to assess these.

#### **5.4 Conclusion**

We found that measures of body fatness,  $\dot{V} O_{2peak}$ , and moderate-to-vigorous PA significantly correlated with arterial function in 10-11 year olds. However, PA above 8km.hr<sup>-1</sup> was the most important variable in terms of influence on those with impaired vascular function. Assuming these findings can be replicated, they suggest that future intervention strategies in children should prioritise increases in high intensity PA, which should in turn result in a concomitant reductions of fat mass and increases in fitness. Exercise should remain a core component of any lifestyle intervention undertaken in children or adults.

#### **Normalization of FMD for the eliciting shear rate stimulus**

Although we have presented FMD data normalised for the eliciting shear rate stimulus in the above study, recent data from our group (Atkinson et al. 2009) have highlighted that the normalisation of FMD for the shear rate stimulus may result in the violation of several statistical assumptions. We therefore believe the question of the most appropriate methods to normalise FMD is an open one, and indeed, the question of whether to normalise FMD at all remains controversial. We have hence forth presented

FMD data only. If the data from the above chapter pertaining to FMD is analysed for raw FMD% only, the principle findings from the whole cohort are altered slightly, associations between vascular function, physical activity and fitness are no longer evident. However, when data is examined by tertile for FMD% there is little difference in the principle outcome, that is, depressed endothelial function is predicted by high intensity PA only.



# Study aims and outcomes

## Chapter 4

**Rationale** – Cardiac literature implies scaling vascular outcomes may be necessary

**Aim** - Address the issue of scaling vascular outcomes for body composition variables

## Outcome

- Possible need to scale artery diameter for lean mass variables
- Scaling artery function is not necessary.

## Chapter 5

**Rationale** – Few studies explore determinants of vascular health in children

**Aim** – Assess relative impact of modifiable risk factors on VF

## Outcome

- Weak associations between VF, measures of PA and fat and  $VO_{2peak}$ .
- High intensity PA was the only significant predictor of depressed VF

## Chapter 6

**Rationale** – No study has assessed whether seasonal variation exists in FMD.

**Aim** – Explore the relationships between seasonal  $\Delta$ FMD and  $\Delta$  PA and body composition.

## Outcome

- FMD and PA significantly decreased between summer and late autumn.
- Change in high intensity PA was the only significant predictor of  $\Delta$ FMD

## Chapter 7

**Rationale** – Despite an increase in sedentary behaviour, no evidence links SB with VF.

## Outcome

- FMD is not associated with SB
- Seasonal decline in FMD is not related to the increase in SB

## Chapter 8

**Rationale** – In adults heritability of FMD appears to be relatively low. No data exists in children

**Aim** – Explore the genetic contribution to FMD using

## Outcome

- FMD is under moderate genetic control, heritability was estimated at 0.44
- Fat and  $VO_{2peak}$  are highly genetically

## Chapter 9

**Rationale** – The heredity of exercise induced change in FMD is yet to be determined

## Outcome

- Change in FMD, fat and  $VO_{2peak}$  are under a large amount of genetic control
- Heritability of FMD was estimated at 0.72



## Chapter 6

# **ASSOCIATIONS BETWEEN SEASONAL DECLINE IN VASCULAR FUNCTION AND PHYSICAL ACTIVITY**

**Based on the article of the same title under revision at MSSE**



## **6 Introduction**

The prevalence of overweight and obesity has increased in recent decades in many western countries (Hossain et al. 2007; Stratton et al. 2007). Accompanied by physical inactivity (Pate et al. 1995), these changes have led to speculation that the beneficial cardiovascular impact of public health interventions in past decades may be compromised in future and that life expectancy may decrease in line with the rising impact of obesity and type 2 diabetes on cardiovascular events (Olshansky et al. 2005).

Our data from chapter 5 highlighted the existence of significant correlations between measures of PA and vascular function in pre-pubertal children. However, to our knowledge, there are currently no studies which have collected repeated measurements to address the question of whether variation in the intensity of physical activity affects endothelial function. We therefore aimed to assess relationships between these variables in a group of boys and girls aged 10-11 years studied in summer (June) and again in late autumn (November). We hypothesised that physical activity levels would decrease, and obesity levels increase, between summer and winter in the United Kingdom and that these changes would be associated with decreases in FMD.

### **6.1 Methods**

#### **6.1.1 Participants**

One hundred and forty five subjects (59 male, 86 female) mean ( $\pm$ SD) age  $10.7 \pm 0.3$  years were originally recruited for the current investigation. Subjects were randomly selected from a database of approximately 3000 9-10 year olds participating in the SportsLinx fitness project (Taylor et al. 2004). All subjects were sequentially studied

without specific exclusion criteria and all were healthy, normotensive and not suffering from known cardiovascular or metabolic conditions. Subjects were not using any medications. Ethics approval was obtained from Liverpool John Moores University Ethics Committee. Written and verbal informed consent was obtained from parents/guardians and children prior to participation in the study.

### **6.1.2 Study Design**

Subjects were required to attend a laboratory to complete a number of physiological examinations including anthropometric tests, dual energy X-ray absorptiometry (DXA) assessment, and assessment of conduit vessel function. All subjects were studied at the same time of day to control for the impact of circadian variation. Participants and their parents/guardians were informed that children must fast on the morning of testing and avoid any strenuous PA for 24 hours prior to testing. All measurements were taken in a quiet room. All outcome measures were assessed in June and again in November. Fitness was assessed

### **6.1.3 Anthropometric and body composition measurement**

Prior to exercise testing, body mass (kg), height (m) and sitting height were obtained and used to calculate TPHV. A dual-energy X-ray absorptiometry (DXA; Fanbeam, QDR series discovery A, Hologic, Bedford, Massachusetts, U.S.A.) scan was then taken to assess whole body and regional fat and lean tissue mass. For further details on anthropometric and body composition measurement please refer to section 3.2.5.

### **6.1.4 Assessment of physical activity (PA)**

The accelerometer was programmed to record PA data (raw activity counts and steps) every 5 seconds. On the first day of monitoring, the children underwent a familiarisation



period. At the end of the data collection period, all data were downloaded and checked for compliance. Sustained 20 minutes periods of zero counts were removed. For inclusion in the analyses, children were required to have produced counts for 9 hours a day for at least three days.

In order to individually calibrate PA levels, participants underwent an incremental discontinuous treadmill-based exercise test to determine peak oxygen uptake ( $\dot{V} O_{2peak}$ ) at entry to the study. Detailed descriptions of  $VO_{2peak}$  protocol and methods of individual physical activity threshold calibration can be found in sections 3.2.4 and 3.2.4.

#### **6.1.5 Assessment of conduit vessel function**

Brachial artery internal diameter was measured using high resolution ultrasonography (Acuson, Aspen, Siemens Corp, Malvern, Penn and Terason, t3000, Teratech,). Please see section 3.2.1 and 3.2.2 for further details on FMD methodology.

#### **6.1.6 Statistical analysis**

Statistical analyses were performed using SPSS 14.0 (SPSS) software. Results are expressed as the mean $\pm$ SD. Data distribution was initially examined for normality using the Shapiro-Wilk test. Variables that were not normally distributed were log transformed, for results obtained that remained below the boundary of normality, which was set at 0.05, non parametric equivalent tests were used.

Baseline data for boys and girls were compared using unpaired Student's *t*-tests or the Mann Whitney test. Change over time was assessed using repeated measures GLM. The effect of maturation was assessed by entering TPHV and the dependent variables (DV)

into a correlation analysis, where significant relationships were observed maturation was considered to be a confounder.

Change scores were calculated for all variables (test 1-test 2). The strength of relationships between variables was evaluated using either a partial correlation, with maturation (TPHV) added as a covariate if it was considered a confounder. Depending on normality of the data, Pearson's or Spearman's correlation analyses were used when maturation was not a confounder. A stepwise backwards linear regression was then performed to identify predictors of change in FMD. Predictors entered into the model were chosen on the basis that they were significantly related to FMD. The group was then split according to gender, and the above analysis was completed for boys and girls.

## **6.2 Results**

Of the original 145 children we obtained suitable FMD data from 129 participants at both time points, a further 10 children did not meet inclusion criteria for physical activity measurement, and 6 children were unable to attend the laboratory for post test measurements. Those subjects with incomplete data sets were removed from the analysis, leaving one hundred and sixteen children included in the study.

No differences existed between girls and boys in age, height, body mass, BMI, and systolic and diastolic blood pressures (Table 6.1). In addition, there was no sex difference in years to peak height velocity. Boys had significantly lower fat %, however there were no further differences for any other DXA measures. Girls spent less time at PA<sub>4</sub>, PA<sub>8</sub> and PA<sub>10</sub>. Total physical activity time and daily steps were also higher in



boys. No difference existed between the groups in terms of brachial artery function (FMD).

### **6.2.1 Seasonal effects on variables: cohort**

Between June and November the average daily temperature dropped from 14.1°C to 7.2°C, and the average hours of sunshine for each month was 144 and 52 respectively (Met Office 2010).

#### *Body composition*

From early summer to the end of autumn the cohort demonstrated significant increases in height and body mass (Table 6.1). Further to this, we observed an increase in BMI, fat mass ( $P<0.001$ ), fat % ( $P<0.001$ ) and lean mass ( $P<0.001$ ).

#### *Physical activity*

Physical activity time spent at 6km (PA<sub>6</sub>) showed no change between the two time points (Table 6.1), whereas time spent at PA<sub>4</sub>, PA<sub>8</sub> and PA<sub>10</sub>, total physical activity time and daily steps taken ( $P<0.01$ ) significantly decreased across the cohort.

#### *Vascular measures*

Brachial artery FMD was significantly reduced in the autumn ( $P<0.001$ ). Both systolic and diastolic blood pressure increased ( $P<0.01$ ) over time (Table 6.1).

Table 6.1 Descriptive characteristics, collected in June and November (mean ± SD)

|  | Group (n = 116) |                | Boys (n = 46) |                | Girls (n = 70)             |                |
|--|-----------------|----------------|---------------|----------------|----------------------------|----------------|
|  | June            | November       | June          | November       | June                       | November       |
| Age (y)  | 10.3 ± 0.3      | 10.7 ± 0.3     | 10.4 ± 0.3    | 10.7 ± 0.3     | 10.3 ± 0.3                 | 10.7 ± 0.3     |
| Body Mass (kg)   | 39.8 ± 9.5      | 42.1 ± 10.0**  | 39.7 ± 10.2   | 41.9 ± 10.6**  | 39.8 ± 9.0                 | 42.3 ± 9.7**   |
| Height (m)   | 1.42 ± 0.07     | 1.44 ± 0.07**  | 1.42 ± 0.07   | 1.44 ± 0.08**  | 1.43 ± 0.07                | 1.45 ± 0.07**  |
| TPHV (y)   | -3.4 ± 0.5      | -3.2 ± 0.5**   | -3.4 ± 0.4    | -3.2 ± 0.4**   | -3.4 ± 0.6                 | -3.2 ± 0.6**   |
| BMI (kg/m <sup>2</sup> )                                     | 19 ± 3          | 20.0 ± 3.5**   | 19 ± 4        | 20.0 ± 3.8**   | 19 ± 3                     | 20.0 ± 3.3**   |
| Fat mass (kg)  | 11.64 ± 5.54    | 12.49 ± 5.54** | 11.11 ± 6.34  | 11.63 ± 5.92   | 11.99 ± 4.97               | 13.06 ± 5.45** |
| %fat mass  | 27.59 ± 6.80    | 28.27 ± 6.65** | 25.10 ± 7.58  | 25.94 ± 7.55** | 29.20 ± 5.64 <sup>††</sup> | 29.80 ± 5.91*  |
| Lean mass (kg)   | 27.46 ± 4.97    | 28.75 ± 5.24** | 28.26 ± 5.23  | 29.45 ± 5.33** | 26.93 ± 4.75               | 28.29 ± 5.05** |
| $\dot{V}O_{2peak}$ (ml.kg <sup>-1</sup> .min <sup>-1</sup> ) | 44.2 ± 5.9      | NA             | 47.2 ± 6.0    | NA             | 42.3 ± 5.1 <sup>††</sup>   | NA             |
| PA4 (min/day)  | 48.5 ± 19.3     | 40.0 ± 16.4**  | 51.2 ± 18.5   | 42.8 ± 19.1**  | 46.7 ± 19.7 <sup>†</sup>   | 36.7 ± 14.0**  |
| PA6 (min/day)  | 20.7 ± 14.3     | 19.5 ± 12.3    | 25.0 ± 18.9   | 20.7 ± 14.3    | 17.8 ± 9.4                 | 18.4 ± 10.6    |
| PA8 (min/day)  | 1.9 ± 2.6       | 1.6 ± 2.3*     | 2.7 ± 3.1     | 2.1 ± 2.6      | 1.4 ± 2.0 <sup>††</sup>    | 1.2 ± 2.0*     |
| PA10 (min/day)   | 6.2 ± 5.6       | 4.9 ± 4.4*     | 8.0 ± 6.5     | 7.3 ± 5.2      | 4.7 ± 4.2 <sup>††</sup>    | 3.0 ± 2.4*     |
| Total PA (min/day)   | 94.1 ± 34.8     | 77.8 ± 33.7**  | 105.3 ± 38.9  | 87.7 ± 41.3**  | 86.8 ± 30.0 <sup>†</sup>   | 71.2 ± 25.4**  |
| Daily steps  | 9112 ± 3031     | 8302 ± 2434*   | 10475 ± 3231  | 9143 ± 2964*   | 8230 ± 2554 <sup>††</sup>  | 7746 ± 1846    |
| Systolic BP (mmHg)   | 107 ± 10        | 109 ± 10*      | 109 ± 12      | 112 ± 12*      | 106 ± 9 <sup>†</sup>       | 107 ± 9        |
| Diastolic BP (mmHg)  | 64 ± 5          | 65 ± 5*        | 65 ± 6        | 67 ± 5*        | 63 ± 4 <sup>†</sup>        | 64 ± 5         |
| FMD  | 10.0 ± 4.3      | 7.9 ± 3.9**    | 10.2 ± 3.9    | 7.7 ± 3.9**    | 10.0 ± 4.6                 | 8.1 ± 3.8*     |

Significant results – June boys vs. girls, <sup>†</sup>  $p < 0.05$ ; <sup>††</sup> boys vs. girls,  $p < 0.01$ . June vs. November, \*  $p < 0.05$ ; \*\*  $p < 0.01$



### **6.2.2 Sex Differences**

#### *Body composition*

From early summer to the end of autumn both groups demonstrated significant increases in height and body mass (Table 6.1). Further to this, significant increases in BMI, fat % ( $P<0.001$ ) and lean mass ( $P<0.001$ ) were observed in both boys and girls. Girls demonstrated an increase in fat mass (kg) ( $P<0.001$ ).

#### *Physical activity*

Physical activity time spent at 4km (PA<sub>4</sub>) and total PA time decreased significantly over time in both the girls and boys (Table 6.1). Time spent at 6km (PA<sub>6</sub>) showed no change in either group between time points. Physical activity time at 8km and 10km decreased significantly in the girls only, boys demonstrated a trend towards decline for these variables, however this was non significant. Daily steps taken were significantly reduced over time in the boys only.

#### *Vascular measures*

Brachial artery FMD significantly decreased ( $P<0.001$ ) in both groups. Modest but significant increases in systolic and diastolic blood pressures were noted ( $P<0.01$ ) over time in the boys only (Table 6.1).

### **6.2.3 Relationships between change in FMD and other variables**

#### *Whole cohort*

No significant relationships were evident between change in FMD and change in body composition variables (BMI, fat mass, fat% and lean mass). A significant relationship

was evident between FMD and PA<sub>8</sub> ( $r=0.24$ ,  $P=0.03$ ); no other relationships were observed between PA measures and FMD.

As PA<sub>8</sub> was the only variable to significantly correlate with FMD, this was the sole variable entered into the linear regression ( $R^2=0.05$ ,  $\beta=0.47$ ,  $P=0.02$ ).

### *Relationships by sex*

Correlation analyses revealed no significant relationships between change in FMD and body composition variables (BMI, fat mass, and lean mass) in boys, although fat% approached significance ( $r=-0.27$ ,  $P=0.07$ ). A significant relationship was evident between change in FMD and change in PA<sub>8</sub> ( $r=0.40$ ,  $P=0.01$ ), no other associations were observed. Regression analysis revealed that PA<sub>8</sub> significantly predicted FMD ( $R^2=0.05$ ,  $\beta=0.47$ ,  $P=0.02$ ). There were no significant relationships observed between change in FMD and change in any of the PA or body composition variables for girls.

## **6.3 Discussion**

This is the first study, to our knowledge, to assess seasonal variation in FMD, a surrogate marker of endothelial function and vascular health, and relate these changes to those in PA intensities and body composition. Our principal finding was that seasonal change existed in measures of vascular function, body composition and PA in children. Measures collected at the end of autumn in Northern England demonstrated a significant decrease in FMD and PA and an increase in body fat, compared to those collected in summer. The decrease in FMD was related to change in PA<sub>8</sub> (equivalent to  $\sim 81\%$   $\dot{V}O_{2peak}$ ) but not with changes in other PA variables or measures of body composition. These data suggest that high intensity PA may be an important determinant of vascular function and health.



Previous studies suggest that seasonal variation in PA exists in adults and children (Belanger et al. 2009; Matthews et al. 2001; Rowlands et al. 2009). These studies observed decreases in PA levels between summer and winter, in line with the findings of the current study. Seasonal changes in adult PA levels have been associated with negative changes in physiological variables such as blood pressure (Woodhouse et al. 1993) and metabolic and homeostatic coronary risk factors (Mavri et al. 2001). Our data add to this previous literature in that we have observed concomitant changes in PA levels and vascular function in a paediatric population. Furthermore, we have been able to associate changes in vascular function to changes in daily time spent performing high intensity PA.

Although this is the first study to associate seasonal variation in FMD to changes in PA, evidence from the literature linking high intensity PA to vascular function is beginning to emerge. Rakabowchuk et al. (Rakobowchuk et al. 2008) demonstrated that 6 weeks of 'all out' training involving Wingate tests (total exercise time across 6 weeks = approx 45 min), induced an improvement in popliteal endothelial function equal to that achieved by completing 6 weeks of endurance training (total exercise time approx 1500 minutes). In addition, recent studies, albeit in adults, suggest that high intensity interval training may be equally, or more effective, than continuous moderate or low intensity exercise programs for improvement in endothelial function in subjects with established CV disease (Munk et al. 2009; Wisloff et al. 2007). Mechanistically, it has been suggested that high intensity exercise may enhance endothelial function via improvement in antioxidant capacity and reduction in oxidative stress. Alternatively, higher intensity training may induce greater augmentation of eNOS gene expression (Green et al. 2004a; Hambrecht et al. 2003). Interval training methods used in adult

populations are similar to children's PA patterns, which typically consist of short bouts of high intensity PA interspersed with longer periods of low-to-moderate intensity activity (Baquet et al. 2007). Thus it is possible that a decrease in high intensity PA, as observed in the current study, could impact negatively upon FMD as the beneficial changes in oxidative stress, antioxidant capacity and eNOS gene expression are lost.

No significant associations between change in FMD and body composition were observed in the present study. Our data from chapter 5 and previous cross sectional studies have demonstrated a negative relationship between fat mass and endothelial function (Arcaro et al. 1999) whilst others have observed a reduction in body fat with concomitant improvement in endothelial function in obese children as a result of an exercise training intervention (Meyer et al. 2006). The current study demonstrated an inverse relationship between change in FMD and change in fat mass in boys, which approached statistical significance ( $r=-0.27$ ,  $p=0.07$ ). The absence of significant relationships between change in body fatness and FMD in the present study may be due, at least in part, to the fact that our cohort was not, on average, overweight or obese and that changes in fatness were relatively modest.

Sub-group analysis by sex revealed that the group differences we observed were predominately determined by the data collected in boys. Although FMD and high intensity physical activity both declined in the girls, no significant relationship was observed between change scores in these variables, a finding which contrasted to that in boys. It is unlikely that this disparity is related to variation in maturation and development between the sexes as there was no significant difference between the rates of change in TPHV. Alternatively, it is possible that no relationship was observed between change in FMD and change in high intensity PA in the girls as they did not



amass enough daily high intensity or total PA to impact upon FMD. There is clearly a dose-response relationship between overall physical activity and CVD risk (Andersen 1995; Berlin and Colditz 1990; Whaley and Blair 1995), and in adults it has been suggested that a high volume of exercise training/PA may be required to have a beneficial effect on endothelial function in subjects who possess normal endothelial function a priori (Green et al. 2004a). In the current study the girls performed less high intensity PA/day and ~20 min less total PA than the boys in both the summer and winter (Table 1). A previous cross sectional study by Pahkala et al. (Pahkala et al. 2008) examined the impact of gender on the relationship between FMD and leisure time PA, assessed by questionnaire. In concordance with the current findings, they demonstrated a significant relationship between FMD and PA in the boys but not the girls, and also that PA was lower in girls than boys. A sub-group analysis carried out in a selection of boys and girls who had the same activity level revealed no gender difference for FMD, suggesting that differences in PA levels, not gender per se, were responsible for the differences between boys and girls. Our findings add substantially to those of Pahkala et al. (Pahkala et al. 2008) in that we directly measured physical activity and the intensity of physical activity, and also demonstrated the relationship between *changes* in PA and body composition and changes in vascular function. We suggest that, like Pahkala et al. (Pahkala et al. 2008) the lack of relationship between changes in FMD and PA observed in girls was due to their lower PA level.

The findings of the current study are limited in their application to other populations and can only be generalised to populations of pre-pubertal children. Accounting for the different stages of maturity of children in the cohort is problematic. The use of Tanner stages is the preferred method for describing pubertal development in children and adolescents. Although we did not use Tanner stages to record the physical maturity of

the cohort, we controlled for differences in development via calculation of TPHV for each child (Mirwald et al. 2002), a widely acknowledged and accepted approach, which was factored into our analyses where necessary. A final limitation worthy of note is that our chosen method of PA measurement is capable of measuring PA during ambulatory movement only, thus total PA levels could have been underestimated.

#### **6.4 Conclusion**

We observed a seasonal decrease in conduit artery endothelial function and PA in both boys and girls. The decrease in endothelial function was associated with a seasonal reduction in time spent performing high intensity physical activity. No relationships were evident between change in FMD and body composition variables. Our findings were particularly apparent in boys. We can infer from these results that high intensity physical activity plays a role in terms of modulating vascular health outcomes. Assuming these findings can be replicated and extended, they suggest that future intervention strategies targeting improving cardiovascular outcomes in children should prioritise increasing high intensity PA, especially during the autumn/winter months which are associated with lower levels of physical activity.



# Study aims and outcomes

## Chapter 4

**Rationale** – Cardiac literature implies scaling vascular outcomes may be necessary

**Aim** – Address the issue of scaling vascular outcomes for body composition variables

## Outcome

- Possible need to scale artery diameter for lean mass variables
- Scaling artery function is not necessary.

## Chapter 5

**Rationale** – Few studies explore determinants of vascular health in children

**Aim** – Assess relative impact of modifiable risk factors on VF

## Outcome

- Weak associations between VF, measures of PA and fat and  $VO_{2peak}$ .
- High intensity PA was the only significant predictor of depressed VF

## Chapter 6

**Rationale** – No study has assessed whether seasonal variation exists in FMD.

**Aim** – Explore the relationships between seasonal  $\Delta$ FMD and  $\Delta$  PA and body composition.

## Outcome

- FMD and PA significantly decreased between summer and late autumn.
- Change in high intensity PA was the only significant predictor of  $\Delta$ FMD

## Chapter 7

**Rationale** – Despite an increase in sedentary behaviour, no evidence links SB with VF.

**Aim** – Investigate the associations between FMD and SB, and  $\Delta$ FMD and  $\Delta$ SB.

## Outcome

- FMD is not associated with SB
- Seasonal decline in FMD is not related to the increase in SB

## Chapter 8

**Rationale** – In adults heritability of FMD appears to be relatively low. No data exists in children

**Aim** – Explore the genetic contribution to FMD using mono- and di-zygotic twins

## Outcome

- FMD is under moderate genetic control, heritability was estimated at 0.44
- Fat and  $VO_{2peak}$  are highly genetically determined

## Chapter 9

**Rationale** – The heredity of exercise induced change in FMD is yet to be determined

**Aim** – Determine the heritability of exercise induced change in FMD, body composition and fitness

## Outcome

- Change in FMD, fat and  $VO_{2peak}$  are under a large amount of genetic control.
- Heritability of FMD was estimated at 0.72



## Chapter 7

# **VASCULAR FUNCTION AND SEDENTARY BEHAVIOUR**

**Based on the article under consideration at the European Journal  
of Cardiovascular Prevention and Rehabilitation  
See appendices for a copy of the submitted version**



## **7 Introduction**

The term sedentary behaviour (SB) encompasses a diverse range of behaviours that are considered "inactive". However, sedentary behaviour is not synonymous with inactivity and researchers prefer to define SB as behaviours where sitting or lying are the dominant postures and energy expenditure is  $<1.5$  METs (Pate et al. 2008). The recent increase in prevalence of sedentary behaviour (SB) has resulted in the emergence of this variable as a novel CV risk factor, which has been independently associated with cardiovascular disease mortality (Dunstan et al. 2010).

Recent paediatric studies suggest that sedentary behaviour may be associated with cardiovascular and metabolic risk factors such as obesity (Rennie et al. 2005), systolic and diastolic blood pressure, fasting glucose and insulin, and triacylglycerol concentrations (Ekelund et al. 2007). In addition, it has been suggested that the association between sedentary behaviour and CV risk may be independent of that between physical activity and risk. However, no previous studies have assessed the relationship between sedentary behaviour and directly measured indices of arterial function and health. The purpose of this study was, therefore, to investigate the association between FMD and sedentary behaviour in a cross section of healthy children. Furthermore, we collected repeated measurements to address the question of whether change in sedentary behaviour correlated with change in endothelial function.

## **7.1 Methods**

### **7.1.1 Participants**

Details of the study population are identical to those used in chapter 6, please see section 6.1.1 for more details. Ethics approval was obtained from the institutional ethics committee. Written and verbal informed consent was obtained from parents/guardians and children prior to participation in the study.

### **7.1.2 Study Design**

Subjects were required to attend a laboratory for assessment of conduit vessel function. All subjects were studied at the same time of day to control for the impact of circadian variation. Participants and their parents/guardians were informed that children must fast on the morning of testing and avoid any strenuous PA for 24 hours prior to testing. All measurements were taken in a quiet room. Sedentary behaviour was assessed over 7 days using accelerometry. Outcome measures were assessed in June and again in November.

### **7.1.3 Assessment of conduit vessel function**

Brachial artery internal diameter was measured using high resolution ultrasonography (Acuson, Aspen, Siemens Corp, Malvern, Penn and Terason, t3000, Teratech,). Please see section 3.2.1 and 3.2.2 for further details on FMD methodology.

### **7.1.4 Assessment of sedentary behaviour**

Sedentary behaviour was objectively measured using a hip-mounted uni-axial accelerometer (GT1M model, Actigraph, Florida, USA) for seven consecutive days. Please refer to section 3.2.4 for further details.



### **7.1.5 Statistical analysis**

Statistical analyses were performed using SPSS 17.0 (SPSS) software. Results are expressed as the mean $\pm$ SD.

Data distribution was examined for normality using the Shapiro-Wilk test. Variables that were not normally distributed were log transformed, where results remained below the boundary of normality, which was set at 0.05, non parametric equivalent tests were used. To assess whether outcome variables were affected by differences in gender and maturational status, forward stepwise linear regressions were performed for the whole cohort with age and TPHV entered as independent variables. Where significant relationships were observed, standardised residuals were used in place of the original data for the subsequent analyses.

Baseline sex differences were examined using an independent *t*-test or a Mann Witney test. Differences between tests in June and November were assessed using a repeated measures GLM. Spearman's correlation analyses were used to assess the strength of relationships between vascular outcomes and SB. Change scores were calculated for all variables (test 2 - test 1). Spearman's correlation analysis was used to assess the strength of relationships between change in FMD and SB variables.

## **7.2 Results**

Of the original 145 children, FMD data was obtained for 129 participants at both time points. A further 10 children did not meet inclusion criteria for SB measurement and 3 children were unable to attend the laboratory for post test measurements. Those subjects

with incomplete data sets were removed from the analysis, leaving 116 children (46 male, 70 female) included in the study.

No differences existed between girls and boys in age, height, body mass, or years to peak height velocity (Table 7.1). In addition, there were no sex differences for FMD, SB or accelerometer wear time.

### **7.2.1 Relationship between FMD and SB**

There was no significant relationship between FMD and SB in the whole cohort, furthermore no relationships were observed between these variables in either sub-group (Table 7.2, Figure 7.1). As no significant correlations were observed, regression analysis was not performed.

### **7.2.2 Time effects on variables**

Height and body mass increased significantly over time. Whilst there was no difference in accelerometer wear time ( $714.7 \pm 70.9$  to  $703.1 \pm 68.4$  min), SB significantly increased ( $499.2 \pm 103.5$  to  $559.9 \pm 81.6$  min). Brachial artery FMD was significantly reduced ( $10.0 \pm 4.3$  to  $7.9 \pm 3.9$ ) within the whole cohort, and in both boys and girls (Table 7.1). However, no significant relationships were evident between change in FMD and change SB for the whole cohort or the sex sub-groups (Table 7.2, Figure 7.2).



Table 7.1 Descriptive characteristics

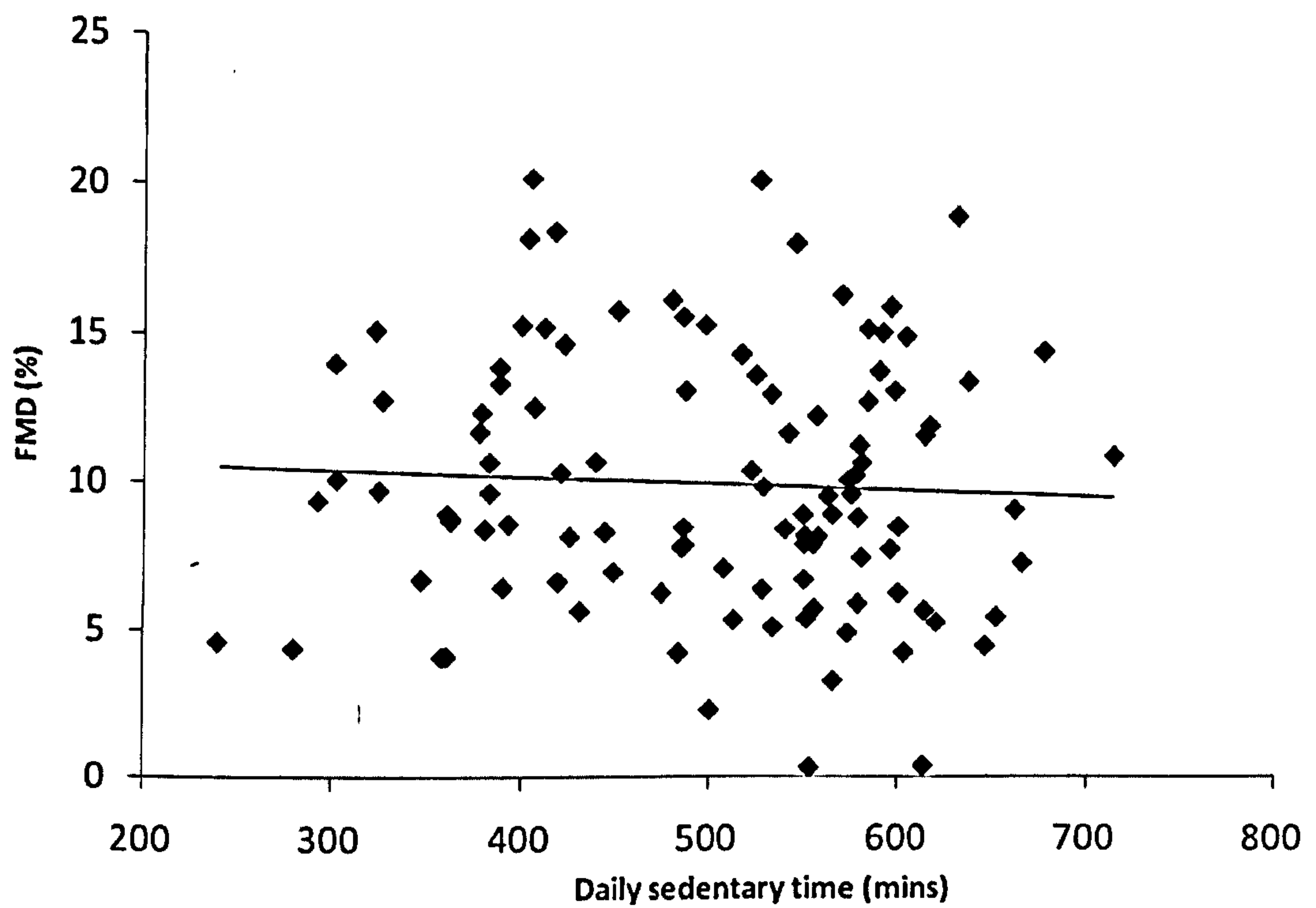
|                                       | Group (n = 116) |                | Boys (n = 46) |                | Girls (n = 70) |                |
|---------------------------------------|-----------------|----------------|---------------|----------------|----------------|----------------|
|                                       | June            | November       | June          | November       | June           | November       |
| Age (y)                               | 10.3 ± 0.3      | 10.7 ± 0.3     | 10.4 ± 0.3    | 10.7 ± 0.3     | 10.3 ± 0.3     | 10.7 ± 0.3     |
| Body Mass (kg)                        | 39.8 ± 9.5      | 42.1 ± 10.0**  | 39.7 ± 10.2   | 41.9 ± 10.6**  | 39.8 ± 9.0     | 42.3 ± 9.7**   |
| Height (m)                            | 1.42 ± 0.07     | 1.44 ± 0.07**  | 1.42 ± 0.07   | 1.44 ± 0.08**  | 1.43 ± 0.07    | 1.45 ± 0.07**  |
| TPHV (y)                              | -3.4 ± 0.5      | -3.2 ± 0.5**   | -3.4 ± 0.4    | -3.2 ± 0.4**   | -3.4 ± 0.6     | -3.2 ± 0.6**   |
| FMD (%)                               | 10.0 ± 4.3      | 7.9 ± 3.9**    | 10.2 ± 3.9    | 7.7 ± 3.9**    | 10.0 ± 4.6     | 8.1 ± 3.8*     |
| Daily sedentary behaviour (min)       | 499.2 ± 103.5   | 559.9 ± 81.6** | 480.8 ± 111.0 | 546.3 ± 93.9** | 511.3 ± 97.2   | 569.0 ± 71.5** |
| Average accelerometer wear time (min) | 714.7 ± 70.9    | 703.1 ± 68.4   | 719.9 ± 63.6  | 703.1 ± 67.2   | 712.8 ± 75.7   | 702.4 ± 70.5   |

June vs. November ANOVA, \* P < 0.05, \*\*P < 0.01

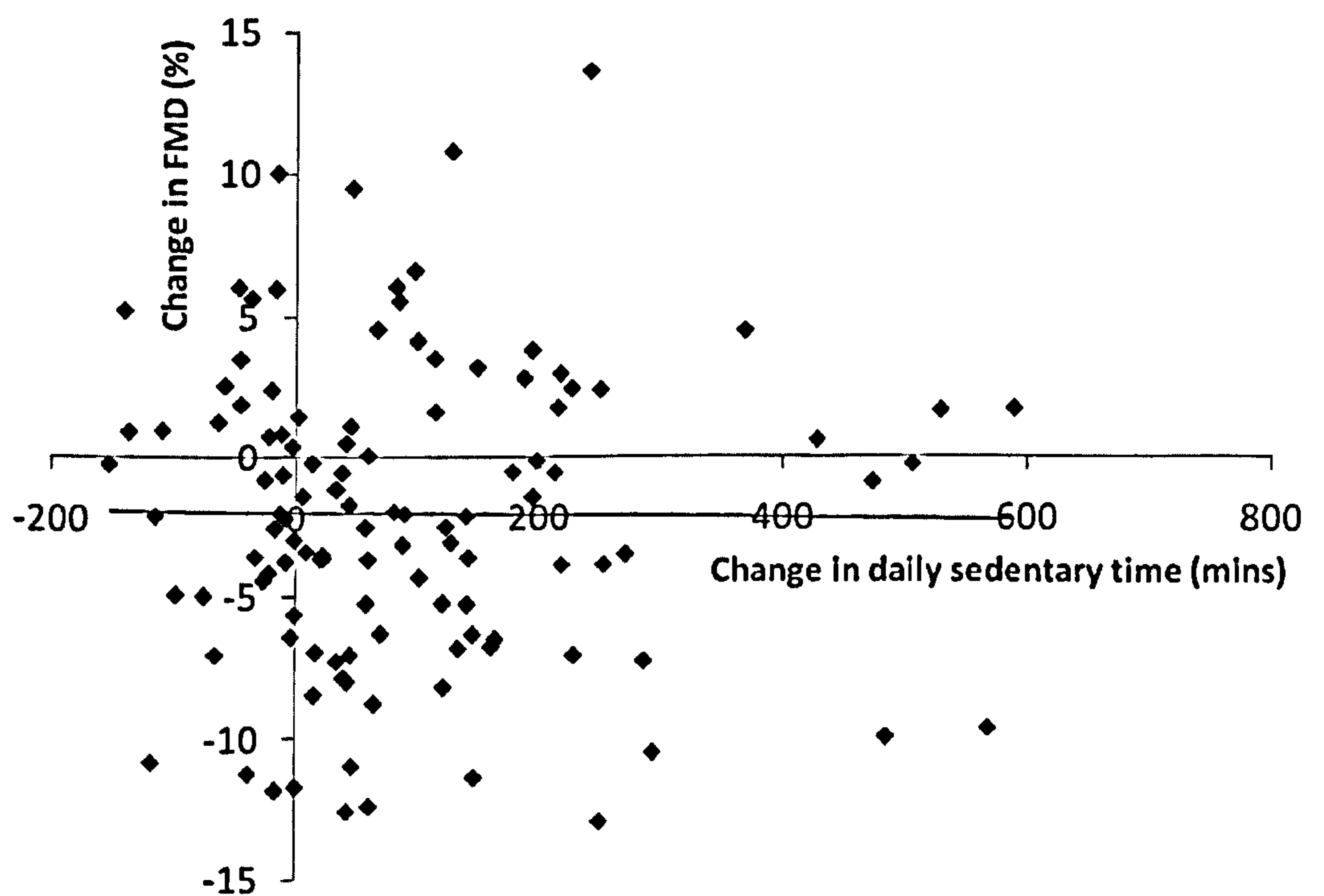
**Table 7.2** Spearman's correlations between baseline SB and vascular outcomes and change in SB and change in vascular outcomes

|                               | Whole cohort |      |          |      | Boys     |      |          |      | Girls    |      |          |      |
|-------------------------------|--------------|------|----------|------|----------|------|----------|------|----------|------|----------|------|
|                               | Baseline     |      | $\Delta$ |      | Baseline |      | $\Delta$ |      | Baseline |      | $\Delta$ |      |
|                               | r            | P    | r        | P    | r        | P    | r        | P    | r        | P    | r        | P    |
| Baseline artery diameter (mm) | -0.06        | 0.56 | 0.15     | 0.13 | -0.28    | 0.07 | 0.28     | 0.15 | 0.12     | 0.34 | 0.03     | 0.80 |
| FMD (%)                       | -0.08        | 0.43 | -0.06    | 0.51 | 0.03     | 0.87 | -0.23    | 0.07 | -0.10    | 0.42 | 0.01     | 0.97 |





**Figure 7.1** Baseline relationship between FMD and sedentary behaviour



**Figure 7.2** Relationship between change in sedentary time and change in FMD.

### **7.3 Discussion**

This is the first study, to our knowledge, to assess the relationship between FMD, a surrogate marker of endothelial function and vascular health, and sedentary behaviour. Furthermore, we examined seasonal variation in SB and related these changes to those in FMD. Our principal findings were that there was no significant relationship observed between basal FMD and SB and, although sedentary behaviour increased and FMD decreased between summer and autumn, changes in these variables were unrelated. There were no significant differences between the sex-sub groups. These data suggest that endothelial function is not related to changes in sedentary behaviour in this cohort.

Sedentary behaviour has recently become the focus of numerous epidemiological studies examining determinants of CV risk factors in children (Ekelund et al. 2007; Ekelund et al. 2006). Technological advances have resulted in an increase in screen-based media time, such as television viewing and computer games, and this has led researchers to suggest that the increase in sedentary behaviour observed in recent years may displace time spent engaging in physical activity (Marshall et al. 2004). Furthermore, excess time spent engaging in sedentary behaviours has been implicated as one of the determinants of childhood obesity (Dietz and Gortmaker 1993; Gortmaker et al. 1996). Although there is little evidence relating sedentary time to other markers of CV risk, Ekelund *et al.* (Ekelund et al. 2006) were able to demonstrate that time spent watching television was significantly associated with both adiposity and fasting insulin levels. The same investigators have also observed weak associations between total sedentary behaviour, systolic and diastolic blood pressure and clustered metabolic risk in children (Ekelund et al. 2007). Despite this evidence that SB may contribute to negative CV risk profiles, the results from the current study imply that endothelial function, a harbinger of atherogenic development, is independent of SB.



Some investigators have suggested that sedentary behaviour is associated with increased CV risk and that this relationship is independent of the association between PA and CV risk. This implies some unique impact of sedentary behaviour separate from PA. The evidence for this is epidemiological and no previous study has measured vascular function or health, or changes in vascular function or health, and related these to SB. In the present study, we observed no relationships between SB and vascular function. In contrast, we have reported data from this cohort of children pertaining to the impact of PA levels on FMD (chapters 5 and 6). These findings indicate that high intensity physical activity, and changes in high intensity physical activity, are significantly correlated with endothelial function. These data, utilising direct and objective measures of SB, PA and vascular health, suggest that vigorous PA level may be a more important variable to modulate with regard to the improvement of endothelial and vascular health in young people.

The current investigation presents some evidence to suggest a role of seasonality in sedentary behaviour of children. Merrill *et al.* (Merrill et al. 2005) have suggested that weather conditions can strongly promote or deter physical activity behaviours, and there is evidence to suggest that seasonal variation in PA exists in adults and children (Belanger et al. 2009; Matthews et al. 2001; Rowlands et al. 2009). Seasonal changes in PA levels have been associated with negative changes in physiological variables such as blood pressure (Woodhouse et al. 1993) and metabolic and homeostatic coronary risk factors (Mavri et al. 2001). As stated above, we have previously reported that the seasonal decline in vigorous physical activity time was a significant predictor of the seasonal decrease in FMD in children (chapter 6). However, the findings of the present study suggest that the decrease in FMD is not associated with the increase in sedentary

behaviour observed between summer and autumn. Taken together our data suggest that, providing sedentary behaviour does not displace vigorous physical activity, sedentary behaviour reduction *per se* should not be the principal target of interventions aimed at improving vascular health in children. Both exercise training (Watts et al. 2004a; Watts et al. 2005) and physical activity levels (Pahkala et al. 2008) are known to modulate endothelial and vascular health in children, we submit that interventions should primarily aim to increase physical activity or exercise.

The findings of the current study are limited in their application to other populations and can only be generalised to populations of pre-pubertal children. As with chapters 5 and 6 the impact of maturity is difficult to rule out, we have however attempted to control for developmental differences where possible. Although accelerometry provides an objective measure of physical activity and sedentary behaviour, it does not allow discrimination between different types of sedentary behaviour, for example time spent engaged in screen based media, which may be related to markers of vascular health. Nonetheless, the strength of our study lies in the repeated collection of direct, objective, physiological and behavioural measurements.

## **7.4 Conclusion**

There was no association between FMD and sedentary behaviour observed in our cohort of children, and the seasonal decrease in conduit artery endothelial function we observed was not associated with the increase in sedentary behaviour. Taken together with our findings from chapters 5 and 6, and assuming these findings can be replicated and extended, they suggest that reduction in sedentary behaviour should not be the primary target of future intervention strategies aimed at improving cardiovascular outcomes in children.



# Study aims and outcomes

## Chapter 4

**Rationale** – Cardiac literature implies scaling vascular outcomes may be necessary

**Aim** - Address the issue of scaling vascular outcomes for body composition variables

## Outcome

- Possible need to scale artery diameter for lean mass variables
- Scaling artery function is not necessary.

## Chapter 5

**Rationale** – Few studies explore determinants of vascular function (VF) in children

**Aim** – Assess relative impact of modifiable risk factors on VF

## Outcomes

- Weak associations between VF, measures of PA and fat and  $VO_{2peak}$ .
- High intensity PA was the only significant predictor of depressed VF

## Chapter 6

**Rationale** – No study has assessed whether seasonal variation exists in FMD.

**Aim** – Explore the relationships between seasonal  $\Delta$ FMD and  $\Delta$  PA and body composition.

## Outcome

- FMD and PA significantly decreased between summer and late autumn.
- Change in high intensity PA was the only significant predictor of  $\Delta$ FMD

## Chapter 7

**Rationale** – Despite an increase in sedentary behaviour, no evidence links SB with VF.

**Aim** – Investigate the associations between FMD and SB, and  $\Delta$ FMD and  $\Delta$ SB.

## Outcome

- FMD is not associated with SB
- Seasonal decline in FMD is not related to the increase in SB

## Chapter 8

**Rationale** – No study has examined heritability of VF in children

**Aim** – Explore the genetic contribution to FMD using mono- and di- zygotic twins.

## Outcome

- FMD is under moderate genetic control, heritability was estimated at 0.44
- Fat and  $VO_{2peak}$  are highly genetically determined

## Chapter 9

**Rationale** – The heredity of exercise induced change in FMD is yet to be determined

**Aim** – Determine the heritability of exercise induced change in FMD, body composition and fitness

## Outcome

- Change in FMD, fat and  $VO_{2peak}$  are under a large amount of genetic control,
- Heritability of FMD was estimated at 0.72



## Chapter 8

# **HEREDITY OF VASCULAR FUNCTION, PHYSICAL ACTIVITY, FITNESS AND BODY COMPOSITION**

**Based on the article entitled  
“Heritability of arterial function, fitness and physical activity in youth:  
A study of mono- and di-zygotic twins.” under consideration at the  
Journal of Paediatrics, and the article “Impact of exercise training on  
body composition in youth: A study of mono- and di-zygotic twins”  
under consideration at the Journal of Applied Physiology.  
(Please see appendices for copies of the submitted articles)**



## **8 Introduction**

It is clear from previous research that cardiovascular disease risk is largely influenced by modifiable risk factors, such as those examined in chapters 5-7 of this thesis (Berlin and Colditz 1990; Francis 1996; Katzmarzyk et al. 2009; Khan et al. 2003; Lee et al. 1999; Whaley and Blair 1995). However, the relative contribution of genetics to CVD risk and mortality is an area which has received comparatively less attention. Studies indicate that the genetic heritability of CHD mortality is moderate and suggest a clear role for some genetic stimuli for the development of CVD (Zdravkovic et al. 2002).

Despite the clinical relevance and potential prognostic value of the FMD assessment, no previous study to our knowledge has addressed the question of genetic heritability of this measure in a paediatric population. Studies of monozygotic (MZ) and dizygotic (DZ) twins offer a method of examining genetic and environmental sources of variance in quantitative traits. In the present study we used a twin design to investigate the relative contribution of genetics and environment on FMD. We also assessed fitness, body composition and physical activity levels in both twin subsets.

### **8.1 Methods**

#### **8.1.1 Participants**

A cross section of 22 cohabiting twin pairs (11 monozygotic, 2♂, 9♀,  $13.3 \pm 1.6$  yrs and 11 di-zygotic, 5♂, 6♀,  $13.6 \pm 1.6$  yrs) were recruited from the community. Zygosity was determined using a previously validated questionnaire (Goldsmith 1991). Participants were healthy, normotensive and not suffering from cardiovascular or metabolic conditions. None were taking any vasoactive medications, nor were they smokers. Ethics approval was obtained from Liverpool John Moores University Ethics

Committee. Informed parental consent and child assent were obtained prior to participation in the study.

### **8.1.2 Experimental design and procedures**

Subjects were required to attend the laboratory on one occasion for assessment of conduit artery function, assessment of body composition and a  $\dot{V}O_{2peak}$  test (in that order). All subjects were studied at the same time of day in a quiet temperature controlled room. Subjects were informed they must fast for 4 hours and avoid strenuous physical activity for 24 hours prior to testing. Physical activity and sedentary behaviour were measured over 7 days using uniaxial accelerometry. Please refer to section 3.2 for detail on methodologies.

### **8.1.3 Statistical Analysis**

Statistical analyses were performed using SPSS 17.0 (SPSS) and STATA 11 software. All data are reported as group means  $\pm$  SD, statistical significance was assumed at  $P < 0.05$ . The assumptions of normality and equal variance were tested a priori. Data in violation of normality underwent log transformation. Between group differences were assessed using independent samples *t*-test using either raw or log transformed data where appropriate. To assess whether outcome variables were affected by differences in gender and maturational status, forward stepwise linear regressions were performed for the whole cohort with age, age<sup>2</sup>, gender, gender\*age<sup>2</sup>, and TPHV entered as independent variables. Where significant relationships were observed, standardized residuals were then used in place of the original data for the subsequent analyses. Intraclass correlation coefficients (*r*) were used to compare the variability between twin pairs in the MZ and DZ groups. A heritability estimate (*h*<sup>2</sup>) was calculated for FMD using the equation:  $h^2 = 2(r_{MZ} - r_{DZ})$ .



## **8.2 Results**

The groups were similar across all measured variables including general demographic data, cardiorespiratory fitness level, vascular function and PA levels (Table 8.1). All physical activity variables violated the assumptions of normality and were therefore log transformed. Standardized residuals were calculated for  $\dot{V}O_{2\text{peak}}$  and total PA and were used in subsequent analysis.

### **8.2.1 Intraclass Correlations**

The intraclass correlation coefficients for raw data, and also sex and maturity adjusted data, are shown in Table 8.2. As MZ twins are genetically identical and DZ twins share, on average, 50% of their genes, the ICC coefficient between MZ twins should theoretically be 1 and the correlation between DZ twins 0.5 (half of the MZ value) if genetic factors are solely responsible for variation in a certain phenotype (Maia et al. 2002). If correlation coefficients remain in the same ratio (2:1), but are lower than these values, then both genes and also non-shared environmental factors are responsible for the variance. If the 2:1 ratio is not evident, then genes, non-shared environmental factors and shared environmental factors may all be contributing to the variation.

#### *Fitness and physical activity*

$\dot{V}O_{2\text{peak}}$  was significantly related in the MZ group but not the DZ twins (table 8.2), Heritability was estimated at 0.8.

Sedentary time and total PA were highly correlated in the MZ twins and showed a moderate correlation between DZ twins. Significant relationships were observed in both the MZ and DZ groups for time spent performing light physical activity. Time spent

performing moderate and vigorous PA were significantly correlated in the MZ twins only.

**Table 8.1** Descriptive characteristics for MZ and DZ twins

|  | <b>MZ</b>           | <b>DZ</b>      | <b>P value</b> |
|--|---------------------|----------------|----------------|
|  | <b>n = 11 pairs</b> | <b>n = 11</b>  |                |
| <b>Age</b>   | 13.3 ± 1.6          | 13.6 ± 1.6     | 0.65           |
| <b>TPHV (years)</b>                                | -1.03 ± 0.56        | -0.94 ± 1.33   | 0.79           |
| <b>Mass (kg)</b>                                   | 55.25 ± 13.82       | 57.39 ± 13.56  | 0.61           |
| <b>Stature (m)</b>                                 | 1.60 ± 0.11         | 1.62 ± 0.10    | 0.41           |
| <b>BMI (kg.m<sup>2</sup>)</b>                      | 22.1 ± 3.8          | 21.9 ± 3.5     | 0.90           |
| <b>%FM</b>   | 25.0 ± 8.2          | 26.6 ± 9.0     | 0.54           |
| <b>%AbFM</b>                                       | 20.6 ± 7.8          | 21.7 ± 9.4     | 0.66           |
| <b>%LM</b>   | 71.3 ± 8.9          | 72.3 ± 7.4     | 0.70           |
| <b>VO<sub>2peak</sub> (ml.kg.min<sup>-1</sup>)</b> | 21 ± 4              | 22 ± 4         | 0.83           |
| <b>Sedentary time (min)</b>                        | 567.03 ± 76.76      | 572.95 ± 51.92 | 0.77           |
| <b>Light PA (min)</b>                              | 153.88 ± 38.64      | 179.55 ± 48.06 | 0.06           |
| <b>Moderate PA (min)</b>                           | 23.51 ± 13.65       | 20.76 ± 10.06  | 0.50           |
| <b>Vigorous PA (min)</b>                           | 0.97 ± 1.32         | 2.78 ± 5.07    | 0.12           |
| <b>Total PA (min)</b>                              | 179.99 ± 41.75      | 206.32 ± 47.34 | 0.08           |
| <b>SBP (mmHg)</b>                                  | 109 ± 9             | 115 ± 15       | 0.10           |
| <b>DBP (mmHg)</b>                                  | 63 ± 6              | 65 ± 4         | 0.13           |
| <b>Flow mediated dilation (%)</b>                  | 7.07 ± 3.08         | 6.17 ± 3.07    | 0.34           |

### *Body composition*

Body mass index was strongly correlated in the MZ group and showed a moderate correlation that approached significance (P=0.07) in the DZ twins. The ratio of correlation coefficients for BMI was close to 2Mz:1Dz, suggesting a strong genetic contribution. Percentage body fat, %AbFM and %LM were significantly related in the MZ group, whereas no such relationships were evident in the DZ group.



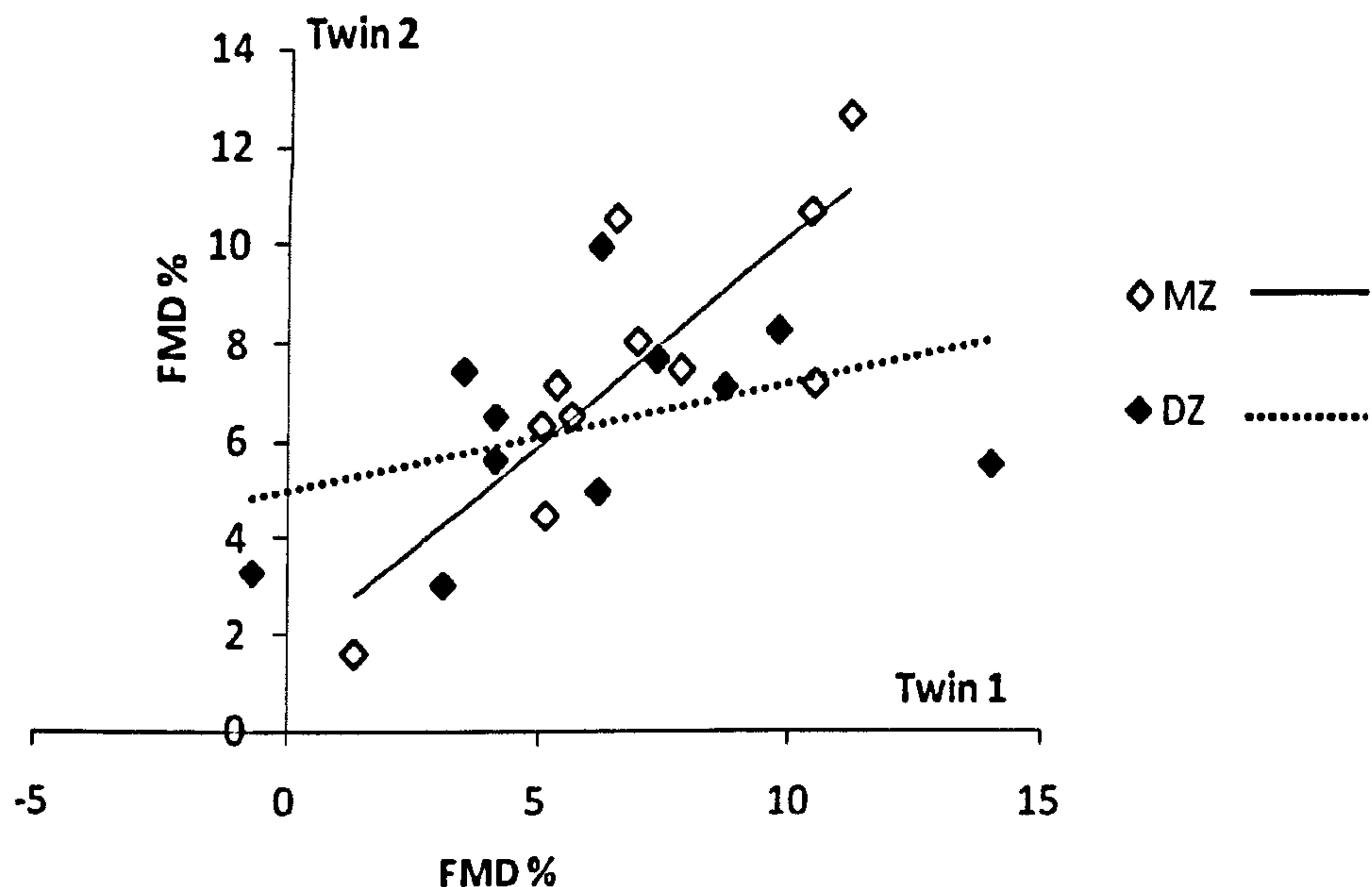
### *Vascular outcomes*

FMD was significantly correlated within the MZ twins, but not in the DZ twins (Table 8.2). The relationship for FMD in each pair of MZ and DZ twins is shown in a scatter plot (Figure 8.1). The heritability estimated for FMD was 0.44.

**Table 8.2. Intraclass correlation coefficients**

|  | <b>MZ</b>         |          | <b>DZ</b>         |          |
|--|-------------------|----------|-------------------|----------|
|  | <b>n=11 pairs</b> |          | <b>n=11 pairs</b> |          |
|  | <b>r</b>          | <b>P</b> | <b>r</b>          | <b>P</b> |
| <b>Mass</b>  | 0.88              | <0.01    | 0.31              | 0.23     |
| <b>Height</b>  | 0.94              | <0.01    | 0.65              | 0.04     |
| <b>BMI</b>   | 0.83              | <0.01    | 0.45              | 0.07     |
| <b>%FM</b>   | 0.90              | <0.01    | 0.22              | 0.24     |
| <b>%AbFM</b>   | 0.89              | <0.01    | 0.23              | 0.23     |
| <b>%LM</b>   | 0.93              | <0.01    | 0.39              | 0.10     |
| <b>VO<sub>2</sub> (ml.kg.min<sup>-1</sup>)</b>           | 0.84              | <0.01    | 0.44              | 0.07     |
| <b>Adjusted* VO<sub>2</sub> (ml.kg.min<sup>-1</sup>)</b> | 0.72              | <0.01    | 0.32              | 0.14     |
| <b>Sedentary time (min)</b>                              | 0.86              | <0.01    | 0.49              | 0.05     |
| <b>Light PA (min)</b>                                    | 0.80              | <0.01    | 0.63              | 0.02     |
| <b>Moderate PA (min)</b>                                 | 0.78              | <0.01    | 0.18              | 0.29     |
| <b>Vigorous PA (min)</b>                                 | 0.55              | 0.03     | 0.51              | 0.06     |
| <b>Total PA (min)</b>                                    | 0.77              | <0.01    | 0.58              | 0.02     |
| <b>Adjusted Total PA (min)</b>                           | 0.68              | <0.01    | 0.60              | 0.02     |
| <b>Flow mediated dilation (%)</b>                        | 0.60              | 0.02     | 0.38              | 0.10     |

\*Values are adjusted for sex and/age and/maturation



**Figure 8.1.** Relationship between FMD in each pair of MZ and DZ twins

### **8.3 Discussion**

This is the first study, to our knowledge, to assess vascular function, cardiorespiratory fitness and physical activity in MZ and DZ twins. The principle finding in this study of 22 twin pairs was that FMD, a surrogate marker of endothelial function and vascular health, was more strongly correlated within MZ twins than DZ twins. This novel data suggests that, although genetics play some role in the determination of endothelial function during adolescence, environmental factors may exert a larger influence. Secondary findings were that measures of fitness, body composition and sedentary behaviour also appeared to be under substantial genetic control, whereas light and vigorous PA were more influenced by environmental factors.

The heritability estimate calculated for FMD in the current study suggests that around 40% of the variance in FMD was accounted for by genetics, leaving ~60% of the variance attributable to environmental factors. Our estimate of heritability is in line with those reported for numerous biological parameters such as blood pressure (Hong et al.



those reported for numerous biological parameters such as blood pressure (Hong et al. 1994) and IMT (Duggirala et al. 1996). Although this is the first study to investigate FMD heritability in a paediatric population, previous studies examining the heritability of FMD in adults have yielded estimates ranging from 0.14-0.39 (Benjamin et al. 2004; Jartti et al. 2002).

One possible reason for the variability in previous estimates of the FMD heritability relates to measurement techniques. FMD assessment is technically demanding and requires specialised training and careful analysis. Despite recent attempts to standardize the technique (Corretti et al. 2002), assessment protocols vary substantially and this can impact upon the derived results (Atkinson et al. 2009; Black et al. 2008). Lower measurement precision will produce an inflated environmental component, in turn yielding a lower heritability estimate. In the present study, we adopted state-of-the-art technical approaches and analysis methodology, which we have extensively validated.

(Thijssen et al. 2009; Woodman et al. 2001). We are therefore confident that the measurement error within the current study was minimised and that our results are valid.

The principal implication of our finding pertaining to FMD is that, although some genetic contribution to the FMD response is apparent, endothelial function can be modified by environmental factors. Many previous studies in the paediatric literature have demonstrated that factors such as cigarette smoking (Celermajer et al. 1992), lipid levels (Engler et al. 2003), diet (Woo et al. 2004), body fatness (Arcaro et al. 1999), cardiorespiratory fitness (Meyer et al. 2006) and PA levels (Abbott et al. 2002) can modify FMD. Furthermore, impaired FMD is known to be responsive to exercise training or increases in PA (Watts et al. 2004a; Watts et al. 2004b). Taken together with

the findings of the present study, these data illustrate that although genes may influence FMD, risk factor modification strongly impacts upon endothelial function.

We found that mass, height, BMI, and measures of percentage body fat and lean mass were strongly correlated in the MZ twins. In DZ twins variables were less strongly and not significantly correlated. In line with previous findings, these data infer that there is a large genetic influence in the determination of body composition variables (Keller et al. 2003). Although data examining genetic contribution to body composition is scant in paediatric populations, numerous studies have examined heredity of BMI in older populations. In agreement with our findings, these studies have typically reported a strong genetic component in its variance (Allison et al. 1994; Bodurtha et al. 1990). Furthermore, Faith *et al.* (Faith *et al.* 1999) using bioelectrical impedance, showed higher MZ correlation coefficients for % fat, similar to those in the current study. However, this method of body composition analysis is not generally considered to be as robust as DXA assessment (Curtin et al. 1997; Morabia et al. 1999). The few studies that have used DXA to measure the intra pair relationships in adult twins have demonstrated stronger  $r$  values for MZ twins than DZs (Malis et al. 2005; Nguyen et al. 1998). Both studies concluded that fat and lean mass are under strong genetic regulation. Our data lend support to the notion of a genetic dominance effect acting on measures of body composition in young people.

We have reported  $\dot{V}O_{2peak}$  intraclass correlations that suggest cardiorespiratory fitness is highly genetically determined. Our findings are comparable to those previously observed in the literature. In a study conducted to examine the heritability of the adaptation of maximal aerobic power to exercise training, the sensitivity of  $\dot{V}O_{2max}$  to exercise training was under considerable genetic control (MZ $r$ = 0.74,  $P<0.01$ )



(Prud'homme et al. 1984). It is possible that the relatively high heritability of fitness measures is a reflection of the genetic contribution to somatic factors such as enzyme concentrations, which contribute to functional capacity. However, another possibility is that exposure to the behavioural stimulus which increases fitness, physical activity, may be genetically determined. However, previous studies pertaining to the genetic determination of physical activity levels reveal mixed results, with estimates that between ~30 and ~80% of the variance in total PA levels can be explained by genetic factors (Eriksson et al. 2006; Joosen et al. 2005; Maia et al. 2002). Methodological differences in PA measurement may explain some of this disparity. For example, subjective assessment of PA levels via questionnaire have significant limitations. In the present study, we used objective measures of PA which were individually calibrated against maximal treadmill exercise tests. Furthermore, ours is the first study to assess different PA intensities in twin subsets. Our findings indicate that PA outcomes such as moderate PA and sedentary behaviour possessed intraclass correlations in monozygotic twins which were higher than those observed in dizygotic twins. This suggests some genetic contribution to paediatric physical activity and sedentary behaviour, which may in turn contribute to the high heritability previously reported in behaviourally modifiable physical capacities such as  $\dot{V}O_{2max}$ .

Our findings can only be generalised to paediatric populations. The cohort was comprised of subjects who varied in biological maturation and included same sex twin pairs of both genders. It is therefore possible that maturation and sex differences may have confounded the results, although we attempted to control for these variables by reporting sex and maturity adjusted results where necessary. In keeping with previous recommendations regarding the use of complex modelling analyses in relatively small twins studies (Christian et al. 1995), we specifically avoided the use of unsuitable

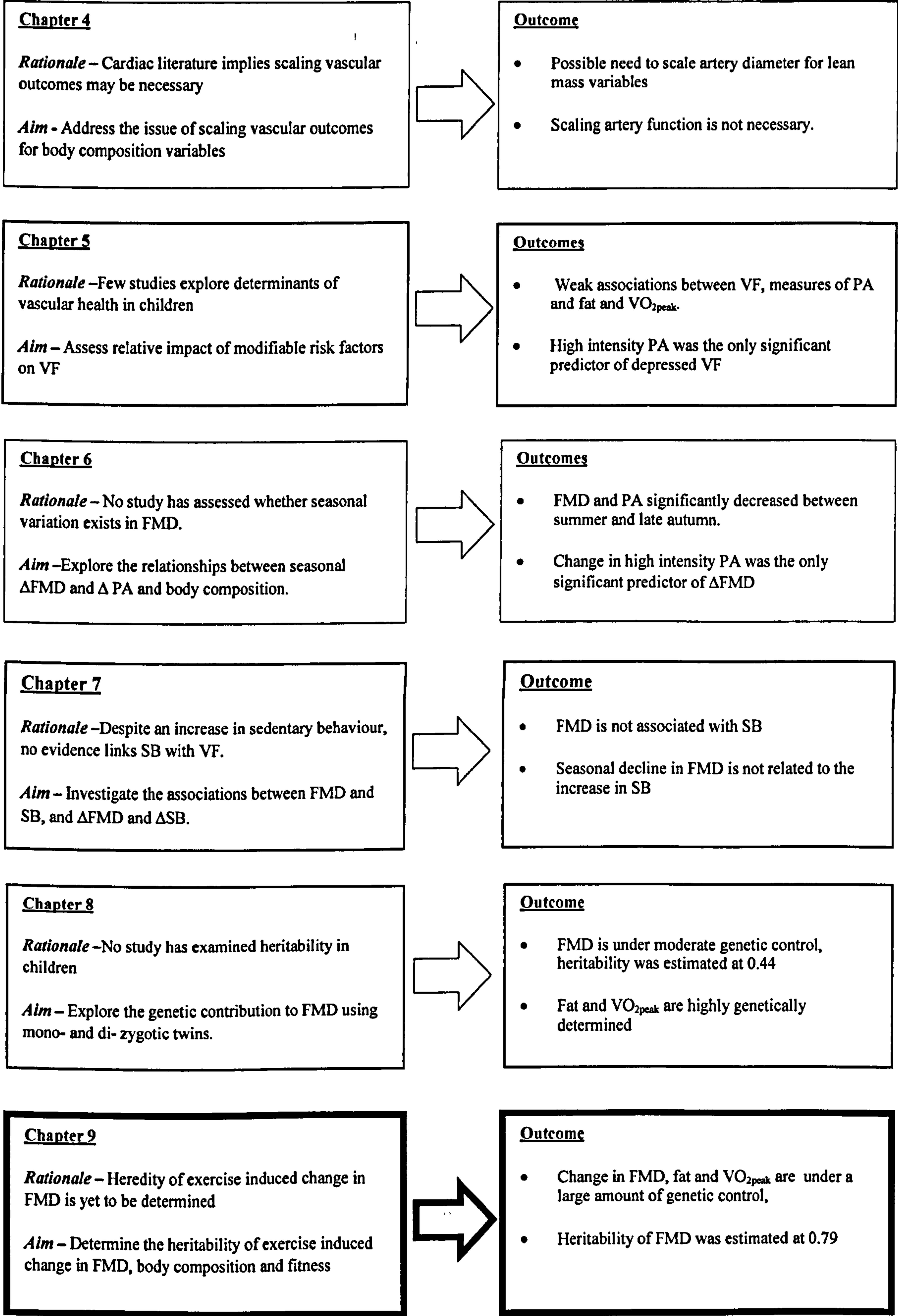
statistical modelling procedures which may give misleading results in such a relatively small cohort. In small samples such as those in the present study, the most transparent and appropriate approach is to present the intraclass correlations for each twin subset, without attempting to partition out the relative contributions of the unique and common environments to the variation in each. Nonetheless we were able to calculate a crude heritability estimate of the genetic contribution to FMD and we collected a range of complex and valid physiological measures which have not previously been assessed using twin pair cohorts. We therefore present our findings as exploratory, with the intention of generating hypotheses which drive future investigations of vascular function, fitness and physical activity behaviours in young people.

#### **8.4 Conclusion**

We observed that FMD was more strongly correlated in MZ than DZ twins, suggesting some genetic contribution to endothelial function in young people. However, the relative strength of each twin subset correlation suggests that environmental factors play a predominant role in determining endothelial function. FMD is therefore modifiable and an emphasis on lifestyle factors to decrease CV risk is recommended. In agreement with previous findings, the current study suggests that body composition variables and  $\dot{V}O_{2peak}$  were largely genetically determined. We found conflicting results for PA variables, whilst both sedentary behaviour and moderate intensity PA seem to be primarily influenced by genetic factors, light, vigorous and very vigorous PA are more heavily influenced by environmental factors. These data may suggest that physical activity may be a more desirable variable to modify and integrate into interventions aimed at improving endothelial function in adolescents.



# Study aims and outcomes



## Chapter 9

# **HEREDITY OF EXERCISE INDUCED CHANGES IN VASCULAR FUNCTION, FITNESS AND BODY COMPOSITION**

**Based on the article “Impact of exercise training on body composition in youth: A study of mono- and di-zygotic twins” under consideration at the Journal of Applied Physiology, and the article “Impact of exercise training on endothelial function, and fitness in young people: A study of mono- and di-zygotic twins” under consideration at Arteriosclerosis, Thrombosis and Vascular Biology.**

**(Please see appendices for copies of the submitted articles)**



## **9 Introduction**

Acute release of NO in the vascular endothelium causes vasodilatation in an attempt to normalise shear during periods of increased flow induced by exercise, for example (Hutcheson and Griffith 1991). This has led to the investigation of the possible upregulation of the NO dilator system through repeated bouts of exercise, such studies have demonstrated an augmentation of NO mediated endothelial function in adults (Clarkson et al. 1999) and children (Meyer et al. 2006; Watts et al. 2004a; Watts et al. 2004b; Woo et al. 2004) in response to exercise training.

Although it is clear that exercise training is capable of modulating endothelial function, little is known about the role of genetics in the adaptive response of FMD to exercise training. Studies of monozygotic (MZ) and dizygotic (DZ) twins offer a method of examining genetic and environmental sources of variance in quantitative traits. Although a small number of studies have utilized this methodology to assess the genetic contribution to FMD response (Benjamin et al. 2004; Jartti et al. 2002), to our knowledge none have examined changes in FMD in response to exercise training. The purpose of the present study was therefore to assess conduit artery endothelial function in mono and di-zygotic twin pairs before and after an eight week exercise training program. We also assessed change in fitness and body composition in both twin subsets. We hypothesised that changes in all variables in response to exercise training would be highly correlated in monozygotic twins, and modestly related in di-zygotic twins, indicating a high degree of heritability.

## **9.1 Methods**

### **9.1.1 Participants**

Twelve cohabiting twin pairs, 6 monozygotic (1♂, 5♀, 13.5±0.8 yrs), 6 di-zygotic (2♂, 4♀, 13.4±0.8 yrs) were recruited from the community. Zygosity was determined using a previously validated questionnaire.(Goldsmith 1991) Participants were healthy, normotensive and not suffering from known cardiovascular or metabolic conditions. None were taking any vasoactive medications, nor were they smokers. Ethics approval was obtained from the institutional ethics committee. Informed parental consent and child assent were obtained prior to participation in the study.

### **9.1.2 Experimental design and procedures**

At baseline and following eight weeks of exercise training conduit artery function, body composition and a cardiorespiratory fitness (in that order) were assessed. For detailed methodologies please refer to section 3.2. From the DXA output percentage body fat mass (%FM), percentage abdominal fat mass (%AbFM) and percentage body lean mass (%LM) were reported as preferred outcome measures for intra pair comparisons.

### **9.1.3 Exercise training**

The eight week intervention consisted of three 45 minute gym based sessions per week, involving a combination of cycling, running and cross training (figure 9.1). All twins received identical training stimuli. During week one all twins exercised at 65% HR max, this was increased to 70% for the following 2 weeks, 80% HR max for weeks 4 and 5 and 85% HR max for the final 3 weeks. Training was continually supervised and monitored. Participants wore a HR monitor (Polar, Kempele, Finland) during all sessions. The gym equipment used monitors and interacts with individual exercising HR, and will adjust the speed/resistance in order to maintain target HR training zones.





**Figure 9.1** Twins undertaking the exercise training intervention

#### **9.1.4 Statistical Analysis**

Statistical analyses were performed using SPSS 17.0 (SPSS) software. All data are reported as group means  $\pm$  SD, statistical significance was assumed at  $P < 0.05$ . Data distribution was initially examined for normality using the Shapiro-Wilk test.

A mixed design GLM was used to assess group differences following the exercise intervention. Raw data change scores were calculated for all variables (test 2-test 1). Intraclass correlation coefficients (ICCs) were used to evaluate the strength of intra-twin change score relationships. A heritability estimate ( $h^2$ ) was calculated for  $\Delta$ FMD using the equation:  $h^2 = 2(r_{MZ} - r_{DZ})$ .



## **9.2 Results**

All data were normally distributed. The groups were similar across all demographic and body composition variables at baseline (table 9.1).

The mean compliance to the exercise training intervention was 94%. There was a significant main effect of time for increase in mass, height, maturation, %FM and %AbFM ( $P < 0.01$ ). A significant main effect of time for baseline diameter was observed ( $P = 0.04$ ). The main time effect for FMD was  $P = 0.055$ , although FMD improved in both subgroups, neither change achieved statistical significance. There were no other significant changes observed between groups for pre and post test (table 9.1).

### **9.2.1 Intraclass Correlations**

The intraclass correlation coefficients for change scores in MZ and DZ are shown in table 9.2. Change in FMD was significantly correlated in the MZ twins, but not in the DZ twins. The relationship for  $\Delta\text{FMD}$  in each pair of MZ and DZ twins is shown in a scatter plot (Figure 9.2). Heritability of change in FMD was estimated at 0.79.

The intrapair relationships for  $\Delta \dot{V}\text{O}_{2\text{peak}}$  were non significant in both groups. Change in body mass index was highly correlated in the MZ group as was %FM. The relationships between %LM approached statistical significance in the MZ twins. There were no significant relationships observed between any body composition variables within the DZ cohort



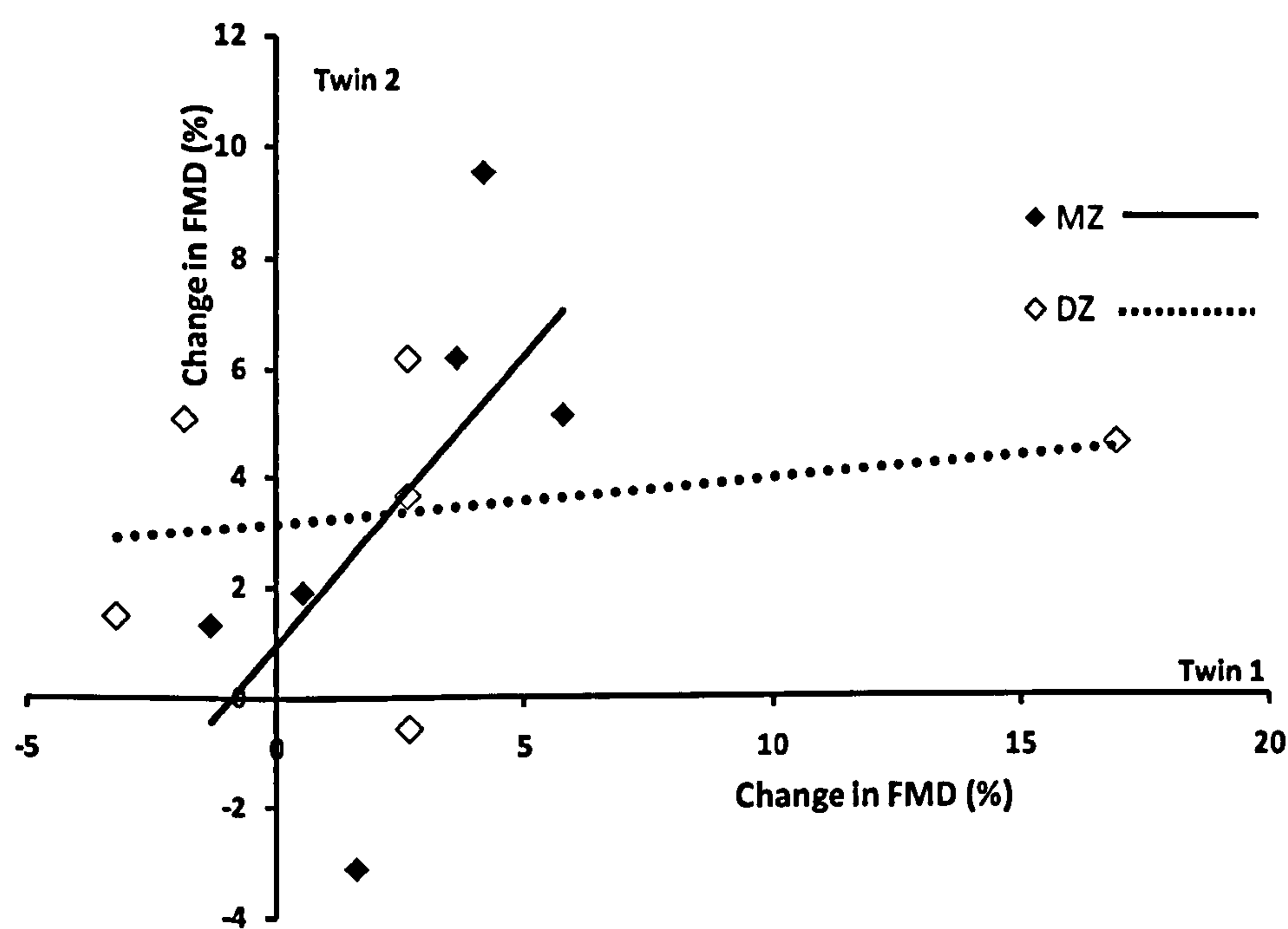
Table 9.1. Pre and post exercise data in mono- and dizygotic twins

|  | MZEx         |              | DZEx          |               |
|--|--------------|--------------|---------------|---------------|
|  | Pre          | Post         | Pre           | Post          |
| Age  | 13.47 ± 0.81 | 13.84 ± 0.79 | 13.39 ± 0.77  | 13.74 ± 0.69  |
| TPHV (years)                               | -0.86 ± 0.53 | -0.99 ± 0.52 | -1.65 ± 0.59  | -1.07 ± 0.85  |
| Mass (kg)                                  | 59.0 ± 11.5  | 60.8 ± 10.4  | 58.9 ± 12.6   | 59.9 ± 11.4   |
| Stature (m)                                | 1.65 ± 0.52  | 1.66 ± 0.47  | 1.63 ± 0.10   | 1.65 ± 0.09   |
| BMI (kg.m <sup>2</sup> )                   | 21.5 ± 3.5   | 22.0 ± 3.0   | 22.0 ± 3.7    | 22.0 ± 3.8    |
| %FM  | 27.08 ± 6.90 | 26.28 ± 7.76 | 26.03 ± 11.27 | 24.93 ± 11.74 |
| %AbFM                                      | 22.71 ± 8.91 | 21.42 ± 6.94 | 21.33 ± 10.60 | 20.97 ± 11.46 |
| %LM  | 69.89 ± 6.75 | 70.68 ± 7.67 | 69.73 ± 9.79  | 72.18 ± 11.19 |
| VO <sub>2</sub> (ml.kg.min <sup>-1</sup> ) | 44.38±8.05   | 45.69±8.09   | 48.35±11.67   | 47.44±8.81    |
| Baseline artery diameter (mm)              | 3.2±0.3      | 3.4±0.3      | 3.1±0.3       | 3.3±0.3       |
| Flow mediated dilation (%)                 | 6.99±2.27    | 8.36±3.47    | 6.08±3.06     | 8.83±3.38     |

Time to peak height velocity (TPHV), body mass index (BMI), percentage body fat mass (%FM), percentage abdominal fat mass (%AbFM), percentage body lean mass (%LM)

**Table 9.2.** Intraclass correlation coefficients performed on change scores for each twin subset

|   | MZEx      |        | DZEx      |      |
|---|-----------|--------|-----------|------|
|   | n=6 pairs |        | n=6 pairs |      |
|   | r         | P      | r         | P    |
| $\Delta$ Mass                                       | 0.89      | <0.01* | -0.04     | 0.52 |
| $\Delta$ Height                                     | 0.39      | 0.17   | 0.32      | 0.22 |
| $\Delta$ BMI  | 0.81      | <0.01* | 0.57      | 0.08 |
| $\Delta$ %FM  | 0.63      | 0.05   | 0.31      | 0.23 |
| $\Delta$ %AbFM                                      | 0.30      | 0.24   | 0.08      | 0.42 |
| $\Delta$ %LM  | 0.52      | 0.10   | 0.34      | 0.21 |
| $\Delta$ VO <sub>2</sub> (ml.kg.min <sup>-1</sup> ) | 0.43      | 0.17   | 0.21      | 0.34 |
| $\Delta$ Flow mediated dilation (%)                 | 0.74      | 0.02*  | 0.34      | 0.21 |
| Baseline diameter (mm)                              | -0.59     | 0.91   | 0.10      | 0.40 |



**Figure 9.2** Relationship between change in FMD in MZ and DZ twins



### **9.3 Discussion**

The aim of the current study was to assess the within pair relationship for exercise induced changes in flow mediated dilation in mono- and dizygotic twins. Few studies have used a twin design to examine the genetic contribution to FMD, and to our knowledge this is the first study to assess *changes* in endothelial function in response to exercise training. Our rationale for studying changes with training relates to the fact that baseline differences between twin pairs reflect variability between subjects, whereas an analysis of changes within subjects may reveal important new insights into the heritability of training responses. Our principal finding was that  $\Delta$ FMD is more strongly correlated within MZ twins than DZ twins. This novel data suggests that, although environmental factors contribute to  $\Delta$ FMD, the degree of improvement in endothelial function following exercise training appears to be highly genetically determined. Regarding the impact of exercise training on changes in  $VO_{2peak}$ , we found that, although the ratio of  $r$  values implies a genetic dominance effect, correlations were modest and non-significant for both subsets which also suggests considerable environmental influence. Furthermore we were able to demonstrate that exercise induced  $\Delta$ BMI and  $\Delta$ %FM were highly correlated in MZ twins, whilst  $\Delta$ BMI was modestly correlated in the DZ twins. These data infer a large genetic component to change in BMI and %FM. .

This is the first study, to our knowledge, to address the question of genetic heritability of changes in endothelial function with exercise training. Change in FMD in our young cohort appears to be largely genotype dependent, with a heritability estimate of 0.79. Studies examining the heritability of FMD in cross sectional analyses of adults have yielded estimates ranging from 0.14-0.39 (Benjamin et al. 2004; Jartti et al. 2002), and our data from chapter 8 suggests that the heritability for a one-off FMD measurement

was 0.44 in young people. The variability in previous estimates of FMD heritability based on cross sectional assessments could relate to issues with measurement. FMD assessment is technically demanding and requires specialised training and careful analysis. Despite recent attempts to standardize the technique (Corretti et al. 2002), assessment protocols vary substantially and this can impact upon the derived results (Atkinson et al. 2009; Black et al. 2008). As discussed in chapter 8, lower measurement precision will result in an inflated environmental component, in turn yielding a lower heritability estimate. In the present study, we adopted state-of-the-art technical approaches and analysis methodology, which we have extensively validated (Thijssen et al. 2009; Woodman et al. 2001). Furthermore, by undertaking a follow up FMD, the measurement imprecision associated with taking a one-off measurement is reduced, therefore giving a more accurate indication of FMD.

Our heritability estimate implies that ~20% of the variance in  $\Delta$ FMD is a result of environmental factors. Previous paediatric studies have demonstrated that factors such as cigarette smoking (Celermajer et al. 1992), lipid levels (Engler et al. 2003), diet (Woo et al. 2004), body fatness (Arcaro et al. 1999), cardiorespiratory fitness (Meyer et al. 2006) and PA levels (Abbott et al. 2002) all modulate FMD. Furthermore exercise training, physical activity and macronutrient intake are known to modify endothelial function in children (Watts et al. 2004a; Watts et al. 2004b; Woo et al. 2004). Taken together with the current findings, these data suggest that, despite a large genetic dominance,  $\Delta$ FMD in response to exercise training is affected to some extent by changes in risk factors, including exercise.

Prud'Homme *et al.* (Prud'homme et al. 1984) previously sought to examine the contribution of genes to exercise induced change in maximal aerobic power in adults.



Following a 20 week endurance training programme the investigators observed a large similarity in the  $\Delta \dot{V}O_{2\max}$  in monozygotic twins (MZr=0.74). The authors concluded that the sensitivity of maximal aerobic power to exercise training was largely genotype-dependent, although dizygotic twins were not studied and compared to monozygotic findings. In concurrence with Prud'Homme *et al.*, (Prud'homme et al. 1984) we were able to demonstrate a high intraclass correlation coefficient for MZ twins ( $r=0.84$ ) in chapter 8. However, the MZr reported for  $\Delta VO_{2\text{peak}}$  in the current study suggest that the  $VO_{2\text{peak}}$  response to training may be more variable than previously reported. This discrepancy may be indicative of the different age or maturational status between the cohorts. Little is known about how the genotypic expression of physiological variables tracks from childhood into adulthood, and given that the heredity of numerous personality traits can vary longitudinally (McGue et al. 1993; Viken et al. 1994), it is reasonable to suggest that the expression of genes that govern  $\Delta$ physiological variables, such as  $VO_{2\text{peak}}$ , may differ during specific developmental stages. Furthermore, the different duration of exercise training undertaken in each study (8 Vs 20 weeks) may also contribute to the variation between MZr values. Similarity within twin pairs for exercised induced response of enzyme activities were mainly detected in the second half of a 15 week training programme (Hamel et al. 1986). It is possible that the time course of the intra-pair  $VO_{2\text{peak}}$  adaptation is similar to that observed by Hamel *et al.* (Hamel et al. 1986), implying that the within pair change over the first 7 weeks of exercise is relatively heterogeneous compared to the response after this. Nonetheless, our findings for  $VO_{2\text{peak}}$  suggest that changes in this parameter are more environmentally influenced than changes in FMD.

In the current study a significant relationship was observed for  $\Delta\%FM$  for the MZ twins only, suggesting that the exercise training induced reduction in  $\%FM$  may be largely

determined by genes. Although, the correlation for  $\Delta\%FM$  in MZ twins of 0.6 also infers some environmental contribution was present. These findings imply that, whilst exercise training is an effective behavioural strategy for body composition management, one challenge in treating and preventing paediatric obesity may be overcoming a genetic predisposition that actively resists perturbations in energy balance. Although stronger correlations were evident for  $\Delta\%LM$  in MZ twins compared to DZs, these relationships were not high and failed to reach statistical significance. However the ratio between MZr and DZr would suggest that genes have a more modest role in the determination of  $\Delta LM$  than is evident for  $\Delta FM$ . Taken together these data imply that whilst  $\Delta\%FM$  is strongly genetically determined and therefore possibly resistant to exercise-induced change,  $\Delta\%LM$  may be more amenable to environmental stimuli such as exercise training. This is an important finding, as improvement in LM is related to an improved glucose disposal and enhanced glycaemic control (Dunstan et al. 2002; Shaibi et al. 2006).

The results from the current study can only be generalised to paediatric populations similar to those studied. Furthermore, the cohort varied in age and biological maturity. It is common practice in the literature to remove the influence of possible confounders such as maturation, by accounting for these variables in statistical analyses, however we did not perform this analysis due to limited subject numbers. In accordance with previous recommendations, we have also avoided complex statistical modelling that can partition the genetic and environmental components of a phenotype.(Christian et al. 1995) Future studies in larger twin cohort could conceivably address such issues.



#### **9.4 Conclusion**

We observed that exercised induced changes in FMD and %FM were more highly correlated in MZ than DZ twins, suggesting a strong genetic component to exercise induced change in endothelial function, whilst changes in  $\text{VO}_{2\text{peak}}$  and %LM appears to be under more modest genetic control. Our data suggest that, whilst all measured variables are under some degree of genetic influence, they are amenable to exercise induced change and the influence of environmental factors.

## **Chapter 10**

### **Synthesis**



## **10 Aims and Objectives**

The primary aim of this thesis was to investigate childhood associations between endothelial function and a range of modifiable and non-modifiable CV risk factors. By addressing a specific series of research questions we were able to provide novel information regarding the relationships between physical activity, sedentary behaviour, body composition, cardiorespiratory fitness and the heritability of endothelial function. In addition, we sought to explore the technical issue of the scaling of vascular outcomes for body size and composition.

Study one (Chapter 4, a technical study) addressed the question of scaling, by evaluating the efficacy of scaling for different body composition variables using allometric and ratio procedures, with the aim of producing size independent vascular indices. Our data demonstrated significant relationships between baseline brachial artery diameter and measures of both total and lean mass, however, the use of allometric scaling removed the influence of these body composition variables. These findings imply that, if between-group comparisons of baseline brachial artery diameter are to be undertaken, appropriate scaling may need to be adopted. The implications drawn from our findings pertaining to scaling arterial function were less succinct. Although allometric scaling procedures were undertaken for both whole arm and forearm fat mass and FMD, the application of such scaling is debatable as the initial associations between variables were very weak. Whilst further research is recommended to explore relationships between body composition and FMD in a variety of populations, the data from the current thesis cannot be used to support the use of scaling for FMD outcomes.

Studies 2 and 4 (Chapters 5 and 7) aimed to assess the cross-sectional relationships between FMD and modifiable CV risk factors in young people. We examined associations between FMD and objective measurements of body composition, cardiorespiratory fitness, physical activity levels and sedentary behaviour. We observed a weak association between percentage body fat and FMD and no further relationships across cohort. However, depressed endothelial function was significantly related to, and predicted by, high intensity physical activity. The relationship between FMD and high intensity PA was further reinforced by the findings from studies 3 and 4 (Chapters 6 and 7). These observational studies demonstrated that seasonal decline in vascular function was associated with, and predicted by, change in high intensity PA. No other variable, including sedentary behaviour, correlated with decline in vascular function. These findings demonstrate, for the first time, that high-intensity PA may be an important determinant of vascular dys/function in children.

Finally, studies 5 and 6 (Chapters 8 and 9) explored the role of genetics in the modulation of FMD. We adopted a classic twin study design, involving assessment of relationships in mono- and dizygotic twins. We concluded that, although a one-off measurement of endothelial function is under some level of genetic control, environmental factors may have a larger influence in the determination of FMD in young people (study 5). In study 6, twins undertook 8 weeks of aerobic exercise training. In this unique study, intra-twin differences in the adaptation of FMD were compared between MZ and DZ twins. This analysis highlighted a greater similarity between MZ twins than DZ twins in the change in FMD, suggesting that exercise-induced improvements in FMD may be largely genetically determined.



In summary, the main findings from this thesis were:

1. Scaling brachial artery function for body composition or size does not appear to be necessary in pre-pubertal children. However, it may be necessary to scale brachial artery diameter for measures of total mass or lean mass using an allometric approach.
2. FMD was weakly, inversely, associated with percent body fat in young people. No relationships were observed between FMD and other measures of body composition, fitness, sedentary behaviour or intensities of physical activity.
3. Impaired endothelial function was associated with high intensity PA. High intensity PA was the only significant predictor of depressed FMD.
4. FMD is significantly reduced between summer and late autumn, implying seasonal variation exists in endothelial function in children.
5. Seasonal decline in FMD was associated with, and predicted by the seasonal decline in high intensity PA only. There was no association between seasonal variation in body composition or sedentary behaviour and vascular function.
6. FMD was more strongly correlated in monozygotic than dizygotic twins, implying moderate genetic control of endothelial function. Heritability was estimated at 0.44.
7. Exercise induced change in FMD appears to be predominantly governed by genes. Heritability of change in FMD was estimated at 0.79.

## **10.1 General discussion**

### **10.1.1 Scaling issues**

Previous research indicates that comparison of measurements of cardiac structure and function between, and sometimes within subjects, can be confounded by differences in body size and composition. Consequently, it is now commonplace to normalise or scale measures of cardiac dimension and function to improve the validity of scientific and clinical interpretation. However, there are no extant data exploring the scaling of vascular outcomes for body size parameters.

The rationale for addressing the question of scaling arterial diameter is clear; numerous studies have demonstrated differences in arterial structure between groups that vary in body size and composition, including able-bodied and disabled athletes, and able-bodied and disabled sedentary participants (de Groot et al. 2006; de Groot et al. 2004; Huonker et al. 2003). For example, studies have shown enlarged vessel diameters in endurance trained athletes whose vasculature is exposed to chronic increases in blood flow as a result of training. Conversely, the diameter of unconditioned vessels subject to chronically reduced blood flow in paraplegic and disabled athletes are significantly smaller compared to the arteries of able bodied control subjects. One mechanism proposed to mediate differences in arterial structure across these groups is exposure to blood flow and shear stress, which is partially determined by the metabolic demand of the tissue distal to the vessel. However, the possibility remains that differences between these groups may relate to generic differences in body size or composition. The rationale for the examination of scaling issues pertaining to FMD is illustrated by the example of the impact of obesity on vascular function. Recent studies indicate that excess body fat has a negative impact on endothelial function and FMD (Arkin et al. 2008; Oflaz et al. 2003), and that FMD is consistently impaired in obese subjects (Watts



et al. 2004a; Watts et al. 2004b; Woo et al. 2004). However, obese subjects possess systematically larger body size and composition characteristics and these have not been considered when comparisons between groups of subjects have been undertaken.

Previous studies infer that vascular outcomes may be, in part, determined by body size parameters. In an attempt to address this issue and account for body size differences, Olive *et al.* (Olive et al. 2003) corrected femoral artery diameter for muscle volume in a between-groups comparison of healthy and spinal cord injured participants using a simple ratio scaling approach. Scaling in this manner decreased the magnitude of between group differences in femoral artery diameter and maximal hyperaemic blood flow response. Despite this study representing a conceptual “step-in-the-right-direction”, it is important to emphasize that the relationship between artery diameter (a one-dimensional measure) and muscle volume (a 3-dimensional measure) would be unlikely to meet the criteria for simple ratio scaling.

Our results, reported in chapter 4, indicate that baseline brachial artery diameter was associated with measures of total and lean mass. These findings infer that, in our cohort of pre-pubertal children, scaling procedures may need to be undertaken to allow valid intra- and inter- group comparisons to be made. As such, we undertook exploratory analyses to determine which scaling procedure, if any, should be employed to normalise measure of artery diameter. The results clearly indicate that allometric scaling procedures should be employed if meaningful comparisons of brachial artery diameter are to be made. It would appear from our data that similar results are obtained whether total lean mass or total body mass are used as the scalar variable. The implications of these findings are that valid scaling of absolute measures of artery diameter in normal weight young populations does not necessitate the use of DXA derived body

composition data, but can be performed adequately using simple measurement of total body mass. These findings have important consequences for the interpretation of previous studies that involve the comparison of arterial diameters between groups that differ significantly in mass.

In terms of arterial function, we observed weak associations between FMD and segmental measures of fat mass (whole arm and forearm). Whilst allometric scaling procedures removed the influence of these variables, the justification for scaling FMD for segmental measures of fat mass is questionable in this cohort. The associations between vascular function and measures of fat were weak, and confidence intervals came close to crossing zero. Furthermore, the measurement of whole arm and forearm fat mass can be problematic and impractical, and crucially, the rationale for scaling for these local fat compartments is unclear. It can therefore be surmised that scaling vascular function for measures of body composition is currently unnecessary in cohorts of pre-pubertal children. However we advocate that individual studies/researchers generate their own cohort-specific scaling data in future.

#### **10.1.2 Physical activity, fitness, body composition, sedentary behaviour and FMD in children**

Previous studies have demonstrated links between fitness, physical activity levels, body composition, sedentary behaviour and adverse cardiac outcomes in adults (Katzmarzyk et al. 2009; Lee et al. 1999; Wessel et al. 2004). Whilst these findings in adults emphasise the importance of modifiable risk factors in the development of cardiovascular disease, there is no data available for the relative impact of these variables on the development of cardiovascular disease in children or adolescents. Studies of this nature would ultimately require careful longitudinal assessment of the



prevalence of fitness, fatness, PA and SB in young cohorts, followed by the assessment many decades later of the incidence of cardiovascular mortality and morbidity in these groups. Such studies are obviously extremely challenging to undertake. In their absence, it may be possible to gain insight into the question using surrogate markers of the early development of cardiovascular disease. Assessment of vascular endothelial health using FMD provides a means of quantifying the risk of future cardiovascular disease during childhood (Celermajer et al. 1992).

Numerous adult cardiovascular risk factors have been associated with endothelial dysfunction during childhood. In addition to measures of adiposity, as evidenced in chapter 4, childhood endothelial health has been previously linked to smoking (Celermajer et al. 1992), hypercholesterolemia, insulin resistance, type 2 diabetes (Aggoun et al. 2005) and physical activity (Abbott et al. 2002; Pahkala et al. 2008). In addition, impaired endothelial function can be normalised by exercise training (Watts et al. 2004b; Woo et al. 2004). Chapters 5 and 7 addressed the question of the relative impact of measures of physical activity, body composition, fitness and sedentary behaviour on FMD in a cross-section of healthy children. We were able to provide evidence that percentage body fat is the only variable significantly associated with FMD in pre-pubertal children, albeit weakly. This finding suggests that maintenance of body fat and prevention of excess adipose accumulation *per se*, may play a role in the preservation of normal endothelial function.

The lack of meaningful relationships between endothelial function and our measured variables may be partially explained when one considers the results from chapter 8, which conclude that FMD is under a relatively large amount of genetic control. We estimate that ~45% of the variance in endothelial health is determined by genes. Chapter

8 also highlights that measures of body composition, fitness, sedentary behaviour and physical activity, with the exception of high intensity PA, are largely genetically determined. Taken together, these data suggest that although FMD may be modulated by environmental risk factors, genetics play an important role in the determination of vascular function.

Perhaps a more important finding from chapter 5 was uncovered when the cohort was split according to FMD. In the group with the poorest endothelial function, measures of high intensity PA ( $PA_8$  and  $PA_{10}$ ) were significantly associated with FMD. High intensity PA ( $PA_{10}$ ) predicted ~13% of the variance in endothelial function in this group. Furthermore, we were able to confirm that the seasonal decline observed in endothelial function observed between summer and late autumn (a novel finding) was associated with high intensity physical activity only. Moreover, the decrease in high intensity physical activity was the only predictor of the decrease in FMD (chapter 6). High intensity interval exercise training is beginning to emerge in the adult literature as an important mediator of vascular health (Rakobowchuk et al. 2008). Mechanistically, it has been suggested that high intensity exercise may enhance endothelial function via improvement in antioxidant capacity and reduction in oxidative stress, or by inducing greater augmentation of eNOS gene expression (Green et al. 2004a; Hambrecht et al. 2003). It is intuitive to suggest that a decrease in high intensity physical activity will act conversely on these mechanisms.

Separate gender analyses undertaken in chapter 6 revealed a strong relationship between change in FMD and change in high intensity PA in boys, whilst no relationships were evident for the girls. We suggest that this discrepancy is due to the difference between the amount of physical activity undertaken by the girls and boys. During both summer



and autumn the girls engaged in significantly less total and high intensity PA than boys. As there is clearly a dose response relationship between physical activity and CVD risk (Andersen 1995; Berlin and Colditz 1990; Whaley and Blair 1995), it is possible that the girls did not amass enough PA to impact upon endothelial function. In accordance with the current findings, Pahkala *et al.* (Pahkala et al. 2008) examined the impact of gender on the relationship between FMD and PA. They demonstrated a significant relationship between FMD and PA in the boys but not the girls, and also that PA was lower in girls than boys. A sub-group analysis carried out in a selection of boys and girls who had the same activity level revealed no gender difference for FMD, suggesting that differences in PA levels, not gender *per se*, were responsible for the differences between boys and girls.

When taken together, our results suggest that while body fat may play a relatively small role in the maintenance of vascular function in normal weight children, high intensity physical activity may exert a stronger influence over endothelial function. The ramifications of these findings are that interventions aimed at improving vascular health in children may need to be refocused to bring about a progressive increase in physical activity, specifically high intensity physical activity, rather than reducing obesity or sedentary time *per se*. High intensity PA may be an attractive variable to modify as it seems to be the only PA variable that is not under genetic control (see below), implying that high intensity PA may be more easily modified than other PA variables. Furthermore, our findings suggest that within populations of apparently healthy children, those at most risk of future cardiovascular risk may be those who engage in the least amount of high intensity physical activity.

### 10.1.3 Heritability of FMD

We estimated the heritability of FMD in our cohort of young twins at 0.44. This figure implies that ~45% of the variance in FMD may be notionally under genetic control, indicating that genes have an important influence over the determination of vascular function in young people. Nonetheless, we suggest that endothelial health can be modified by environmental factors such as high intensity physical activity and adiposity. Our heritability estimate for FMD is slightly elevated in comparison to previous estimates of 0.14-0.39 (Benjamin et al. 2004; Jartti et al. 2002). The slight divergence may arise as a result of technical or methodological differences in the measurement of FMD between studies. Alternatively, the difference may be related to maturational or developmental issues influencing the expression of genes during adolescence.

In chapter 9 we observed a non significant improvement in FMD in both the MZ and DZ twins following 8 weeks of aerobic exercise training. The intra-twin pair difference in this improvement was more closely related in the MZ than the DZ twins. The heritability of the exercise induced change in endothelial function was estimated at 0.79. We therefore conclude that the improvement in FMD was largely genotype dependent.

The discrepancy between the heritability estimates for chapters 8 and 9, that is the heritability of baseline versus changes in FMD, may be related in some way to the relative imprecision of taking a "once-off" measure. Repeated measures of FMD and analysis of change within subjects, rather than comparisons between groups of subjects, are likely to provide a more accurate FMD measure and, in turn, a greater heritability estimate. In addition, our inflated heritability estimate for change in FMD seemingly fits the hypothesis, originally proposed by Bouchard (Bouchard 1983), that human



adaptability has a genetic basis. The weaker correlation observed for the exercise induced adaptation of FMD in the DZ twins, who share ~50% of their genes, taken with the strong correlation between MZ twins, who are presumed to be genetically identical, is a reflection that there is great heterogeneity in individual responses to exercise. In a review article examining individual differences in the response to chronic exercise, Bouchard and Rankinen (Bouchard and Rankinen 2001) concluded that the heterogeneity in the response of numerous physiological indicators of risk factors to regular physical activity is not random, and is actually characterized by familial aggregation. Our findings add to this data as we have attempted to empirically determine the genetic contribution to the exercise induced adaptation of FMD.

Secondary findings from chapter 9 illustrate a genetic dominance effect acting on  $\text{VO}_{2\text{peak}}$  although correlations were modest and non-significant for both MZ and DZ twins, which also suggests considerable environmental influence. Our data in children are unique and indicate a much greater environmental influence over  $\text{VO}_{2\text{peak}}$  adaptation than previously reported in adults. Prud'Homme *et al.* (Prud'homme et al. 1984) examined the contribution of genes to exercise induced change in maximal aerobic power in adults. Following a 20 week endurance training programme the investigators observed a large similarity in the  $\Delta \dot{V}\text{O}_{2\text{max}}$  in monozygotic twins ( $\text{MZr}=0.74$ ). In contrast to our findings the authors concluded that the sensitivity of maximal aerobic power to exercise training was largely genotype-dependent. The difference between the results from this thesis and those reported by Prud'Homme *et al.* (Prud'homme et al. 1984) may be a reflection of the different durations of the 2 exercise interventions (8 vs. 20 weeks). Alternatively they may pertain to the different ages and developmental stages of the participants studied. Taken together these data may be indicative of a greater trainability of  $\text{VO}_{2\text{peak}}$  during childhood/adolescence than adulthood.

Previous studies that have used DXA to measure the intra-pair relationships in adult twins have demonstrated stronger  $r$  values for MZ than DZ twins (Malis et al. 2005; Nguyen et al. 1998). Both studies concluded that fat and lean mass are under strong genetic regulation. Our data from chapter 8 lend support to the notion of a genetic dominance effect acting on measures of body composition in young people. However, the impact of exercise training on the heritability of body composition has not previously been assessed. We were able to demonstrate that exercise induced change in BMI and percentage body fat were highly correlated in MZ twins, whilst modest correlations were observed in the DZ twins. These data infer a large genetic component to change in BMI and percentage body fat in young people. This is novel data examining the genetic influence to change in body composition within subjects following a training regime and it provides an important new insight into heritability compared to previously collated baseline analyses which are likely impacted by a much larger set of variables. These findings imply that, whilst exercise training is an effective behavioural strategy for body composition management, one challenge in treating and preventing paediatric obesity may be overcoming a genetic predisposition that actively resists perturbations in energy balance.

The data from chapter 9 may go some way to aid the interpretation of our findings regarding the seasonal variation in FMD and relationships between change in FMD, body composition and physical activity. In chapter 9 we induced improvements in FMD via an increase in PA, specifically high intensity PA. Conversely, but consistently, chapter 6 suggests that the decrease in FMD was related to the decrease in high intensity PA. We propose that ~20% of the variation in change in FMD was determined by environmental factors, and that on average ~5% of the variation in change in FMD can



be predicted by high intensity PA. This leaves a small proportion of the variance in change in FMD unaccounted for. Considering that the measurement of important CV risk factors such as the impact of passive smoking, dietary intake, and blood borne CVD biomarkers was beyond the scope of this thesis, the observation that changes in adiposity and sedentary behaviour are not closely related to change in FMD in normal weight children appear to be reasonable findings.

## **10.2 Limitations and future directions**

This thesis is limited in that our sole measure of future CV risk was endothelial health, assessed using FMD. The use of additional measures of arterial function and structure, such as IMT and arterial stiffness would have provided further prognostic information and possibly given further insight into CV risk in apparently healthy children.

The measurement of CV risk factors including caloric and macronutrient intake, lipid profiles and exposure to passive smoking was beyond the scope of this thesis. Measurement of these variables would, however, have provided a clearer and more complete picture of the modulators of vascular health in children.

As with the examination of any paediatric population, differences between participants maturational status are liable to influence results. In the current thesis we used time to peak height velocity to determine somatic maturity, and where possible this variable was entered into statistical analyses in an attempt to prevent confounding results. Similarly, in chapters 8 and 9, the cohort was comprised of same sex twins of both genders. It is therefore possible that gender confounded results, although we attempted to control for gender differences by reporting adjusted results where necessary.

Although our data suggest high intensity PA may be an important modulator of endothelial health in children, future studies that seek to investigate the exact 'dose' of physical activity required to produce the most effective improvements of endothelial function should be undertaken. Such studies may examine the effect of interventions that prioritise high intensity interval training vs. moderate continuous exercise for example. Little is known about the predictive value of FMD measurement taken during childhood. Although this would ultimately require challenging longitudinal studies to be undertaken, information may be acquired from investigations that examine childhood FMD and the development of structural changes that are associated with future CVD such as IMT.

### **10.3 Summary**

The primary aims of this thesis were to evaluate the strength of the relationships between endothelial function and modifiable CV risk factors, and examine the role genetics plays in endothelial health. We found that in terms of a single, basal measure of FMD, moderate genetic control exists, however environmental factors appear to be of greater importance. Our data highlight an association between percentage body fat and FMD, albeit a weak one. However, high intensity physical activity may be the most important of our measured variables in terms of prevention of endothelial dysfunction in children. No relationships were evident between FMD and fitness or sedentary behaviour, implying that during childhood FMD is independent of fitness and sedentary behaviour.

Exercise induced changes in FMD appear to be more genotype dependent. Nevertheless, our data suggest that the contribution of environmental influences should not be



underestimated. Although neither changes in body composition nor sedentary behaviour were related to the seasonal decline in FMD, we have demonstrated that the attenuation of FMD was predicted solely by the seasonal decline in high intensity PA. Furthermore, exercise training seems to be capable of modulating FMD in young people that possess normal endothelial function *a priori*. These data infer that whilst a genetic predisposition to endothelial dysfunction may exist, interventions that aim to increase high intensity physical activity may maximise vascular health in those most at risk of endothelial impairment.

## **11 References**

- Abbott RA, Harkness MA, Davies PS (2002) Correlation of habitual physical activity levels with flow-mediated dilation of the brachial artery in 5-10 year old children. *Atherosclerosis* 160: 233-239
- Aggoun Y, Szezepanski I, Bonnet D (2005) Noninvasive assessment of arterial stiffness and risk of atherosclerotic events in children. *Pediatric research* 58: 173-178
- Alexander RM (1989) Optimization and gaits in the locomotion of vertebrates. *Physiological Reviews* 69: 1199-1227
- Allison DB, Heshka S, Neale MC, Heymsfield SB (1994) Race effects in the genetics of adolescents' body mass index. *Int J Obes Relat Metab Disord* 18: 363-368
- Andersen LB (1995) Physical activity and physical fitness as protection against premature disease or death. *Scandinavian journal of medicine & science in sports* 5: 318-328
- Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC (1995) Close relationship of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 26: 1235-1241
- Arcaro G, Zamboni M, Rossi L, Turcato E, Covi G, Armellini F, Bosello O, Lechi A (1999) Body fat distribution predicts degree of endothelial dysfunction in uncomplicated obesity. *Int J Obesity* 23: 9936-9942
- Arkin JM, Alsdorf R, Bigornia S, Palmisano J, Beal R, Istfan N, Hess D, Apovian CM, Gokce N (2008) Relation of cumulative weight burden to vascular endothelial dysfunction in obesity. *The American journal of cardiology* 101: 98-101
- Atkinson G, Batterham AM, Black MA, Cable NT, Hopkins ND, Dawson EA, Thijssen DH, Jones H, Tinken TM, Green DJ (2009) Is the ratio of flow-mediated dilation and shear rate a statistically sound approach to normalization in cross-sectional studies on endothelial function? *J Appl Physiol* 107: 1893-1899
- Avogaro A, Piarulli F, Valerio A, Miola M, Calveri M, Pavan P, Vicini P, Cobelli C, Tiengo A, Calo L, Del Prato S (1997) Forearm nitric oxide balance, vascular relaxation, and glucose metabolism in NIDDM patients. *Diabetes* 46: 1040-1046
- Baquet G, Stratton G, Van Praagh E, Berthoin S (2007) Improving physical activity assessment in prepubertal children with high-frequency accelerometry monitoring: a methodological issue. *Preventive medicine* 44: 143-147
- Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ (1989) Weight in infancy and death from ischaemic heart disease. *Lancet* 2: 577-580



- Batterham A, George K, Whyte G, Sharma S, McKenna W (1999) Scaling cardiac structural data by body dimensions: a review of theory, practice, and problems. *International journal of sports medicine* 20: 495-502
- Batterham AM, George KP, Mullineaux DR (1997) Allometric scaling of left ventricular mass by body dimensions in males and females. *Medicine and science in sports and exercise* 29: 181-186
- Belanger M, Gray-Donald K, O'Loughlin J, Paradis G, Hanley J (2009) Influence of weather conditions and season on physical activity in adolescents. *Ann Epidemiol* 19: 180-186
- Bell LM, Watt K, Siafarikas A, Thompson A, Ratnam N, Bulsara M, Finn J, O'Driscoll G, Green DJ, Jones TW, Davis EA (2007) Exercise alone reduces insulin resistance in obese children independently of changes in body composition. *J Clin Endocrinol Metab* 92: 4230-4235
- Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF, Jr., Lehman BT, Fan S, Osypiuk E, Vita JA (2004) Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 109: 613-619
- Berlin JA, Colditz GA (1990) A meta-analysis of physical activity in the prevention of coronary heart disease. *American journal of epidemiology* 132: 612-628
- Black MA, Cable NT, Thijssen DHJ, Green DJ (2008) Importance of measuring the time-course of flow-mediated dilation (FMD) in humans. *Hypertension* 51: 203-210
- Blair SN, Church TS (2004) The fitness, obesity, and health equation: is physical activity the common denominator? *Jama* 292: 1232-1234
- Blair SN, Kampert JB, Kohl HW, Barlow CE, Macera CA, Paffenbarger RS, Gibbons LW (1996) Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *JAMA* 276: 205-210
- Boddy LM, Hackett AF, Stratton G (2010) Changes in fitness, body mass index and obesity in 9-10 year olds. *J Hum Nutr Diet* Epub ahead of print
- Bodurtha JN, Mosteller M, Hewitt JK, Nance WE, Eaves LJ, Moskowitz WB, Katz S, Schieken RM (1990) Genetic analysis of anthropometric measures in 11-year-old twins: the Medical College of Virginia Twin Study. *Pediatric research* 28: 1-4
- Bouchard C (1983) Human adaptability may have a genetic basis. In: Landry F (ed) 18th annual meeting of the Society of Prospective Medicine. Canadian Public Health Association, Ottawa, pp. 463-476
- Bouchard C, Rankinen T (2001) Individual differences in response to regular physical activity. *Medicine and science in sports and exercise* 33: S446-451; discussion S452-443

- Brage S, Wedderkopp N, Ekelund U, Franks PW, Wareham NJ, Andersen LB, Froberg K (2004) Features of the metabolic syndrome are associated with objectively measured physical activity and fitness in Danish children: the European Youth Heart Study (EYHS). *Diabetes care* 27: 2141-2148
- Brevetti G, Silvestro A, Schiano V, Chiariello M (2003) Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease. *Circulation* 108: 2093-2098
- Brodersen NH, Steptoe A, Boniface DR, Wardle J (2007) Trends in physical activity and sedentary behaviour in adolescence: ethnic and socioeconomic differences. *British journal of sports medicine* 41: 140-144
- Brown SP, Thompson WR (1987) Standardization indices of cardiac hypertrophy in weight lifters. *J Sports Sci* 5: 147-153
- Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, et al. (1992) Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 359: 641-644
- Catellier DJ, Hannan PJ, Murray DM, Addy CL, Conway TL, Yang S, Rice JC (2005) Imputation of missing data when measuring physical activity by accelerometry. *Medicine and science in sports and exercise* 37: S555-562
- Celermajer DS, Ayer JGJ (2006) Childhood risk factors for adult cardiovascular disease and primary prevention in childhood. *Heart* 92: 1701-1706
- Celermajer DS (2008) Reliable endothelial function testing. At our fingertips? *Circulation* 117: 2428-2430
- Celermajer DS, Adams MR, Clarkson P, Robinson J, McCredie R, Donald A, Deanfield JE (1996) Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. *N Engl J Med* 334: 150-154
- Celermajer DS, Sorensen KE, Georgakopoulos D, Buller C, Thomas O, Robinson J, Deanfield JE (1993) Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 88: 2149-2155
- Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 340: 1111-1115
- Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE (1994) Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol* 24: 471-476



- Dinenno FA, Tanaka H, Monahan KD, Clevenger CM, Eskurza I, DeSouza CA, Seals DR (2001) Regular endurance exercise induces expansive arterial remodelling in the trained limbs of healthy men. *J Physiol* 534: 287-295
- Dollman J, Norton K, Norton L (2005) Evidence for secular trends in childrens physical activity behaviour. *Br J Sports Med* 39: 892-897
- Dollman J, Olds T, Norton K, Stuart D (1999) The Evolution of Fitness and Fatness in 10- 11-Year-Old Australian Schoolchildren: Changes in Distributional Characteristics Between 1985 and 1997. *Pediatric Exercise Science* 11
- Doshi SN, Naka KK, Payne N, Jones CJH, Ashton M, Lewis MJ, Goodfellow J (2001) Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin Sci* 101: 629-635
- Duggirala R, Gonzalez Villalpando C, O'Leary DH, Stern MP, Blangero J (1996) Genetic basis of variation in carotid artery wall thickness. *Stroke; a journal of cerebral circulation* 27: 833-837
- Dull RO, Davies PF (1991) Flow modulation of agonist (ATP)-response ( $\text{Ca}^{2+}$ ) coupling in vascular endothelial cells. *Am J Physiol* 261: H149-H154
- Dunstan DW, Barr EL, Healy GN, Salmon J, Shaw JE, Balkau B, Magliano DJ, Cameron AJ, Zimmet PZ, Owen N (2010) Television viewing time and mortality: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). *Circulation* 121: 384-391
- Dunstan DW, Daly RM, Owen N, Jolley D, De Courten M, Shaw J, Zimmet P (2002) High-intensity resistance training improves glycemic control in older patients with type 2 diabetes. *Diabetes care* 25: 1729-1736
- Dunstan DW, Salmon J, Owen N, Armstrong T, Zimmet PZ, Welborn TA, Cameron AJ, Dwyer T, Jolley D, Shaw JE (2004) Physical activity and television viewing in relation to risk of undiagnosed abnormal glucose metabolism in adults. *Diabetes care* 27: 2603-2609
- Eisenmann JC, Wickel EE, Welk GJ, Blair SN (2005) Relationship between adolescent fitness and fatness and cardiovascular disease risk factors in adulthood: the Aerobics Center Longitudinal Study (ACLS). *American heart journal* 149: 46-53
- Ekelund U, Anderssen SA, Froberg K, Sardinha LB, Andersen LB, Brage S (2007) Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. *Diabetologia* 50: 1832-1840
- Ekelund U, Brage S, Froberg K, Harro M, Anderssen SA, Sardinha LB, Riddoch C, Andersen LB (2006) TV viewing and physical activity are independently associated with metabolic risk in children: the European Youth Heart Study. *PLoS medicine* 3: e488
- Engler MM, Engler MB, Malloy MJ, Chiu EY, Schloetter MC, Paul SM, Stuehlinger M, Lin KY, Cooke JP, Morrow JD, Ridker PM, Rifai N, Miller E, Witztum JL,

- Mietus-Snyder M (2003) Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. *Circulation* 108: 1059-1063
- Enos WF, Holmes RH, Beyer J (1953) Coronary disease among United States soldiers killed in action in Korea: preliminary report. *JAMA* 152: 1090-1093
- Eriksson M, Rasmussen F, Tynelius P (2006) Genetic factors in physical activity and the equal environment assumption-- the Swedish young male twins study. *Behavior genetics* 36: 238-247
- Eskurza I, Kahn ZD, Seals DR (2006) Xanthine oxidase does not contribute to impaired peripheral conduit artery endothelium-dependent dilatation with ageing. *The Journal of physiology* 571: 661-668
- Eskurza I, Myerburgh LA, Kahn ZD, Seals DR (2005) Tetrahydrobiopterin augments endothelium-dependent dilatation in sedentary but not in habitually exercising older adults. *The Journal of physiology* 568: 1057-1065
- Fagard RH (2001) Exercise characteristics and the blood pressure response to dynamic physical training. *Med Sci Sports Exerc* 33: S484-S492
- Faith MS, Pietrobelli A, Nunez C, Heo M, Heymsfield SB, Allison DB (1999) Evidence for independent genetic influences on fat mass and body mass index in a pediatric twin sample. *Pediatrics* 104: 61-67
- Farrell SW, Braun L, Barlow CE, Cheng YJ, Blair SN (2002) The relation of body mass index, cardiorespiratory fitness, and all-cause mortality in women. *Obesity research* 10: 417-423
- Farrell SW, Kampert JB, Khol H, Barlow CE, Macera CA, Paffenbarger RS, Gibbons L, Blair SN (1998) Influences of cardiorespiratory fitness levels and other predictors on cardiovascular disease mortality in men. *Medicine and Science in Sport and Exercise* 30: 899-905
- Fathi R, Haluska B, Isbel N, Short L, Marwick TH (2004) The relative importance of vascular structure and function in predicting cardiovascular events. *J Am Coll Cardiol* 43: 616-623
- Fernhall B, Agiovlasitis S (2008) Arterial function in youth: window into cardiovascular risk. *J Appl Physiol* 105: 325-333
- Fleck SJ, Henke C, Wilson W (1989) Cardiac MRI of elite junior Olympic weight lifters. *International journal of sports medicine* 10: 329-333
- Francis K (1996) Physical activity in the prevention of cardiovascular disease. *Physical therapy* 76: 456-468
- Freedman DS, Patel DA, Srinivasan SR, Chen W, Tang R, Bond MG, Berenson GS (2008) The contribution of childhood obesity to adult carotid intima-media thickness: the Bogalusa Heart Study. *International journal of obesity* (2005) 32: 749-756



- Frick M, Suessenbacher A, Alber HF, Dichtl W, Ulmer H, Pachinger O, Weidinger F (2005) Prognostic value of brachial artery endothelial function and wall thickness. *J Am Coll Cardiol* 46: 1006-1010
- Furchgott RF, Jothianandan D (1991) Endothelium-dependent and -independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. *Blood vessels* 28: 52-61
- Geilen S, Hambrecht R (2004) The obesity epidemic: Impact on endothelial function. *Circulation* 109: 1911-1913
- George KP, Batterham AM, Jones B (1998) The impact of scalar variable and process on athlete-control comparisons of cardiac dimensions. *Med Sci Sports Exerc* 30: 824-830
- George KP, Whyte GW, Sharma S, Batterham AM, McKenna WJ (2001) The relationship between echocardiographic indices of cardiac size and body dimensions: An allometric analysis in 453 elite junior athletes. *Clin Sci* 100: 47-54
- Gerhard M, Roddy MA, Creager SJ, Creager MA (1996) Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. *Hypertension* 27: 849-853
- Gocke N, Keaney JF, Hunter LM, Watkins MT, Menzoian JO, Vita JA (2002a) Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* 105: 1567-1572
- Gocke N, Keaney JF, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, Vita JA (2003) Predictive value of non-invasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *Am J Cardiol* 41: 1769-1775
- Gocke N, Vita JA, Bader DS, Sherman DL, Hunter LM, Holbrook M, O'Malley C, Keaney JF, Balady GJ (2002b) Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. *Am J Cardiol* 90: 124-127
- Goldsmith HH (1991) A zygosity questionnaire for young twins: a research note. *Behavior genetics* 21: 257-269
- Gortmaker SL, Must A, Sobol AM, Peterson K, Colditz GA, Dietz WH (1996) Television viewing as a cause of increasing obesity among children in the United States, 1986-1990. *Archives of pediatrics & adolescent medicine* 150: 356-362
- Green DJ (2005) Point-Counterpoint: Flow-mediated dilation does reflect nitric oxide-mediated endothelial function. *J Appl Physiol* 99: 1233-1234
- Green DJ, Cable NT, Joyner MJ, O'Driscoll G (2008) Exercise and cardiovascular risk reduction: Updating the rationale for exercise. *J Appl Physiol* 105: 766-768

- Green DJ, Maiorana AJ, O'Driscoll G, Taylor R (2004a) Topical Review: Effects of exercise training on vascular endothelial nitric oxide function in humans. *J Physiol (Lond)* 561: 1-25
- Green DJ, Walsh JH, Maiorana A, Best M, Taylor RR, O'Driscoll JG (2003) Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: a pooled analysis of diverse patient populations. *Am J Physiol* 285: H2679-2687
- Green DJ, Walsh JH, Maiorana AJ, Burke V, Taylor RR, O'Driscoll G (2004b) Comparison of resistance and conduit vessel nitric oxide-mediated vascular function in vivo: Effects of exercise training. *J Appl Physiol* 97: 749-755
- Groves P, Kursz S, Just H, Drexler H (1995) Role of endogenous bradykinin in human coronary vasomotor control. *Circulation* 92: 3424-3430
- Hambrecht R, Adams V, Erbs S, Linke a, Krankel N, Shu Y, Baither Y, Geilen S, Thiele H, Gummert JF, Mohr FW, Schuler G (2003) Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 107: 3152-3158
- Hamel P, Simoneau JA, Lortie G, Boulay MR, Bouchard C (1986) Heredity and muscle adaptation to endurance training. *Medicine and science in sports and exercise* 18: 690-696
- Harrison DG (1996) Endothelial control of vasomotion and nitric oxide production. A potential target for risk factor management. *Cardiology Clinics* 14: 1-15
- Healy GN, Dunstan DW, Salmon J, Cerin E, Shaw JE, Zimmet PZ, Owen N (2007) Objectively measured light-intensity physical activity is independently associated with 2-h plasma glucose. *Diabetes care* 30: 1384-1389
- Healy GN, Dunstan DW, Salmon J, Shaw JE, Zimmet PZ, Owen N (2008) Television time and continuous metabolic risk in physically active adults. *Medicine and science in sports and exercise* 40: 639-645
- Hecker M, Mülsch A, Bassenge E, Busse R (1993) Vasoconstriction and increased flow: two principal mechanisms of shear stress dependent endothelial autacoid release. *Am J Physiol* 265: H828-H833
- Hogikyan RV, Galecki AT, Pitt B, Halter JB, Greene DA, Supiano MA (1998) Specific impairment of endothelium-dependent vasodilation in subjects with type 2 diabetes independent of obesity. *The Journal of clinical endocrinology and metabolism* 83: 1946-1952
- Hong Y, de Faire U, Heller DA, McClearn GE, Pedersen N (1994) Genetic and environmental influences on blood pressure in elderly twins. *Hypertension* 24: 663-670



- Hossain P, Kavar B, El Nahas M (2007) Obesity and diabetes in the developing world-- a growing challenge. *N Engl J Med* 356: 213-215
- Huonker M, Schmid A, Schmid-Truckass A, Grathwohl D, Keul J (2003) Size and blood flow of central and peripheral arteries in highly trained able-bodied and disabled athletes. *J Appl Physiol* 95: 685-691
- Huotari PR, Nupponen H, Laakso L, Kujala UM (2009) Secular trends in aerobic fitness and its determinants in Finnish 13- to 18-year-old adolescents from 1976 and 2001. *British journal of sports medicine*
- Hutcheson IR, Griffith TM (1991) Release of endothelium-derived relaxing factor is modulated both by frequency and amplitude of pulsatile flow. *Am J Physiol* 261: H257-H262
- Ignarro LJ, Adams JB, Horwitz PM, Wood KS (1986) Activation of soluble guanylate cyclase by NO-hemoproteins involves NO-heme exchange. *J Biol Chem* 261: 4997-5002
- Janssen I, Katzmarzyk P, Srinivasan S, Chen W, Malina R, Bouchard C, Berenson G (2005) Utility of childhood BMI in the prediction of adulthood disease: comparison of national and international references. *Obes Res* 13: 1106-1115
- Jartti L, Ronnema T, Kaprio J, Jarvisalo MJ, Toikka JO, Marniemi J, Hammar N, Alfredsson L, Saraste M, Hartiala J, Koskenvuo M, Raitakari OT (2002) Population-based twin study of the effects of migration from Finland to Sweden on endothelial function and intima-media thickness. *Arterioscler Thromb Vasc Biol* 22: 832-837
- Joannides R, Haefeli WE, Linder L, Richard V, Bakkali E, Thuillez C, Lüscher TF (1995) Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 91: 1314-1319
- Joosen AM, Gielen M, Vlietinck R, Westerterp KR (2005) Genetic analysis of physical activity in twins. *The American journal of clinical nutrition* 82: 1253-1259
- Karatzis EN, Ikonomidis I, Vamvakou GD, Papaioannou TG, Protogerou AD, Andreadou I, Voidonikola PT, Karatzi KN, Papamichael CM, Lekakis JP (2006) Long-term prognostic role of flow-mediated dilatation of the brachial artery after acute coronary syndromes without ST elevation. *The American journal of cardiology* 98: 1424-1428
- Katzmarzyk PT, Church TS, Craig CL, Bouchard C (2009) Sitting time and mortality from all causes, cardiovascular disease, and cancer. *Medicine and science in sports and exercise* 41: 998-1005
- Keller KL, Pietrobelli A, Faith MS (2003) Genetics of food intake and body composition: lessons from twin studies. *Acta diabetologica* 40 Suppl 1: S95-100
- Khan F, Green F, C., Forsyth S, Greene S, A., Morris A, D., Belch J, J, F. (2003) Impaired microvascular function in normal children: effects of adiposity and poor glucose handling. *Journal of Physiology* 551: 705-711

- Kitta Y, Nakamura T, Kodama Y, Takano H, Umetani K, Fujioka D, Saito Y, Kawabata K-i, Obata J-e, Ichigi Y, Mende A, Kugiyama K (2005) Endothelial Vasomotor Dysfunction in the Brachial Artery Is Associated With Late In-Stent Coronary Restenosis. *J Am Coll Cardiol* 46: 648-655
- Kitta Y, Obata JE, Nakamura T, Hirano M, Kodama Y, Fujioka D, Saito Y, Kawabata K, Sano K, Kobayashi T, Yano T, Nakamura K, Kugiyama K (2009) Persistent impairment of endothelial vasomotor function has a negative impact on outcome in patients with coronary artery disease. *J Am Coll Cardiol* 53: 323-330
- Kooijman M, Thijssen DHJ, de Groot PCE, Bleeker MWP, van Kuppevelt HJM, Green DJ, Rongen GA, Smits P, Hopman MTE (2008) Flow-mediated dilatation in the femoral artery is nitric oxide mediated in humans. *Journal of Physiology (London)* 586: 1137-1145
- Kopelman P, G. (2000) Obesity as a medical problem. *Nature* 404: 635-643
- Kristensen PL, Moller NC, Korsholm L, Wedderkopp N, Andersen LB, Froberg K (2008) Tracking of objectively measured physical activity from childhood to adolescence: the European youth heart study. *Scandinavian journal of medicine & science in sports* 18: 171-178
- Laurson K, Eisenmann JC, Moore S (2008) Lack of association between television viewing, soft drinks, physical activity and body mass index in children. *Acta Paediatr* 97: 795-800
- Lee CD, Blair SN, Jackson AS (1999) Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. *Am J Clin Nutrition* 69: 373-380
- Leon AS, Sanchez OA (2001) Response of blood lipids to exercise training alone or combined with dietary intervention. *Medicine and science in sports and exercise* 33: S502-515; discussion S528-509
- Lobstein T, Frelut ML (2003) Prevalence of overweight among children in Europe. *Obes Rev* 4: 195-200
- Maia JA, Thomis M, Beunen G (2002) Genetic factors in physical activity levels: a twin study. *American journal of preventive medicine* 23: 87-91
- Maiorana A, O'Driscoll G, Dembo L, Goodman C, Taylor RR, Green DJ (2001) Exercise training, vascular function and functional capacity in middle-aged subjects. *Med Sci Sports Exerc* 33: 2022-2028
- Maiorana A, O'Driscoll GJ, Taylor RR, Green DJ (2003) Exercise and the nitric oxide vasodilator system. *Sports Medicine* 33: 1013-1035
- Malina RM (1996) Tracking of physical activity and physical fitness across the lifespan. *Research quarterly for exercise and sport* 67: S48-57



- Malis C, Rasmussen EL, Poulsen P, Petersen I, Christensen K, Beck-Nielsen H, Astrup A, Vaag AA (2005) Total and regional fat distribution is strongly influenced by genetic factors in young and elderly twins. *Obesity research* 13: 2139-2145
- Marshall S, Welk G (2008) Definitions and measurement. In: Smith A, Biddle S (eds) *Youth physical activity and sedentary behavior: Challenges and solutions*. Human Kinetics, Champaign, IL, pp. 3-29
- Marshall SJ, Biddle SJ, Gorely T, Cameron N, Murdey I (2004) Relationships between media use, body fatness and physical activity in children and youth: a meta-analysis. *Int J Obes Relat Metab Disord* 28: 1238-1246
- Matthews CE, Freedson PS, Hebert JR, Stanek EJ, 3rd, Merriam PA, Rosal MC, Ebbeling CB, Ockene IS (2001) Seasonal variation in household, occupational, and leisure time physical activity: longitudinal analyses from the seasonal variation of blood cholesterol study. *American journal of epidemiology* 153: 172-183
- Mattocks C, Ness A, Leary S, Tilling K, Blair SN, Shield J, Deere K, Saunders J, Kirkby J, Smith GD, Wells J, Wareham N, Reilly J, Riddoch C (2008) Use of accelerometers in a large field-based study of children: protocols, design issues, and effects on precision. *Journal of physical activity & health* 5 Suppl 1: S98-111
- Mavri A, Guzic-Salobir B, Salobir-Pajnic B, Keber I, Stare J, Stegnar M (2001) Seasonal variation of some metabolic and haemostatic risk factors in subjects with and without coronary artery disease. *Blood Coagul Fibrinolysis* 12: 359-365
- McGill HC, Jr., McMahan CA, Herderick EE, Tracy RE, Malcom GT, Zieske AW, Strong JP (2000) Effects of coronary heart disease risk factors on atherosclerosis of selected regions of the aorta and right coronary artery. PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. *Arterioscler Thromb Vasc Biol* 20: 836-845
- McGue M, Bacon S, Lykken D, T (1993) Personality stability and change in early adulthood: A behavioural genetic analysis. *Developmental Psychology* 29: 96-109
- Merrill RM, Shields EC, White GL, Jr., Druce D (2005) Climate conditions and physical activity in the United States. *American journal of health behavior* 29: 371-381
- Meyer A, Kundt G, Lenschow U, Schuff-Werner P, Kienast W (2006) Improvement of early vascular changes and cardiovascular risk factors in obese children after a six-month exercise program *J Am Coll Cardiol* 48: 1865 - 1870
- Meyer B, Mortl D, Strecker K, Hulsmann M, Kulemann V, Neunteufl T, Pacher R, Berger R (2005) Flow-mediated vasodilation predicts outcome in patients with chronic heart failure: comparison with B-type natriuretic peptide. *J Am Coll Cardiol* 46: 1011-1018

- Minetti AE (2001) Walking on other planets. *Nature* 409: 467, 469
- Mirwald RL, Baxter-Jones ADG, Bailey DA, Beunen GP (2002) An assessment of maturity from anthropometric measurements. *Medicine and science in sports and exercise* 34: 689-694
- Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R (2002) Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *JACC* 40: 505-510
- Mora S, Cook N, Buring JE, Ridker PM, Lee IM (2007) Physical activity and reduced risk of cardiovascular events: Potential mediating mechanisms. *Circulation* 116: 2110-2118
- Morabia A, Ross A, Curtin F, Pichard C, Slosman DO (1999) Relation of BMI to a dual-energy X-ray absorptiometry measure of fatness. *The British journal of nutrition* 82: 49-55
- Mossberg H, O. (1989) 40 year follow up of overweight children. *The Lancet* II: 491-493
- Mullen MJ, Kharbanda RK, Cross J, Donald AE, Taylor M, Vallance P, Deanfield JE, MacAllister RJ (2001a) Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo. *Circ Res* 88: 145-151
- Munk PS, Staal EM, Butt N, Isaksen K, Larsen AI (2009) High-intensity interval training may reduce in-stent restenosis following percutaneous coronary intervention with stent implantation A randomized controlled trial evaluating the relationship to endothelial function and inflammation. *American heart journal* 158: 734-741
- Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, Palinski W (1997) Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *The Journal of clinical investigation* 100: 2680-2690
- Naylor L, O'Driscoll G, Fitzsimons M, Arnold L, Green DJ (2006) Effects of training resumption on conduit arterial diameter in elite rowers. *Medicine and science in sports and exercise* 38: 86-92
- Neunteufl T, Heher S, Katzenschlager R, Wolfl G, Kostner G, Maurer G, Weidinger F (2000) Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. *Am J Cardiol* 86: 207-210
- Nguyen TV, Howard GM, Kelly PJ, Eisman JA (1998) Bone mass, lean mass, and fat mass: same genes or same environments? *American journal of epidemiology* 147: 3-16



- Oflaz H, Ozbey N, Mantar F, Genchellac H, Mercanoglu F, Sencer E, Molvalilar S, Orhan Y (2003) Determination of endothelial function and early atherosclerotic changes in healthy obese women. *Diabetes Nutr Metab* 16: 176-181
- Oleson SP, Clapham DE, Davies PF (1988) Haemodynamic shear stress activates a  $K^+$  current in vascular endothelial cells. *Nature (Lond)* 331: 168-170
- Olive JL, Dudley GA, McCully KK (2003) Vascular remodeling after spinal cord injury. *Medicine and science in sports and exercise* 35: 901-907
- Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, Hayflick L, Butler RN, Allison DB, Ludwig DS (2005) A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med* 352: 1138-1145
- Pahkala K, Heinonen OJ, Lagstrom H, Hakala P, Simell O, Viikari JS, Ronnema T, Hernelahti M, Sillanmaki L, Raitakari OT (2008) Vascular endothelial function and leisure-time physical activity in adolescents. *Circulation* 118: 2353-2359
- Palinski W, Napoli C (1999) Pathophysiological events during pregnancy influence the development of atherosclerosis in humans. *Trends in cardiovascular medicine* 9: 205-214
- Palmer RMJ, Rees DD, Ashton DS, Moncada S (1988) L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem Biophys Res Commun* 153: 1251-1256
- Papadakis M, Whyte G, Sharma S (2008) Preparticipation screening for cardiovascular abnormalities in young competitive athletes. *Bmj* 337: a1596
- Pate RR, O'Neill JR, Lobelo F (2008) The evolving definition of "sedentary". *Exercise and sport sciences reviews* 36: 173-178
- Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, Buchner D, Ettinger W, Heath GW, King AC, et al. (1995) Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *Jama* 273: 402-407
- 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
- Prentice AM, Jebb SA (1995) Obesity in Britain: gluttony or sloth? *BMJ* 311
- Prud'homme D, Bouchard C, Leblanc C, Landry F, Fontaine E (1984) Sensitivity of maximal aerobic power to training is genotype-dependent. *Medicine and science in sports and exercise* 16: 489-493
- Pyke KE, Tschakovsky ME (2005) The shear stress stimulus FMD relationship: Implications for FMD as an assessment of endothelial function. *J Physiol (Lond)* 568: 357-369

- Pyke KE, Tschakovsky ME (2007) Peak vs. total reactive hyperemia: which determines the magnitude of flow-mediated dilation? *J Appl Physiol* 102: 1510-1519
- Raitakari M, Ilvonen T, Ahotupa M, Lehtimäki T, Harmoinen A, Suominen P, Elo J, Hartiala J, Raitakari OT (2004) Weight reduction with very-low-caloric diet and endothelial function in overweight adults: role of plasma glucose. *Arterioscler Thromb Vasc Biol* 24: 124-128
- Rakobowchuk M, Tanguay S, Burgomaster KA, Howarth KR, Gibala MJ, MacDonald MJ (2008) Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans. *Am J Physiol Regul Integr Comp Physiol* 295: R236-242
- Rennie KL, Johnson L, Jebb S (2005) Behavioural determinants of obesity. *Childhood Obesity* 19: 343-358
- Rowlands AV, Pilgrim EL, Eston RG (2009) Seasonal changes in children's physical activity: an examination of group changes, intra-individual variability and consistency in activity pattern across season. *Annals of human biology* 36: 363-378
- Samdal O, Tynjälä J, Roberts C, Sallis JF, Villberg J, Wold B (2007) Trends in vigorous physical activity and TV watching of adolescents from 1986 to 2002 in seven European Countries. *European journal of public health* 17: 242-248
- Schlicker SA, Borra ST, Regan C (1994) The weight and fitness status of United States children. *Nutrition reviews* 52: 11-17
- Schretzenmayr A (1933) Über kreislaufregulatorische Vorgänge an den großen Arterien bei Muskelarbeit. *Pflügers Arch Ges Physiol* 232: 743-748
- Sciacqua A, Candigliota M, Ceravolo R, Scozzafava A, Sinopoli F, Corsonello A, Sesti G, Perticone F (2003) Weight loss in combination with physical activity improves endothelial dysfunction in human obesity. *Diabetes care* 26: 1673-1678
- Shaibi GQ, Cruz ML, Ball GD, Weigensberg MJ, Salem GJ, Crespo NC, Goran MI (2006) Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. *Medicine and science in sports and exercise* 38: 1208-1215
- Shige H, Ishikawa T, Suzukawa M, Ito T, Nakajima K, Higashi K, Ayaori M, Tabata S, Ohsuzu F, Nakamura H (1999) Endothelium-dependent flow-mediated vasodilation in the postprandial state in type 2 diabetes mellitus. *The American journal of cardiology* 84: 1272-1274, A1279
- Shimai S, Yamada F, Masuda K, Tada M (1993) TV game play and obesity in Japanese school children. *Perceptual and motor skills* 76: 1121-1122
- Shimbo D, Grahame-Clarke C, Miyake Y, Rodriguez C, Sciacca R, Di Tullio M, Boden-Albala B, Sacco R, Homma S (2007) The association between endothelial dysfunction and cardiovascular outcomes in a population-based multi-ethnic cohort. *Atherosclerosis* 192: 197-203



- Sinoway LI, Hendrickson C, Davidson WRJ, Prophet S, Zelis R (1989) Characteristics of flow-mediated brachial artery vasodilation in human subjects. *Circ Res* 64: 32-42
- Skilton MR, Evans N, Griffiths KA, Harmer JA, Celemajer DS (2005) Aortic wall thickness in newborns with intrauterine growth restriction. *Lancet* 365: 1484-1486
- Sproston K, Primatesta P (2003) Health Survey for England. The Stationery Office, London
- Srinivasan S, Bao W, Wattigney W, A., Berenson G (1996) Adolescent overweight is associated with adult overweight and related multiple cardiovascular risk factors: the Bogalusa Heart Study. *Metabolism* 45: 235-240
- Stry HC (1989) Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. *Arterioscler* 9: 19-32
- Stry HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Jr., Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW (1995) A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 92: 1355-1374
- Stratton G, Canoy D, Boddy LM, Taylor SR, Hackett AF, Buchan IE (2007) Cardiorespiratory fitness and body mass index of 9-11-year-old English children: a serial cross-sectional study from 1998 to 2004. *International Journal of Obesity* 31: 1172-1178
- Strong JP, McGill HCJ (1969) The pediatric aspects of atherosclerosis. *The Journal of Atherosclerosis Research* 9: 251-265
- Takase B, Hamabe A, Satomura K, T. A, Uehata A, Ohsuzu F, Ishihara M, Kurita A (2005) Close relationship between the vasodilator response to acetylcholine in the brachial and coronary artery in suspected coronary artery disease. *Int J Cardiol* 105: 58 - 66
- 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. *Am J Cardiol* 82: 1535-1539
- Taylor S, Hackett A, Stratton G, Lamb L (2004) SportsLINX: improving the health and fitness of Liverpools youth. *Education and Health* 22: 11-15
- Telama R (2009) Tracking of Physical Activity from Childhood to Adulthood: A Review. *Obes Facts* 2: 187-195

- Telama R, Yang X, Laakso L, Viikari J (1997) Physical activity in childhood and adolescence as predictor of physical activity in young adulthood. *American journal of preventive medicine* 13: 317-323
- Telama R, Yang X, Viikari J, Valimaki I, Wanne O, Raitakari O (2005) Physical activity from childhood to adulthood: a 21-year tracking study. *American journal of preventive medicine* 28: 267-273
- Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris R, A, Parker B, Widlansky M, E, Tschakovsky M, E, Green DJ (In press) Methodological and technical considerations related to assessment of flow mediated dilation (FMD). *The Applied Journal of Physiology*
- Thijssen DH, Dawson EA, Tinken TM, Cable NT, Green DJ (2009) Retrograde flow and shear rate acutely impair endothelial function in humans. *Hypertension* 53: 986-992
- Thompson PD, Buchner D, Pina IL, Balady GJ, Williams MA, Marcus BH, Berra K, Blair SN, Costa F, Franklin B, Fletcher GF, Gordon NF, Pate RR, Rodriguez BL, Yancey AK, Wenger NK (2003) Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. A statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation* 107: 3109-3116
- Thompson PD, Crouse SF, Goodpaster B, Kelley D, Moyna N, Pescatello L (2001) The acute versus the chronic response to exercise. *Medicine and science in sports and exercise* 33: S438-445; discussion S452-433
- Tinken TM, Thijssen DH, Black MA, Cable NT, Green DJ (2008) Time course of change in vasodilator function and capacity in response to exercise training in humans. *The Journal of physiology* 586: 5003-5012
- Title LM, Cummings PM, Giddens K, Nassar BA (2000) Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. *J Am Coll Cardiol* 36: 2185-2191
- Tomkinson GR, Olds TS (2007) Secular changes in pediatric aerobic fitness test performance: the global picture. *Medicine and sport science* 50: 46-66
- Tomkinson GR, Olds TS, Gulbin J (2003) Secular trends in physical performance of Australian children. Evidence from the Talent Search program. *The Journal of sports medicine and physical fitness* 43: 90-98
- Tounian P, Aggoun Y, Dubern B, Varille V, Guy-grand B, Sidi D, Girardet J, Bonnet D (2001) Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. *Lancet* 358: 1400-1404



- Treuth MS, Schmitz K, Catellier DJ, McMurray RG, Murray DM, Almeida MJ, Going S, Norman JE, Pate R (2004) Defining accelerometer thresholds for activity intensities in adolescent girls. *Medicine and science in sports and exercise* 36: 1259-1266
- Troiano RP, Briefel RR, Carroll MD, Bialostosky K (2000) Energy and fat intakes of children and adolescents in the united states: data from the national health and nutrition examination surveys. *The American journal of clinical nutrition* 72: 1343S-1353S
- Viken RJ, Rose RJ, Kaprio J, Koskenvuo M (1994) A developmental genetic analysis of adult personality: extraversion and neuroticism from 18 to 59 years of age. *J Pers Soc Psychol* 66: 722-730
- Vita JA, Keaney JF (2002) Endothelial function: a barometer for cardiovascular risk? (Editorial). *Circulation* 106: 640-642
- Wang X, Guo F, Li G, Cao Y, Fu H (2009) Prognostic role of brachial reactivity in patients with ST myocardial infarction after percutaneous coronary intervention. *Coronary artery disease* 20: 467-472
- Watts K, Beye P, Siafarikas A, Davis EA, Jones TW, O'Driscoll G, Green DJ (2004a) Exercise training normalises vascular dysfunction and improves central adiposity in obese adolescents. *J Am Coll Cardiol* 43: 1823-1827
- Watts K, Beye P, Siafarikas A, Jones T, Davis E, Green DJ (2004b) Exercise training in obese children: Effects on vascular function and body composition. *J Pediatrics* 144: 620-625
- Watts K, Davis E, Jones T, Green DJ (2005) Effect of exercise training in obese children and adolescents. *Sports Med* 35: 1-18
- Wessel TR, Arant CB, Olson MB, Delia Johnson B, Reis SE, Sharaf BL, Shaw LJ, Handberg E, Sopko G, Kelsey SF, Pepine CJ, Noel Bairey Merz C (2004) Relationship of physical fitness vs body mass index with coronary artery disease and cardiovascular events in women. *JAMA* 292: 1179-1187
- West GB, Brown JH, Enquist BJ (1997) A general model for the origin of allometric scaling laws in biology. *Science* 276: 122-126
- Whaley MH, Blair SN (1995) Epidemiology of physical activity, physical fitness and coronary heart disease. *Journal of cardiovascular risk* 2: 289-295
- Wisloff U, Stoylen A, Loennechen JP, Bruvold M, Rognmo O, Haram PM, Tjonna AE, Helgerud J, Slordahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen O, Skjaerpe T (2007) Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* 115: 3086-3094
- Woo KS, Chook P, Yu CW, Sung RY, Qiao M, Leung SS, Lam CW, Metreweli C, Celermajer DS (2004) Effects of diet and exercise on obesity-related vascular dysfunction in children. *Circulation* 109: 1981-1986

- Woodhouse PR, Khaw KT, Plummer M (1993) Seasonal variation of blood pressure and its relationship to ambient temperature in an elderly population. *J Hypertens* 11: 1267-1274
- Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Green D (2001) Improved analysis of brachial artery ultrasound images using a novel edge-detection software system. *J Appl Physiol* 91: 929-937
- Xavier F, Sunyer P (2002) The obesity epidemic: pathophysiology and consequences of obesity. *Obes Res* 10: 97-104
- Yeboah J, Crouse JR, Hsu F-C, Burke GL, Herrington DM (2007) Brachial flow-mediated dilation predicts incident cardiovascular events in older adults. The cardiovascular health study. *Circulation* 115: 2390-2397
- Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, Lima JA, Crouse JR, Herrington DM (2009) Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. *Circulation* 120: 502-509
- Zdravkovic S, Wienke A, Pedersen NL, Marenberg ME, Yashin AI, De Faire U (2002) Heritability of death from coronary heart disease: a 36-year follow-up of 20 966 Swedish twins. *Journal of internal medicine* 252: 247-254
- Zeppilli P, Vanniculli R, Dello Russo A, Palmieri V, Cameli S, Corsetti S, Pietrangeli L (1995) Echocardiographic size of conductance vessels in athletes and sedentary people. *Int J Sports Med* 16: 38-44
- Zhao J, Cheema FA, Reddy U, Bremner JD, Su S, Goldberg J, Snieder H, Vaccarino V (2007) Heritability of flow-mediated dilation: a twin study. *J Thromb Haemost* 5: 2386-2392