THE UNIVERSITY OF HULL

Endothelial function response to different modes of acute and chronic exercise in both health and diseased populations

A thesis submitted for the degree of Doctor of Philosophy

To the Faculty of Science and Engineering at the University of Hull

By

Richard James Thompson Kirk BSc (Hons), MSc

September 2014

Table of Contents

Acknowledgements **6**

Dissemination **8**

List of Figures **10**

List of Tables **15**

Nomenclature **18**

Abstract **21**

Chapter 1: General Introduction **25**

1.1 General Introduction **26**

Chapter 2: Literature Review **30**

2.1 The endothelium **31**

2.1.1 Endothelial dysfunction (ED) 32

2.1.2 Coagulation in ED 33

2.1.3 Assessment of endothelial function (EF) 34

2.1.4 Flow-Mediated Dilation (FMD) 36

2.1.5 EndoPAT 37

2.2 Microparticles (MP) **38**

2.2.1 MP formation and release 39

2.2.2 Role of MP 43

2.2.3 Detection of MP 44

2.2.4 Endothelial microparticles (EMP) 45

2.2.5 EMP in healthy conditions 47

2.2.6 CD106 48

2.2.7 CD105 49

2.8 Exercise and EF **51**

2.9 Polycystic ovary syndrome **58**

2.9.1 Overview 58

2.9.2 Diagnosis of PCOS 59

2.9.3 Prevalence of PCOS 60

2.9.4 Pathogenesis of PCOS 61

2.9.5 Abnormalities associated with PCOS 62

2.9.6 Obesity and PCOS 63

2.9.7 ED in PCOS 64

2.9.8 Exercise intervention on EF in PCOS 65

Chapter 3: General Methodologies **68**

3.1 Recruitment and screening of participants **69**

3.1.1 Data storage 70

3.2 Anthropometric measurements **70**

3.2.1 Blood pressure 70

3.2.2 Height 71

3.2.3 Body mass 71

3.2.4 Manual waist to hip measurements 72

3.3 Peak power output (PPO) test **72**

3.4 Sub maximal exercise test **73**

3.4.1 Maximal exercise test 75

3.5 Expired gas analysis **75**
3.6 Blood collection **76**
3.6.1 Capillary blood samples 76
3.6.2 Venous blood samples 76
3.7 EMP **77**
3.7.1 Sample analysis of EMP 79
3.8 EF using the EndoPAT-2000 ..... **80**

Chapter 4: Investigation into the effects of repeated supra maximal cycling on the EMP response in healthy males **84**
4.1 Introduction **85**
4.2 Methods **87**
4.2.1 Participant characteristics 87
4.2.2 Experimental design 87
4.2.3 Statistical analysis 89
4.3 Results **89**
4.3.1 Acid base homeostasis 89
4.3.2 PRR 91
4.3.3 CD105+ MP 93
4.3.4 CD106+ MP 95
4.3.5 Overall + MP response to exercise 97
4.4 Discussion **98**

Chapter 5: Investigation into the effects of repeated supra maximal cycling on the EMP response in healthy females **105**
5.1 Introduction **106**
5.2 Methods **111**
5.2.1 Participant characteristics 111
5.2.2 Experimental design 111
5.2.3 Statistical analysis 113
5.3 Results **113**
5.3.1 Acid base homeostasis 113
5.3.2 Performance data 115
5.3.3 SBP, DBP, HR and % HRmax 115
5.3.4 RPE, PRR and RPM and HR during exercise 117
5.3.5 CD105+ MP 119
5.3.6 CD106+ MP 120
5.4 Discussion **121**

Chapter 6: Investigation into the effect of a long duration intermittent cycling protocol on the EMP release in healthy males **125**
6.1 Introduction **126**
6.2 Methods **129**
6.2.1 Participant characteristics 129
6.2.2. Experimental design 129
6.2.3 Blood sampling 130
6.2.4 Statistical analysis 130
6.3 Results **131**
6.3.1 Acid-base homeostasis 131
6.3.2 HR data 132
6.3.3 PRR and RPE and during exercise 133

6.3.4 CD105+ MP 135
6.3.5 CD106+ MP 136
6.4 Discussion 138

Chapter 7: EF response to acute exercise in healthy, sedentary individuals measured using EMP and EndoPAT-2000 143
7.1 Introduction 144
7.2 Methods 147
7.2.1 Subject characteristics 147
7.2.2 Experimental design 147
7.2.3 Blood sampling 148
7.2.4 Exercise protocol 148
7.2.5 Statistical Analysis 149
7.3 Results 149
7.3.1 Baseline characteristics 149
7.3.2 Effects of exercise on blood marker measurements 150
7.3.3 CD105+ MP 151
7.3.4 CD106+ MP 152
7.3.5 Correlation of EndoPAT-2000 data with EMP 154
7.3.6 CD105+ MP 154
7.3.7 CD106+ MP 155
7.4 Discussion 155

Chapter 8: The effects of an 8 week moderate intensity exercise programme on EF in women with and without PCOS 160
8.1 Introduction 161
8.2 Methods 163
8.2.1 Participant Characteristics 163
8.2.2 Experimental design 164
8.2.3 Baseline assessment 166
8.2.4 Exercise training programme 167
8.2.5 Mid-point assessment 168
8.2.6 Final assessment 168
8.2.7 Venous blood samples 168
8.2.8 Statistical analysis 169
8.3 Results 170
8.3.1 Baseline characteristics 170
8.3.2 Effect of exercise on measured variables 172
8.3.3 Blood marker measurements 174
8.3.4 CD105+ MP 174
8.3.5 CD106+ MP 175
8.3.6 Correlations of anthropometric data with EMP data 177
8.4 Discussion 179

Chapter 9: Combination of data from previous chapters 187
9.1 Introduction 188
9.2 Methods 188
9.2.1 Statistical analysis 189
9.3 Results 189
9.3.1 CD105+ MP baseline data 189
9.3.2 CD106+ MP baseline data 191

9.3.3 Effects of gender on EMP 192
9.3.4 Effects of acute exercise on EMP 193
9.4 Discussion 194
Chapter 10: General Discussion 197
10.1 Introduction 198
10.2 CD105+ MP and CD106+ MP responses to exercise 201
10.3 Biomarkers of EF 203
10.4 Recommendations for future research 204
Chapter 11: References 206

Acknowledgements

I am enormously grateful to a number of people who have helped me in the completion of this doctoral thesis. Thanks must be given first of all to Professor Stephen Atkin and Dr Paul Potrac who gave me the initial opportunity to study at the University of Hull. I also wish to thank Professor Sean Carroll for your continued support throughout my time here, both academically and personally, I really appreciate everything you have done for me. Special thanks must be given to Dr Leigh Madden, who over the course of my studies has been invaluable in various aspects, not only with biochemical measurements, but also as a main supervisor and mentor, I cannot thank you enough. None of what I have been able to achieve would have been possible without my primary supervisor, Dr Rebecca Vince. I am entirely grateful for your great patience and generosity, and the stimulating ideas you helped to provide helped make this entire project possible, thank you, for everything.

I also must express my appreciation to my fellow post graduate colleagues within the department for their great friendship and advice from the get go; namely Bryna, Chris, Steph and particularly Dan and Will. You all played a huge part of my time here at Hull and you will never be forgotten. Thanks also must be directed at all of those who willingly volunteered to take part in the numerous experiments along the way. I must also take the time out to thank my fellow research colleagues that I have worked with along the way, particularly Dr Myint Aye, as without your coherence and great minds, this work would not have been possible.

My final and most important thanks are reserved for my family. To Mam and Dad, words cannot describe the extent of my love and gratitude towards you, particularly over this very long and at times very difficult journey. You have always been there for me when required and always offered the correct advice. I set out on this project with an aim to make you proud and to prove that I could do it, and now I have. I love you both.

Dissemination

Published Research Articles

Kirk, R.J., Peart, D.J., Madden, L.A., Vince, R.V. (2013). Repeated supra-maximal sprint cycling with and without sodium bicarbonate supplementation induces endothelial microparticle release. *European Journal of Sport Science*, **DOI: 10.1080/17461391.2013.785600**

Conference Presentations

Kirk, R.J., Madden, L.A., Atkin, S.L., Aye, M.M., Vince, R.V. (2013). Short-term moderate-intensity exercise independently improves endothelial function in women with polycystic ovary syndrome'. *Young Life Scientists Symposium, London*. **Finalist for best poster presentation: Cardiovascular Medicine: Bridging Basic and Clinical Researchers**

Kirk, R.J., Peart, D.J., Madden, L.A., Vince, R.V. (2012). Repeated supra-maximal sprint cycling with and without sodium bicarbonate supplementation induces endothelial microparticle release. *Annual Conference of the European College of Sports Science, Bruges.* **Nominated for Young Investigator Award**

List of Figures

Chapter 2: Literature Review

Fig 2.1 Electron microscopy of circulating MP from patients with acute coronary syndrome (From : Boulanger, Amabile & Tedgui, 2006)

Fig 2.2 Plasma membrane response to cell stimulation leading to MP release.

Fig 2.3 Phenotypes of cellular biomarkers of endothelial health using EMP

Fig 2.4 Polycystic ovaries assessed on ultrasound

Chapter 3: General Methodologies

Fig 3.1 Representation of the flow cytometry MP gating used to determine MP numbers throughout the entire thesis showing FSC and SSC plots

Fig 3.2 Representation of flow cytometry quantification of EMP

Fig 3.3 EndoPAT machine

Fig 3.4 EndoPAT set up used in experimental trials. Panel A represents the setup of the system including the finger probes on each hand. Panel B shows the blood pressure reading for the occlusion of the brachial artery

Chapter 4: Investigation into the effects of repeated supra maximal cycling on the EMP response in healthy males

Fig 4.1 Performance parameters as measured by PRR in both experimental conditions

Fig 4.2 CD105+ MP expressed at rest and immediately, 90, and 180 min post exercise during the placebo and experimental trials

Fig 4.3 CD106+ MP expressed at rest and immediately, 90, and 180 min post exercise during the placebo and experimental trials

Fig 4.4 Overall group means of CD105+ MP and CD106+ MP expressed at rest, immediately, 90, and 180 min post exercise

Chapter 5: Investigation into the effects of repeated supra maximal cycling on the EMP response in healthy females

Fig 5.1 Performance parameters during exercise as measured by average HR, peak HR, RPE and PRR during exercise

Fig 5.2 CD105+ MP at rest and immediately, 45 min, 90 min and 180 min post exercise

Fig 5.3 CD106+ MP at rest and immediately, 45 min, 90 min and 180 min post exercise

Chapter 6: Investigation into the effect of a long duration intermittent cycling protocol on the EMP release in healthy males

Fig 6.1 Performance parameters during exercise as measured by average HR and peak HR during exercise

Fig 6.2 Performance parameters during exercise as measured by RPE and PRR during exercise

Fig 6.3 CD105+ MP expressed at rest, and immediately, 45 min, 90 min, and 180 min post exercise

Fig 6.4 CD106+ MP expressed at rest, and immediately, 45 min, 90 min, and 180 min post exercise

Chapter 7: EF response to acute exercise in healthy, sedentary individuals measured using EMP and EndoPAT-2000

Fig 7.1 CD105+ MP expressed at pre, post and 60 min post exercise

Fig 7.2 CD106+ MP expressed at pre, post and 60 min post exercise

Fig 7.3 RHI correlated with CD105+ MP

Fig 7.4 RHI correlated with CD106+ MP

Chapter 8: The effects of an 8 week moderate intensity exercise programme on EF in women with and without PCOS

Fig 8.1 Diagram of participant information and study design from initial volunteering of the study to final study completion

Fig 8.2 CD105+ MP expressed at pre, mid, and post exercise intervention in PCOS and control participants

Fig 8.3 CD106+ MP expressed at pre, mid, and post exercise intervention in PCOS and control participants

Chapter 9: Combination of data from previous chapters

Fig 9.1 CD105+ MP expressed in participants from chapter 4 (C4), chapter 5 (C5), chapter 6 (C6), chapter 7 (C7), chapter 8 (controls, C8 Con) and chapter 8 (PCOS, C8 PCOS) at rest/pre exercise.

Fig 9.2 CD106+ MP expressed in participants from chapter 4 (C4), chapter 5 (C5), chapter 6 (C6), chapter 7 (C7), chapter 8 (controls, C8 Con) and chapter 8 (PCOS, C8 PCOS) at rest/pre exercise.

Fig 9.3 CD105+ MP and CD106+ MP expressed in participants from chapter 4 (C4), chapter 5 (C5), and chapter 6 (C6) at rest, and then post, 45, 90 and 180 min post exercise.

List of Tables

Chapter 2: Literature Review

Table 2.1 Most common methods for the evaluation of EF and their advantages and disadvantages

Table 2.2 Summary of exercise trials measuring EMP and/or cell adhesion molecules as a marker of EF

Chapter 4: Investigation into the effects of repeated supra maximal cycling on the EMP response in healthy males

Table 4.1 Acid base characteristics (pH, HCO_3^-, base excess, and H^+) measured pre and post ingestion of $NaHCO_3$ (means ± SD)

Chapter 5: Investigation into the effects of repeated supra maximal cycling on the EMP response in healthy females

Table 5.1 Acid base characteristics (pH, lactate, H^+, Hctc, and base excess) measured at rest, immediately after exercise, and 45 min, 90 min and 180 min post exercise (means ± SD)

Table 5.2 SBP, DBP, HR, and percentage of maximum HR recorded at rest, during, immediately after exercise, and during post exercise recovery (means ± SD)

Chapter 6: Investigation into the effect of long duration intermittent cycling protocol on the EMP release in healthy males

Table 6.1 Acid base characteristics at rest and then 18 min intervals throughout the exercise (means ± SD)

Chapter 7: EF response to acute exercise in healthy, sedentary individuals measured using EMP and EndoPAT-2000

Table 7.1 Baseline characteristics of participants showing the mean body mass, SBP, DBP, VO_{2max}, BMI, RHI, fRHI and AI (means ± SD)

Table 7.2 Characteristics of participants showing mean variables measured from pre exercise to post, and 60 min after exercise ((means ± SD, n = 10).

Chapter 8: The effects of an 8 week moderate intensity exercise programme on EF in women with and without PCOS

Table 8.1 Body mass, SBP, DBP, VO_{2max}, BMI, WHR, WC, CD105+ MP and CD106+ MP expressed at baseline in PCOS and control women

Table 8.2 Body mass, SBP, DBP, VO_{2max}, BMI, WHR, and WC expressed at baseline, mid and post exercise intervention in PCOS and control women

Table 8.3 Correlation of CD105+ MP and CD106+MP with variables measured in control and PCOS patients across the exercise programme

Chapter 9: Combination of data from previous chapters

Table 9.1 Resting/pre exercise + MP concentrations in healthy trained males, healthy trained females, healthy males and healthy females from entire thesis (mean ± SEM)

Nomenclature

ºC	The degree Celsius
µl	Microlitre
µm	Micromolar
Ach	Acetylcholine
AI	Augmentation index
AOX	Antioxidant
BA-FMD	Brachial artery flow mediated dilation
BP	Blood pressure
BMI	Body mass index
BW	Body weight
Ca^{2+}	Calcium ions
CD31	Platelet endothelial cell adhesion molecule (PECAM-1)
CD51	Integrin-αV
CD105	Endoglin
CD106	Vascular cell adhesion molecule-1 (VCAM-1)
CD144	VE-Cadherin
CD146	Melanoma cell adhesion molecule
CD62E	E-selectin
CD62P	P-selectin
CHO	Carbohydrate
CK	Creatine kinase
cm	Centimetre
CO_2	Carbon dioxide
CRP	C-Reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ED	Endothelial dysfunction
EF	Endothelial function
ELISA	Enzyme linked immunosorbant assay
EMP	Endothelial microparticle(s)
ET-1	Endothelin-1
FACS	Fluorescent activated cell sorting
FC	Flow cytometer
FMD	Flow mediated dilation
fRHI	Framingham reactive hyperaemia index
FSC	Forward scatter
g	Gram
H^+	Hydrogen ion
H_2O	Water
HC	Hip circumference
HCO_3^-	Bicarbonate
HDL-C	High density lipoprotein cholesterol
hr	Hour (s)
HR	Heart rate
HR_{max}	Maximum heart rate
HR_{peak}	Peak heart rate
$HR_{recovery}$	Recovery heart rate
HRR	Heart rate reserve

HSP	Heat shock protein
HSP72	Heat shock protein 72
HUVECS	Human umbilical vein endothelial cells
IAT	Individual anaerobic threshold
ICAM-1	Intercellular adhesion molecule-1
IL-1	Interleukin-1
IL-6	Interleukin-6
IR	Insulin resistance
KCl	Potassium chloride
kg	Kilogram
kJ	Kilojoules
km	Kilometre
LH	Luteinising hormone
M	Molar
m^2	Square metres
METS	Metabolic equivalents
min	Minute(s)
mg/dL	Milligram per decilitre
mmHg	Millimetre of mercury
mmol/l	Millimole per litre
MP	Microparticle(s)
MS	Metabolic syndrome
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NAFLD	Non-alcoholic fatty liver disease
NIH	National institute of health
NO	Nitric oxide
O_2	Oxygen
PAI-1	Plasminogen activator inhibitor-1
PAT	Peripheral arterial tone
PCOS	Polycystic ovary syndrome
PFP	Platelet free plasma
PMP	Platelet microparticle(s)
PPO	Peak power output
PRR	Perceived readiness rating
PS	Phosphatidylserine
PWA	Pulse wave amplitude
RHI	Reactive hyperaemia index
RM	Repetition maximum
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPE	Rate of perceived exertion
RPM	Revolutions per minute
s	Second(s)
SBP	Systolic blood pressure
sCD106	Soluble vascular cell adhesion molecule -1
sCD62E	Soluble E-selectin
sICAM-1	Soluble intra-cellular adhesion molecule-1
SSC	Side scatter
TACE	Tumor necrosis factor-α converting enzyme
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

TG	Triglycerides
TM	Thrombomodulin
TNF-α	Tissue necrosis factor-α
VEGF	Vascular endothelial growth factor
VF	Visceral fat
VO_2	Volume of oxygen consumed
VO_{2max}	Maximal oxygen uptake
$VO_{2submax}$	Sub- Maximal oxygen uptake
W	Watt
WC	Waist circumference
WHR	Waist hip ratio

Abstract

Endothelial microparticles (EMP) offer an insight into the state of the endothelium and are known to be elevated in diseases characterised by endothelial dysfunction (ED) (Horstman et al., 2004; Vince et al., 2009a). EMP have also been shown to increase after exercise/endothelial stress in healthy individuals (Sossdorf et al., 2011; Vince et al, 2009) but this area remains relatively novel.

The purpose of the first experiment was to quantify the effects of an acute bout of strenuous exercise on the circulating levels of EMP and to assess if this effect is different after the ingestion of an extensively researched ergogenic aid (sodium bicarbonate, $NaHCO_3$). Seven physically active and apparently healthy males volunteered to perform 10 x 15 second (s) cycle sprints after the ingestion of either 0.03 $g.kg.BW^{-1}$ $NaHCO_3$ or 0.045 $g.kg.BW^{-1}$ of a placebo (sodium chloride, NaCl) in capsules. The ingestion of $NaHCO_3$ induced a pre exercise alkalosis as evidenced by a significantly altered resting acid base status, but had no influence on levels of EMP in healthy males. As a result, the data was combined for the two experimental groups, and the exercise produced a significantly increased level of CD105+ MP (MP; microparticles) at 90 minutes (min) and 180 min when compared with resting levels ($p = 0.010$, $p = 0.043$ respectively). The observed peak value at 90 min was also significantly greater compared to immediately post exercise ($p = 0.019$). CD106+ MP also increased significantly to 90 min from immediately post exercise ($p = 0.020$) and this was still greater at 180 min compared to post exercise ($p = 0.015$). It was concluded that exercise of this nature was sufficient to elicit ED, although the endothelium shows signs of endothelial repair within a matter of hours (hr). Also, it appears that pre exercise alkalosis has no effect on the attenuation of EMP quantity.

Additional work was completed to verify the novel finding that CD105+ MP and CD106+ MP appear markers of endothelial function (EF), and to further examine the quantification of EMP, this time in healthy females. There was also an additional blood draw in order to assess where the maximum level of endothelial stress was occurring post exercise. In the second experiment, 10 healthy females completed the identical repeated sprints protocol as the first experiment, this time without the ingestion of $NaHCO_3$. CD105+ MP were increased 90 min post exercise compared to immediately after exercise ($p = 0.042$). There was again a decline in both markers from 90 min to 180 min, although this was not significant. Furthermore, with the addition of a blood draw at 45 min post exercise, it was suggested that EMP levels appear to be rising between 45 min and 90 min post exercise, speculating this is the time point of greatest endothelial damage. Finally, shear stress was suggested as a key reason behind the increase in endothelial damage as a result of exercise, as indicated by significant changes in variables such as heart rate (HR) and systolic blood pressure (SBP).

The third experiment employed a longer 90 min interval cycling protocol with the purpose of quantifying EF over a greater period of time, allowing investigation into whether the markers of EF were altered in the same way as the previous two experiments. It was also an aim to further assess the possible influence of shear stress factors on ED. Fourteen healthy males completed 90 min of high intensity aerobic exercise, and there were several changes in both CD105+ MP and CD106+ MP. CD105+ MP rose significantly from rest to an observed peak at 90 min ($p = 0.019$). Both of these markers indicated a significant restoration of the endothelium as indicated by a fall from peak values during recovery to 180 min post exercise (CD105+ MP, $p = 0.009$; CD106+ MP, $p = 0.022$). This experiment concluded that the endothelium is greatly affected by highly intense exercise over a prolonged period of time, but is

recovered fully in a time period of 3 hr. The effects of shear stress again appear to be largely influential, but future work must now be conducted in order to build on the findings from this research and examine shear stress closely during exercise and its relationship with EMP quantification.

It was the aim of the next experiment to investigate two separate methods of assessing EF (EMP and EndoPAT-2000), this time in a group of sedentary, but otherwise healthy individuals, in order to monitor the changes as a result of an acute bout of moderate intensity acute exercise. There were no significant differences found in EF as a result of exercise. This was indicated by no significant changes in CD105+ MP concentrations from pre to post exercise ($p = 0.84$) or pre to 60 min post exercise ($p = 0.612$). CD106+ MP concentrations showed a decrease from resting values (2513 CD106+ MP per µl platelet free plasma; PFP) to immediately post exercise (1368 CD106+ MP per µl PFP, $p = 0.09$), and again at 60 min post exercise (1293 CD106+ MP per µl PFP, $p = 0.073$) compared to resting values. Additionally, EndoPAT scores were unaffected by exercise, with values of reactive hyperaemia index (RHI) changing from rest (2.43) to post exercise (2.57), but this was not significant ($p = 0.35$). Correlations were carried out in order to determine and comparisons that may have existed between EMP and EndoPAT score using RHI. Although there was a slight trend for the higher numbers of CD105+ MP to correlate with the lower scores of RHI ($r = 0.327$) this was not significant ($p = 0.171$). CD106+ MP showed no correlations with RHI ($r = -0.087$, $p = 0.717$). This chapter suggested that exercise was not strenuous enough to see any significant changes in EF, and EMP continue to appear efficient markers of EF in a population of sedentary, healthy individuals.

The final experimental chapter investigated the effects of a supervised 8 week moderate intensity exercise programme on women with polycystic ovary syndrome (PCOS) and control women free from any known disease. The aim was to assess if this type of exercise could improve EF in this population, and if there was a relationship with EMP (CD105+ MP and CD106+ MP) to other factors, such as body composition and cardiorespiratory fitness. EF was improved from baseline values to post exercise programme, with CD105+ MP concentrations reducing from 2113 CD105+ MP per µl PFP to 424 CD105+ MP per µl PFP ($p = 0.025$). Furthermore, control women showed no significant change from pre to post exercise programme in CD105+ MP ($p = 0.25$), or CD106+ MP ($p = 0.99$). Further analysis was performed to look for any associations with the changes in EMP compared to body composition changes as a result of exercise, but no significant correlations existed. This study concluded that supervised, moderate intensity exercise independent of substantial weight loss was enough to elicit an00 improvement in EF in women with PCOS compared to healthy control women. Additionally, EMP concentrations appear to be able to effectively map changes in EF across a long period of time in diseased states, adding to the notion that EMP may account for EF. Future work must now build on these findings from this research and examine this response in a larger cohort involving PCOS women with varied phenotypes and body composition.

Chapter 1: General Introduction

1.1 General Introduction

The endothelium is made up of a continuous monolayer of cells known as endothelial cells, and constitutes the inner lining of the blood vessels and the lymphatic system (Johnson-Leger, Aurrand-Lions & Imhof, 2000). The endothelium plays a critical role as a semi selective barrier between the vessel lumen and surrounding tissue, and is the primary sensor of any physical and chemical alterations in the blood (Burger & Touyz, 2012). The endothelium regulates vascular tone (Vita, 2011) and is essential for controlling the passage of fluid into the tissue (Burger & Touyz, 2012).

Endothelial dysfunction (ED) is a term that includes a shift from a normal, healthy endothelium to a stressed/damaged endothelium, characterised by a pro-vasoconstriction, pro-coagulation and pro-inflammatory phenotype (Flammer & Luscher, 2010; van den Oever et al., 2010). It is now well recognised that any alterations in the integrity of the endothelium and physiological functions represent key mechanisms in the initiation and development of vascular diseases (Navasiolava et al., 2010). In humans, ED correlates with disease progression (Halcox et al., 2009), is associated with various disease risk factors (Hamburg et al., 2008a;b), and is a prominent feature of metabolic diseases such as type 2 diabetes mellitus (T2DM) and obesity (Xu & Zou, 2009). To show the true extent to which ED is associated with an array of diseases, it has been involved in hypertension, atherosclerosis, aging, heart and renal failure, coronary syndrome, preeclampsia, type I (T1DM) and T2DM, impaired glucose tolerance, insulin resistance (IR), obesity, inflammation, smoking and mental stress (Feletou & Vanhoutte, 2006).

When subjected to various substances (released by autonomic and sensory nerves or platelets), circulating hormones, and cytokines, as well as to physical and chemical stimuli (e.g. pressure changes, shear stress and pH), endothelial cells synthesise and release various factors that modulate angiogenesis, inflammatory responses, haemostasis, and also vascular tone and permeability (Feletou & Vanhoutte, 2006). ED may be assessed by various techniques in humans, such as biochemically through the release of biomarkers (e.g. adhesion molecules and cytokines) in the blood (Thomson et al., 2012), or functionally by measuring endothelium dependent dilation *in vitro* and *in vivo* either in response to an agonist, or to changes in the flow in the forearm, coronary, or peripheral circulation (Deanfield, Halcox & Rebelink, 2007).

A growing area of research in recent times related to endothelial stress and ED includes the measurement of endothelial microparticles (EMP) (Dignat-George & Boulanger, 2011; Hargett & Bauer, 2013). These cellular biomarkers represent an alternative to other direct measures of endothelial function (EF) (Burger & Touyz, 2012). EMP are fragments of cellular membrane shed from stressed or damaged endothelial cells (Dignat-George & Boulanger, 2011; Horstman et al., 2004). EMP are released under conditions of cell stress/damage, and plasma levels of EMP have been shown to be increased in several cardiovascular (CV) and metabolic diseases, such as T2DM (Gad et al., 2014), hypertension (Preston et al., 2003) and metabolic syndrome (Helal et al., 2011). EMP would therefore appear to be reflective of ED, suggesting that this family of MP are valuable markers of disease.

An area of research gaining heightened attention is the response of EMP to exercise, whether it is acute or chronic exercise, in both health and diseased populations (Chaar et al., 2011; Guiraud et al., 2013; Sossdorf et al., 2010), although it must be said that

research in this area remains highly novel. Individuals participating in vigorous exercise have been observed for their endothelial state by looking at circulating levels of vascular endothelial markers, of which two of the more common markers are Endoglin (CD105) and vascular cell adhesion molecule-1; VCAM-1 (CD106) (Chaar et al., 2011; Chrismas et al., 2010; Madden et al., 2010; Marsh & Coombes, 2005; Vince et al., 2009a;b). Moderate intensity long term exercise is also known to either decrease or steady levels of endothelial adhesion molecules in both health and diseased populations (Adamopoulos et al., 2001; Sjogren et al., 2010; Thomson et al., 2012), adding to the intriguing and novel concept of the use of endothelial adhesion molecules as markers of EF across acute and chronic exercise. CD105 is a transmembrane glycoprotein which is predominantly expressed constitutively on activated vascular endothelial cells (Dallas et al., 2008; Jimanez et al., 2003). The expression of CD105 after exercise has received attention as of late, specifically following acute bouts of exercise/oxidative stress (Chrismas et al., 2010; Madden et al., 2010). CD106 is a transmembrane glucoprotein member of the immunoglobulin super family that is expressed on activated vascular endothelial cells, usually under the influence of inflammatory stimuli (Fotis et al., 2012; Vince et al., 2009b). Specifically, CD106 has been used in order to assess EF after acute bouts of exercise of differing natures (Chaar et al., 2011; Vince et al., 2009a;b). Additionally, when looking into chronic exercise bouts, moderate intensity exercise is known to either decrease or steady levels of endothelial markers in health and diseased populations, showing the diverse potential of such markers making them hugely intriguing to explore deeper (Adamopoulos et al., 2001; Sjogren et al., 2010; Thomson et al., 2012).

There is currently limited research into the effects of different modes and durations of exercise on EF, with many currently only being able to postulate the mechanisms

governing a change in EF (Hallmark et al., 2014). This creates inconsistency, which is in part due to the intensity and duration of the exercise stimulus, the mode of exercise, population studied, time of day for the exercise, and also post exercise evaluations (Hallmark et al., 2014). Furthermore, the use of EMP to assess EF is still known as a relatively novel area, but one gaining in reputation and profound interest (Yong, Koh & Shim, 2013), making it an intriguing topic of research. The purpose of this thesis was to profile the effect of acute and chronic exercise on the endothelial stress response in health and diseased populations, and predominantly to investigate the use of EMP as biomarkers of EF.

Chapter 2: Literature Review

2.1 The endothelium

The healthy endothelium is a monolayer of relatively simple cells, but one of great importance (Deanfield, Halcox, & Rabelink, 2007). The endothelium represents the largest functional organ in the human body, weighing about 1 kg and covering up to 7000 m^2 of the surface area (Wolinsky, 1980) and it lines the vascular system, the serous, heart chamber cavities, and lymph vessels (Malyszko, 2010). The extent of the endothelium is remarkable, with a surface area of several square metres and appreciatively ten trillion (10^{13}) cells (Wolinsky, 1980). The endothelium is able to respond to physical and chemical signals by the regulation of vascular tone, platelet activity, maintenance of blood circulation, leukocyte adhesion, and angiogenesis, therefore it may be said that the endothelium represents an interface between the circulation elements and several of the systems in the body (Ghisi et al., 2010; Wagner & Frenette, 2008). The endothelium is semi-permeable with regards to a role as a barrier, and regulates the transfer of small and large molecules (Galley & Webster, 2004). Furthermore, the endothelium plays an important role in maintaining CV homeostasis (Lovren & Verma, 2013; Widlansky et al., 2003). This CV homeostasis is achieved by the secretion of endothelium derived relaxing and endothelium derived contracting substances (Furchgott & Zawadzki, 1980; Verma & Anderson, 2002). The key endothelium derived relaxing factor is nitric oxide (NO) which plays a crucial role in the maintenance of vascular tone and reactivity (Moncada & Higgs, 1993). Furchgott and Zawadzki (1980) were the first to demonstrate that NO was an endothelium derived relaxing factor (Furchgott & Zawadzki, 1980). This observation came about through chance, when this group elucidated the paradox that acetylcholine (ACh) was a vasodilator *in vivo* in rabbit thoracic aorta (Furchgott & Zawadzki, 1980). NO is not only the main determinant of basal vascular smooth muscle tone, but an inhibitor of

coagulation, inflammation, and oxidative stress (Verma, Buchanan & Anderson, 2003). Additionally to this, a diminished production or lack of availability of NO and/or an imbalance of endothelium-derived relaxing and contracting factors have been associated in ED (Lerman & Burnett, 1992).

2.1.1 Endothelial dysfunction (ED)

ED may be described as the inability of one's artery to adequately dilate in response to an appropriate endothelial stimulus (Moerland et al., 2012). ED indicates an imbalance between endothelium-derived vasodilators and vasoconstrictors, abnormal inflammatory cell endothelial interactions, and an increase in adhesion molecule expression, which all collectively make up ED (Balciunas et al., 2009). Cell adhesion molecules are expressed by the endothelium, and soluble isoforms of these molecules are found in the plasma, thought to be as a result of shedding from cell surfaces as a result of endothelial damage (Gearing & Newman, 1993; Marsh & Coombes, 2005). ED is commonly associated with the development of a wide variety of CV diseases (CVD), such as atherosclerosis (Ross, 1999; Shimokawa, 1999), and measures of ED may offer prognostic information with respect to vascular events (Verma & Anderson, 2002). Indeed, most CV risk factors activate the endothelium that results in the expression of chemokines, cytokines, and adhesion molecules (Tousoulis et al., 2003). ED is characterised by the endothelium displaying a pro-atherogenic phenotype, meaning that it loses its pro-inflammatory/oxidant/vasoconstrictor phenotype (Donato et al., 2009). Activation of the inflammatory process after endothelial injury or stress then leads to the release of primary pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) (Donato et al., 2009). These cytokines then activate endothelial cells with the release of adhesion

molecules that in turn lead to local recruitment of circulating inflammatory cells (Balciunas et al., 2009). Cytokines like TNF-α stimulate the synthesis of endothelial adhesion molecules from endothelial cells, such as CD106, intracellular adhesion molecule-1 (ICAM-1), and selectins (E- and P-; CD62E and CD62P respectively) (Pearson et al., 2003; Verma & Anderson, 2002) which are referred to in greater detail in section 2.2.6 and 2.2.7. Soluble forms of these molecules that are released from the endothelial cell surface can be useful in assessing and indicating ED, and have been shown to be effective markers of diseases prone to ED such as T2DM (Cui & Song, 2009).

2.1.2 Coagulation in ED

Endothelial damage results in the rapid adhesion of platelets at the site of injury, as well as in the activation of the coagulation cascade (Bartzeliotou et al., 2007; Stenina, 2003). ED may alter its ability to participate adequately in both coagulation and fibrinolysis, which may lead to arterial thrombosis (Strukova, 2006). Thrombomodulin (TM) is a biologically active molecule with anti-coagulant activity, which may be released from injured/activated endothelial cells (Dohi et al., 2003; Bartzeliotou et al., 2007; Salomaa & Wu, 1999; Wang et al., 2006). Plasma levels of TM have been shown to be elevated in disease states, such as women with PCOS, who show excess production of testosterone and increased adiposity, when compared with age and BMI (body mass index) matched control women (Oral et al., 2009). This study shows that testosterone and increased adiposity does in fact appear to increase levels of TM, indicating that those with increasing risk of ED have increased TM levels. Levels of TM were recently shown to be affected by exercise in a study by Bartzeliotou et al (2007) who looked at

the effects of a “Spartathlon” race in healthy, well trained individuals. TM was measured in this study, and its levels were significantly elevated at the end of the race compared to resting levels (Bartzeliotou et al., 2007). These increased levels of TM following endothelial damage are speculated to be coming from NO damaged endothelial cells that may constitutively have a higher expression of TM, possibly acting in a protective manner against thrombotic events (Salomaa et al., 1999). Additionally, the returning levels of TM back to basal levels 48 hr post the event imply that in healthy individuals, their endothelium is able to recover efficiently, reinforcing the vasoprotective action of TM.

2.1.3 Assessment of EF

Prolonged and/or repeated exposure to cardiovascular risk factors has an exhaustive effect on the endothelium, and can reverse the protective effect of the anti-inflammatory systems within endothelial cells (Deanfield, Halcox, & Rabelink, 2007). This then leads to a dysfunctional endothelium, with endothelial cells losing their integrity, progressing to their detachment into the circulation (Woywodt et al., 2002). Table 1 refers to the more common methods of analysing EF, and with it their various advantages and disadvantages. As seen in Table 1, the more ‘gold standard’ techniques tend to be invasive by nature, meaning that participation compliance is affected. Added to this, techniques such as coronary epicardial vasoreactivity are expensive, and also challenging to measure unless well trained. One advantage of the EndoPAT device is that it is very easy to perform and is non-invasive, but it is relatively expensive.

Table 2.1 The most common methods for the evaluation of EF and their advantages and disadvantages.

Technique	Vascular bed	Advantages	Disadvantages
Coronary epicardial vasoreactivity	-Epicardial macrovascular -Conduit arteries	-Gold standard -Assessment directly in the coronary vascular bed	-Invasive -Expensive -Time invasive -Limited to those undergoing coronary angiography -Challenging for serial measurements
Venous occlusion plethysmography (changes in forearm blood flow)	-Forearm vasculature -Micro-vasculature	-Easy access -Vasoactive substances infused to generate a dose-response relationship -Contralateral arm as a control	-Invasive (cannulation of the brachial artery) -Time consuming
Coronary microvascular function-Doppler wires	Coronary microvascular Resistance arteries	-Assessed directly in the coronary microvasculature	-Invasive -Expensive -Limited to those undergoing coronary angiography -Time intensive
Ultrasound FMD	-Brachial artery -Conduit artery	-Non-invasive -Easy access -Correlation with invasive epicardial vascular function -Many outcome studies -Inexpensive	-Requires experienced performers -Need for standardisation
EndoPAT	-Finger -Micro-vascular	-Non-invasive -Easy to perform -Automated -Low inter- and intra-observer variability -Correlation with invasive microvascular vascular function	-Expensive after single use of finger probes -PAT signal influenced by non endothelial variables

FMD = Flow-mediated dilation; PAT = Peripheral Arterial Tone

2.1.4 Flow-Mediated Dilation (FMD)

Flow-mediated dilation (FMD) is an endothelium-dependent process that reflects the relaxation of a conduit artery when there is exposure to an increase in blood flow, and therefore shear stress (Moens et al., 2005). Celermajer and colleagues (1992) first described the most popular technique for assessing FMD in humans (Celermajer et al., 1992). This study demonstrated that by increasing blood flow through a superficial conduit artery would induce flow-dependent dilation that could be measured by a non-invasive technique using high-resolution ultrasound. The degree of dilation would then indicate the level of functional integrity of the endothelium. Blood flow was increased in the BA after release of a forearm cuff that had been inflated for 5 min. FMD is usually assessed through large peripheral conduit arteries (brachial, radial and femoral) (Stout, 2009; Takase et al., 1998). The goal of using FMD is basically to create a shear stress stimulus that produces a NO-dependent response so that FMD can be used as direct marker of NO bioavailability (Stout, 2009). In simple terms, a small-FMD response indicates a low NO bio-availability and a possible risk of CVD (Pyke & Tschakovsky, 2005). FMD is operation dependent, but in the best hands the reproducibility can be very good, with a coefficient of variation of less than 2% (Sorensen et al., 1995). This technique is very well used throughout the world and is appealing due to its non-invasive and safe nature, and it can be performed with standard ultrasound and Doppler equipment (Swampillai et al., 2005). There are however several factors that affect dilation, such as differing cuff positions (Doshi et al., 2001). Studies have used the wrist, forearm or upper arm, which is problematic due to dilation varying with different cuff positions (Swampillai et al., 2005). The measure of FMD is technically demanding, requiring specific training, and it is sensitive to factors that may influence vascular function transiently, without having a great importance on long term

CV risk (Celermajer et al., 2008). For example, FMD may be acutely lowered by an inter-current viral illness, it may be impaired after a meal, and varies in a circadian pattern (Celermajer et al., 2008). Finally, given that FMD is measured at one arm only, there are no opportunities to correct for any possible measurement-induced changes in the systematic haemodynamic, such as those resulting in the autonomous nervous system tone (Moerland et al., 2012). It is important to note the differences between endothelial dependent and endothelial independent FMD. Endothelial dependent FMD is created when shear stress occurs within an artery, causing the vascular endothelium to produce NO in order to facilitate arterial dilation (Stout, 2009). On the other hand, endothelial-independent dilation occurs through an NO donor, such as glycerine trinitrate, and so this process is dilation without the production of NO from the endothelial cells (Stout, 2009).

2.1.5 EndoPAT

The EndoPAT device uses a non-invasive measure of vasoreactivity without having to use ultrasound measurements (Moerland et al., 2012). The EndoPAT works by detecting plethysmographic pressure changes in the finger tips caused by the arterial pulse, and then this is translated to a peripheral arterial tone (PAT) (Kuvin et al., 2003; Moerland et al., 2012). This device is easy to use, not operator-dependent, and has comprehensive automatic analysis. The EndoPAT was devised in an attempt to rectify issues with FMD, such as the fact FMD is operator dependent (Vogel, Corretti & Plotnick, 2000). However, there is only limited data on the performance of the EndoPAT for repeated measurements over a relatively short period of time (Moerland et al., 2012). There have however been several studies that have shown a correlation

between the ‘EndoScore’ or RHI and multiple CV risk factors, such as T2DM and cholesterol (Hamburg et al., 2008a; Kuvin et al., 2003). Several studies have also shown an improvement in EF as assessed by the EndoPAT as a result of lifestyle modification (smoking cessation and dietary change) (Aversa et al., 2008; Barringer, Hatcher, & Sasser, 2011).

2.2 Microparticles (MP)

The presence of sub-cellular procoagulant particles in highly centrifuged plasma has been known for many years (Boulanger, Amabile, & Tedgui, 2006). The first depiction of these vesicles was made back in 1967, with the reports of a ‘platelet dust’ (platelet membrane fragments) being present in human plasma, and these were capable of facilitating thrombin generation in the same way as intact platelets (Wolf, 1967). After a more precise description on their origin, composition and function, these vesicles were termed MP, which have been studied more extensively over the past decade (Boulanger & Dignat-George, 2011). MP are sub-micron membrane vesicles that are shed almost spontaneously from the plasma membrane blebs of virtually all cell types under normal homeostasis (Azevedo, 2012; Freyssinet, 2003) and under a number of stressful conditions (Freyssinet, 2003; Shantsila, Kamphuisen, & Lip, 2010). Such sealed fragments typically range in size from 0.1 to 1.0 μm (Brodsky et al., 2004; Dignat-George & Boulanger, 2011; Piccin, Murphy, & Smith, 2007) (Fig 2.1), and are irregularly shaped, consisting of a cell-derived vesicle that is formed from the outward blebbing of the plasma membrane and successive shedding unto the extracellular space (Boulanger, 2010). MP contain cell surface proteins and cytoplasmic components of the cells of their parental origin (Combes et al., 1999; Zwaal & Schroit, 1997) and although

they may be released from almost all cell types, most investigators have focused more specifically on MP from platelets (PMP), leucocytes, vascular smooth muscle cells and endothelial cells (Roos et al., 2010). Despite having this long connotation of being somewhat insignificant as 'platelet dust', MP have been shown to reflect importance in both *in vitro* and *in vivo* studies. They are able to testify to cellular activation occurring *in vivo* under a variety of pathophysiological conditions, as well as in 'healthy' individuals (Berckmans et al., 2001; Freyssinet, 2003).

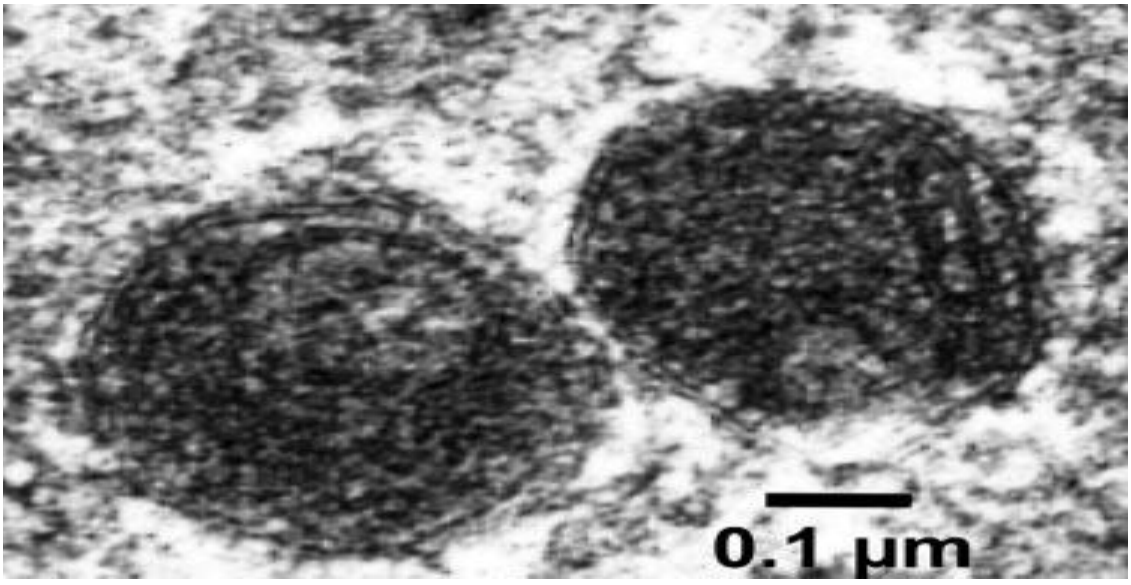


Fig 2.1 Electron microscopy of circulating MP from patients with acute coronary syndrome (From: Boulanger, Amabile & Tedgui, 2006).

2.2.1 MP formation and release

MP formation is a tightly regulated process that occurs in different types of cells, and their composition depends on the stimulus that is applied (Montoro-Garcia et al., 2011).

The generation and shedding of MP occurs through biological processes of some variety, including not only cellular activation following a stimulus of pro-inflammatory, pro-thrombotic or pro-apoptotic substances, or exposure to high shear stress, but also from cellular differentiation or apoptotic cell breakdown (Horstman & Ahn, 1999). It is true that the precise determinants of activated induced MP formation are not yet known, however cytoskeletal reorganisation and alterations in phospholipid symmetry are essential to their formation (Burger et al., 2013). The formation of MP involves the outward blebbing the plasma membrane (Azevedo, 2012; Morel et al., 2011). The activation of a MP producing cell is accompanied by changes in specific membrane appearances, such as phosphatidylserine (PS) (Montoro-Garcia et al., 2011), as well as cytoskeleton disruption and changes in the local concentration of certain intracellular molecules, all of which result in the budding of vesicles that will become MPs (Montoro-Garcia et al., 2011) (Fig 2.2).

The release of MP was first described by George et al. (1982) originating from platelets after they were activated by different stimuli, such as thrombin, collagen, and shear stress (George et al., 1982). Since then, there have been numerous studies, both *in vitro* and *in vivo*, investigating the release of such vesicles from activated and/or apoptotic cells, and their presence in human plasma is eagerly sought after as they are known to correlate with EF (Chrismas et al., 2010; Madden et al., 2010; Vince et al., 2009a;b). The budding of the plasma membrane is driven by cell activation and apoptosis, leading to the release of MP (Montoro-Garcia et al., 2011). It is generally agreed that the plasma membrane is the first structure to be involved in the generation of MP; however, these studies are usually regarding PMP formation (Azevedo, 2012; Montoro-Garcia et al., 2011). Under normal conditions, each of the two leaflets of the membrane bilayer has a specific lipid composition and are known to display an asymmetric distribution of

phospholipids The distribution of these lipids in the membrane is not a fixed process, but active and a tightly regulated energy-dependent process whose equilibrium is governed by three major players; an inward-directed pump, referred to as flippase, an outward-directed pump, known as floppase, and a lipid scramblase that promotes unspecific bidirectional redistribution across the bilayer (Bevers et al., 1999; Hugel et al., 2005; Morel et al., 2011; van Meer, Voelker, & Feigenson, 2008). Following a significant and sustained increase in cytosolic Ca^{2+}, a collapse of the membrane asymmetry by stimulation of scramblase and floppase may occur, and at the same time an inhibition of flippase (Freyssinet & Toti, 2010). The most important change in lipid distribution is the exposure of PS on the surface followed by MP release made possible after cytoskeleton degradation by Ca^{2+} dependent proteolysis and an imbalance in the phospholipid transient mass between the 2 leaflets (Freyssinet & Toti, 2010). Altogether, these aforementioned events lead to plasma membrane budding, formation of membrane blebs and consequently MP release into the extracellular fluid. Of note, there is a suggestion that the MP formed during cellular activation phenotypically and quantitatively differ from those MP that are released through apoptosis from the same cell type (Mause & Weber, 2010). For example, CD62E and CD106 are more markedly expressed on EMP released upon activation (Gelderman & Simak, 2008; Jimenez et al., 2003; Vince et al., 2009a;b) and CD105 is expressed on vascular endothelial cells (Gelderman & Simak, 2008).

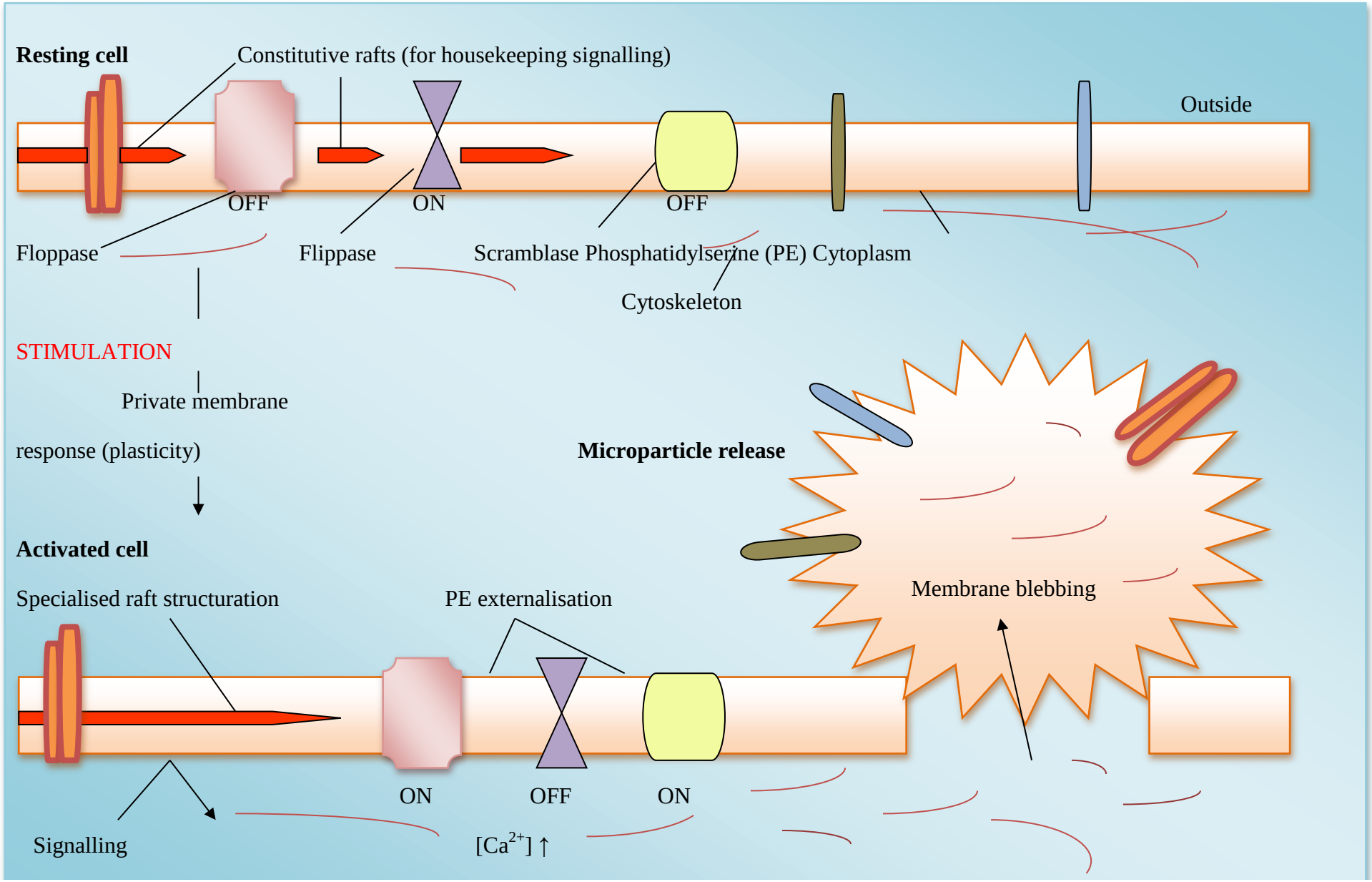


Fig 2.2 The plasma membrane response to cell stimulation leading to MP release. Adapted from Hugel et al (2005).

2.2.2 Role of MP

It would be reasonable to assume that MP are 'bad' in nature due to the deleterious potential associated with them, but a debate is arising that they may indeed be viewed as 'good' (Freyssinet, 2003). 'Good' MP may be those that fulfil a function in general homeostasis (Freyssinet, 2003). This may be illustrated in haemophilia for example, where MP levels are higher than normal, and can be elevated in the case of acute bleeding (Freyssinet, 2003; Proulle et al., 2004), which reflects a permanent mobilisation of the haemostatic system, but importantly without the threat of any thrombosis due to insufficient thrombin being formed (Proulle et al., 2004). Due to their procoagulant state, MP could be used to improve platelet function when it is impaired, for example in diseases such as thrombocytopenia, where high MP levels appear protective (Hugel et al., 1999; Jy et al., 1992). The common assumption that MP are always formed under deleterious circumstances is perhaps an oversimplification (Curtis et al., 2013). MP can hijack functional receptors, proteins, bioactive lipids and genetic material from their parental cell of which they originate, but they are also active sensors, communicators and effectors of the intercellular environment (Curtis et al., 2013). More recently, MP have been gaining profound interest as messengers of biological information having once been considered inert cell debris (Mause & Weber, 2010; Thery, Ostrowski & Segura, 2009). MP have been rationalised as vehicles for the intercellular exchange of biological signals and information (Thery, Ostrowski, & Segura, 2009). There is thought to be two main mechanisms by which MP mediate intercellular signalling of biological information (Mause & Weber, 2010). Firstly, MP may act as circulating signalling modules that affect cellular properties by activation of receptors on the target cell via the presentation of membrane-associated, bioactive

molecules (Mause & Weber, 2010). Secondly, MP may mediate signalling by direct transformation of their content including proteins or RNA to the recipient cell, which possibly leads to cell activation and reprogramming of cell function (Mause & Weber, 2010). Interestingly, due to the fact that MP are found not only in the areas around activated parental cells but also readily circulate in the vasculature, both of the previous mechanisms not only allow signalling to neighbouring cells but also at remarkable distances from the donor cell origin (Mause & Weber, 2010). This allows for MP to communicate specific properties and information amongst cells rapidly and processes such as immune-regulation and the maintenance of homeostasis can be more efficiently managed ((Mause & Weber, 2010; Smalheiser, 2009).

2.2.3 Detection of MP

The most widely used approach for MP detection is flow cytometry (FC) (Piccin, Murphy & Smith, 2007; Ross, 2011). FC examines the fluorescent and light scattering properties of cells and particles in suspension when passed through a stream of fluid (Lacroix et al., 2010). A sample is analysed cytometrically where a laser detects immunofluorescence that is emitted by fluorescent tags on specific antibodies that are attached to antigens on the cells, or in this case MPs (Kim & Ligler, 2010). This procedure produces quantifiable phenomenon called side scatter (SSC) and forward scatter (FSC) (Abcam, *no date*). SS represents the granularity of the cells or particles, and FSC indicates the particle size (Abcam, *no date*). FC can be performed with a high number of events in a very short time period (Jy et al., 2004). There is however a lacking uniform method to enumerate EMP and there is an issue regarding EMP size as it is near the detection limit of most flow cytometers (Chandler, Yeung & Tait, 2011;

Lacroix et al., 2012). There is also no agreement between laboratories on the pre-analytical preparation of plasma samples, making comparisons between studies very difficult (Enjeti, Lincz, & Seldon, 2007; Jy et al., 2004; Lekakis et al., 2011; van lerssel et al., 2010). Several factors have actually been shown to affect the FC recovery of MP including centrifugation technique, storage time and temperature, needle bore size and anticoagulant used (Kim et al., 2002; Lekakis et al., 2011; Shah et al., 2008; van lerssel et al., 2010). A recent study aimed at analysing the impact of centrifugation and storage on EMP expression (van lerssel et al., 2010). CD31+(PECAM-1; Platelet endothelial cell adhesion molecule-1) CD42b-, CD62E+, CD144+ (VE-Cadherin) and CD144+CD105+ MP were assessed as specific EMP markers. Several different variables were altered during testing, such as storage methods and centrifugation, and the main finding from this study was that freezing at -80 °C increased CD62E+ and CD31+CD42b- MP counts and lowered CD144+ MP, whereas storage at 4ºC did not affect EMP quantification (van Ierssel et al., 2010). However, these results do point towards a change in EMP quantification as a result of altered pre analytical treatment of samples can affect MP quantification.

2.2.4 Endothelial microparticles (EMP)

MP originating from endothelial cells (EMP) may represent a small percentage of the overall pool of plasma MP, but it is becoming more and more apparent that circulating levels of EMP constitute an emerging surrogate marker of ED (Lekakis et al., 2011; Vince et al., 2009b). EMP result from the endothelial plasma membrane blebbing and cell surface protein expression allows for the detection of EMP using markers such as, CD105, CD106 and CD62E (Chironi et al., 2009; Shet, 2008) (Fig 2.3). EMP present a

whole host of diverse molecules on their surface, with functions encompassing coagulation, cell survival, inflammation, enhanced oxidative stress, adhesion, proteolysis, remodelling, angiogenesis and tumour growth (Dignat-George & Boulanger, 2011). It should be of great importance to develop strategies that combine multiple endothelial markers in order to exclude any possible contaminating subpopulations, thus leading to a more accurate assessment of the endothelial origin of MP in biological fluids (Dignat-George & Boulanger, 2011). The first study investigating the generation of EMP was carried out by Combes et al (1999) who used human umbilical vein endothelial cells (HUVECs) that were stimulated by TNF-α (Combes et al., 1999). This study included healthy individuals as well as patients with lupus anticoagulant who present a pro-thrombotic coagulation abnormality. Results showed that following TNF-stimulation, EMP were detectable in normal human blood, as well as an increase of EMP in patients with a coagulation abnormality (Combes et al., 1999). It is worthy to note that EMP can also be released through other non-apoptotic stimuli, such as with mediators like pro-inflammatory cytokines, activated platelets or oxidised low density lipoproteins postulated to promote EMP (Leroyer, Tedgui, & Boulanger, 2008). Once released into the circulation, EMP can then exert functional effects on target cells through surface membrane interactions (Curtis et al., 2013). The *in vivo* stimulation of EMP remains relatively untested and thus is an area that appeals to researchers and an aim of this thesis.

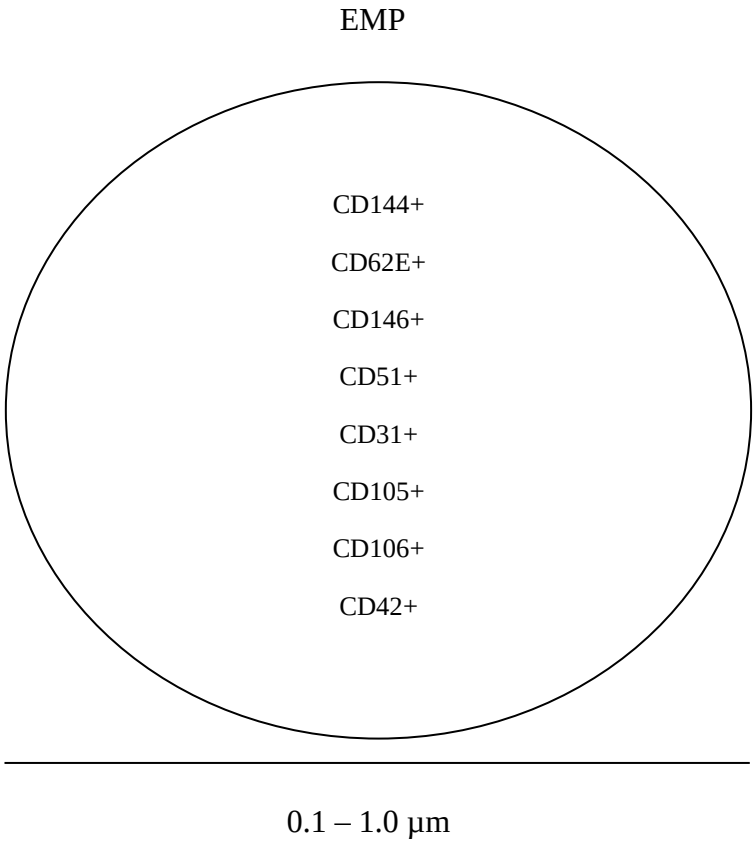


Fig 2.3 Phenotype of cellular biomarkers of endothelial health using EMP. CD144 (VE-Cadherin); CD62E; CD146 (melanoma cell adhesion molecule); CD51 (integrin-αV); CD31 (platelet endothelial cell adhesion molecule); CD105; CD106; CD42 (glycol-protein 1b). Representation adapted from (Chironi et al., 2009; Shet, 2008; Sossdorf et al., 2011).

2.2.5 EMP in healthy conditions

MP are not assessed as often in health compared with disease states, due to the common characteristics and related risk factors of ED in many diseases (Freyssinet, 2003). The few studies that have evaluated the half-life of MP suggest that they are cleared rapidly following introduction into the circulation (Flaumenhaft, 2006; Rand et al., 2006).

These findings suggest that MP would have to be constantly shed from their source into the circulation of healthy individuals in order to produce the concentrations seen of MP circulating in the plasma of health individuals (Italiano, Mairuhu, & Flaumenhaft, 2010). Detectable levels of MP originating from different cells, including endothelial cells, are known to circulate in the plasma of healthy subjects (Dignat-George & Boulanger, 2011; Freyssinet, 2003, Vince et al., 2009a;b). Homeostasis results from a balance between cell proliferation and degenerescence (Freyssinet et al., 1999). Cells undergo differentiation, they expand, fulfil particular functions and then follow a process of programmed death and are finally cleared by phagocytosis (Hugel et al., 2005). It is when the cell is subjected to a variety of stimuli at each stage of its life that the release of MP occurs (Hugel et al., 2005). Vessel homeostasis is ensured in normal conditions, assuming one is 'healthy', through anti-inflammatory, anti-thrombotic and anti-atherogenic properties of the intact endothelial cell monolayer (Chironi et al., 2009). The presence of EMP in plasma reflects an active balance between EMP generation and clearance, but these respective offerings to the circulating levels have not been extensively looked at, perhaps down to the lack of knowledge regarding MP clearance (Dignat-George & Boulanger, 2011; Piccin, Murphy & Smith, 2007). EMP and their changing levels may carry important information in healthy participants as well as those with pathological disorders (Chironi et al., 2009), and warrants further investigation.

2.2.6 CD106

CD106 (commonly referred to as VCAM-1) is a 90-110 kDa trans-membrane glycoprotein that is a member of the immuno-globulin super-family and it is expressed

on activated endothelial cells (Carlos et al., 1990). CD106 has a role of regulating the adhesion of monocytes, lymphocytes, and eosinophils to endothelial cells activated by lipopolysaccharide, interleukin-1 (IL-1) or TNF-α (Golias et al., 2007). The expression of CD106 has been shown to be promoted by cytokines produced in the tissue such as inflammatory molecules TNF-α, shear stress, high levels of reactive oxygen species (ROS) and high glucose (Carter & Wicks, 2001; Kato et al., 2005). CD106 has been advocated in the pathogenesis of various diseases, in atherosclerosis for example, CD106 is the first adhesion molecule to be expressed before the atherosclerotic plaque development (Iiyama et al., 1999).

CD106 is known to be in the plasma in a soluble form (sCD106) and is used as a predictor of diseases, and levels of sCD106 have been found to increase with activation of the endothelium in diseases such as T1DM (Clausen et al., 2000) and atherosclerosis (Peter et al., 1999). CD106 has been commonly used as a marker of ED, particularly in healthy individuals when subjected to stress through exercise (Table 2.2). CD106 tends to be used most exclusively as a marker of ED in exercise trials through its soluble form, but there are instances of its expression as CD106+ MP across exercise trials (Chaar et al., 2011; Vince et al., 2009a;b).

2.2.7 CD105

CD105 (commonly referred to as endoglin) is a 180 kDa transmembrane glycoprotein which is constitutively phosphorylated, with a marked tissue specificity (Blasquez-Medela et al., 2010; Burrows et al., 1995). Supporting this concept, CD105 is

predominantly expressed on activated endothelial cells (Miller et al., 1999) and elevated levels of CD105 expression have been detected on human micro-vascular endothelium (Wong et al., 2000) as well as on vascular endothelial cells in tissues that are undergoing active angiogenesis (Miller et al., 1999). In fact, the importance of the role of CD105 as a promoter of angiogenesis can be characterised by various studies showing that mice lacking CD105 die during embryonic development because of defective angiogenesis (Arthur et al., 2000; Bourdeau, Dumont, & Letarte, 1999; Li et al., 1999). CD105 expression is primarily restricted to cells in the vessel wall, including endothelial cells and monocytes, as well as on mesenchymal tissue from the endocardium, including fibroblasts and vascular smooth muscle cells (Bernabeau, Conley & Vary, 2007; Bourdeau, Dumont & Letarte, 1999; Kapur, Morine, & Letarte, 2013).

It has previously been shown that CD105 plays a role in many pathological processes, such as cancer angiogenesis (Perez-Gomez et al., 2010), as well as metabolic and CVD, including preeclampsia (De Vivo et al., 2008), hypertension and T2DM (Blazquez-Medela et al., 2010; Lopez-Novoa & Bernabeu, 2010). Interestingly, CD105 expression has been increased in vessels during certain pathological situations, such as hypoxia or vascular injury (Chrismas et al., 2010; Madden et al., 2010; Nachtigal et al., 2012). Recently, using a simulated dive in 5 healthy males, it was shown that CD105+ MP were seen to increase post-decompression after breathing in air compared to those breathing in oxygen (O_2) (Madden et al., 2010). Considering that CD105 is a constitutive endothelial marker, and the observation that CD105+ MP only altered in their quantified number, this finding suggests that the increase post-decompression is due to endothelial damage and not a physiological change resulting in MP release (Madden et al., 2010). Another recent study (Chrismas et al., 2010) looked at diving in

5 healthy males, where subjects performed both a control dive and one with an anti-oxidant (AOX) pill separated by 2 weeks. It was found that CD105+ MP were significantly increased from pre dive and at depth to immediately post dive in the control group. This study showed that CD105+ MP release may be attenuated by AOX administration, and further strengthened the notion that a significant rise in CD105+ MP post-dive in controls is associated with a decline in EF.

2.8 Exercise and EF

Considering that this thesis wishes to assess the endothelial response to exercise, particularly on the EMP response, it is important to review the literature on existing data in this field. Table 2 consists of a summary of the main studies that have been conducted with respect to exercise trials that have measured EMP and/or circulating markers of EF, concentrating on acute bouts of exercise in healthy individuals, considering that this is a fundamental aim for the forthcoming experimental chapters. A range of studies have assessed the concentrations of CD105+ MP (Chrismas et al., 2010; Madden et al., 2010) and CD106+ MP (Vince et al., 2009b) following a series of simulated SCUBA dives, proposed to induce ED. Significant increases in both EMP markers was observed in all 3 of the aforementioned studies post dive in specific groups. Vince et al (2009b) found that CD106+ MP was significantly elevated 1 hr post air dive conmpared to the control (breathing O_2). In comparison to these findings, another marker of EF, CD105+ MP also increased post SCUBA dive in two separate studies, again in healthy males participants, suggesting a reliable use of CD105+ MP and CD106+ MP across studies using similar populations (Chrismas et al., 2010; Madden et al., 2010). A recent study looked at assessing the MP response to acute

exercise following strenuous cycling exercise (Chaar et al., 2011). Although this group performed 3 successive maximal exercise tests in healthy male participants, they failed to detect any changes in MP production from endothelial cells as characterised by undetectable levels of CD106+ MP. Additionally to this, plasma levels of sCD106 were unchanged as a result of exercise. In contrast to these findings, exercise was sufficient enough to see significant increases in CD62E+ MP as assessed by FC, in healthy male participants who conducted a steady state (80% of their individual anaerobic threshold; IAT) 90 min cycling exercise (Sossdorf et al., 2011). This study speculated that shear stress mechanisms played an important role in the release of MP, as assessed by blood pressure (BP) and HR throughout exercise. This speculation is consistent with others recently (Jee & Jin, 2012) who have also found a correlation with running speed and the release of sCD106, suggesting an intensity dependent mechanism in the release of endothelial adhesion molecules. Jee and Jin (2012) assessed 24 male marathon runners, who had a mean age of 49.5 years which is a lot higher than other studies, as studies in this area tended to use younger participants in their 20's (Chrismas et al., 2010; Sossdorf et al., 2010; 2011; Vince et al., 2009b). Recently, Jenkins et al (2013) showed *in vivo* that disturbed blood flow acutely induces endothelial activation and apoptosis, as reflected by an increase in the release of activated (CD62E) and apoptotic (CD31+/CD42b) EMP measured by FC. Ten healthy male participants were studied and cuff inflation occurred on the one arm, while the other arm was used as a control arm for a period of 20 min. Relative to baseline, CD62E+ MP increased by approximately 3 fold at 10 min, and approximately 4 fold at 20 min in the experimental arm. CD31+/CD42b- + MP were elevated almost 9 fold compared to rest (0 min) at the 20 min time point. Further to this, there was no change in either of the EMP concentrations in the contralateral arm exposed to normal resting blood flow. This study provides data

of an *in vivo* experiment of disturbed blood flow inducing endothelial damage in healthy humans.

The studies discussed thus far in this section have been acute exercise lasting no more than 90 min in duration and have been high intensity in nature (Sossdorf et al., 2011). The 'spartathlon' is a gruelling, 246 km foot race, and it was during this event that Bartzeliotou et al (2007) investigated the levels of adhesion molecules. Soluble forms of CD106 and CD62E were assessed and both sCD106 and sCD62E were significantly greater at the end of this 246 km race, with levels returning to normal 48 hr after the end of the race (Bartzeliotou et al., 2007). Similarly to this study, Jee and Jin (2012) assessed 24 males participating in a 308 km ultra-marathon. This exercise was evidently longer in duration that Bartzeliotou et al (2007), but this study incorporated blood sampling during exercise itself. Subsequently, significant increases of sCD106 and sCD62E were observed at 100 km. intriguingly, sCD106 was correlated with running speed, suggesting an intensity dependent relationship with endothelial molecules. Additionally, Jee and Jin (2012) assessed 24 male marathon runners, who had a mean age of 49.5 years which is a lot higher than other studies, as studies in this area tended to use younger participants in their 20's (Chrismas et al., 2010; Sossdorf et al., 2010; 2011; Vince et al., 2009b). the studies discussed during this section indicate that the endothelium is highly affected by exercise of a strenuous or prolonged nature, and this is finding backed up by studies using different assessment techniques in healthy participants such as FMD (Hwang et al., 2012; Ramirez-Velez, Aguilar & Ramirez, 2010).

The reasons for such correlating relationship between the endothelial adhesion molecules and exercise intensity are speculated to be multifactorial. Although exercise related stress has been shown to build a protection against diseases such as atherosclerosis, stress from exercise can lead to negative consequences (Marsh & Coombes, 2005), such as increased heat production, HR, leukocyte count, shear stress along with stress hormones for the release of leukocytes and systemic inflammation (Suzuki et al., 1999). Shear stress during exercise is increased due to the increased O_2 demand in working muscles and with it an increase in haemodynamic values such as BP and cardiac output (Jee & Jin, 2012). An increase in exercise intensity also stimulates an increase in resistance or laminar shear force exerted by circulating blood upon the blood vessel walls (Marsh & Coombes, 2005; Stewart et al., 2004), which has been speculated to increase the activation of endothelial adhesion molecules and thus may be the reason for the increases in EMP witnessed post strenuous exercise (Chaar et al., 2011; Sossdorf et al., 2011; Vince et al., 209b).

This section highlights that there is a sufficient lack of consistent data available in this area, but it is one of high promise. The possibility of shear stress and exercise intensity affecting levels of + EMP seems plausible and requires greater understanding. Additionally, more studies using EMP as markers of EF after acute exercise are needed in this area, and so this thesis aims to add to existing literature and strengthen the use of CD105+ MP and CD106+ MP.

Table 2.2 Summary of exercise trials measuring EMP as a marker of EF

Author	Sample size	Subject characteristics	Exercise protocol	Endothelial function measures (detection)	Findings	Limitations
Jenkins et al (2013)	10 healthy males	Means ± SD; 29 ± 1 years 176 ± 1 cm 83 ± 2 kg	2 pneumatic cuffs on experimental arm (a-distal cuff inflated 220mmHg;b-proximal cuff inflated to 40 mmHg) Controls – a-simultaneous blood flow; b-no cuffs	Plasma EMP (CD62E, CD31+/CD42b-) by FC	CD62E ↑ at 10 and 20 min vs baseline in experimental arm. CD31+/CD42b- ↑ at 20 min vs rest. No changes in controls	-small sample size -brachial artery FMD to compare would have been useful - correlation
Jee & Jin, (2012)	24 healthy males	Means; 49.5 years 168 cm 65.5 kg	308 km ultra marathon	Plasma TNF-α, sCD106, sCD62E (ELISA)	↑ sCD106, CD62E, Plasma TNF-α sCD106 correlated with exercise intensity	-continuous measures of haemodynamic values
(Chaar et al., 2011)	7 healthy males	Mean ± SD; 19.8 ± 0.4 years 178.9 ± 2.0 cm 74.9 ± 1.9 kg	3 maximal ramp tests interspersed with 10 min recovery on cycle ergometer – fixed at 70 RPM	Plasma for assays of sCD106, sICAM-1, sL-selectin and sCD62P (ELISA). CD106+ MP (FC)	Undetectable quantity of EMP	-pre-analytical methodology-enumerated MPs that were positive for annexin V staining -small sample size -continuous measures of haemodynamic values -small sample size
(Sossdorf et al., 2011)	16 males (8 trained, 8 untrained)	Untrained; 24.3 ± 3.4 years 47.6 ± 3.9	1 to 2 weeks before single exercise test, VO_{2peak} determined on cycle ergometer;	Plasma for assays of CD62E (FC)	Exercise ↑ HR, plasma lactate, SBP & DBP in both groups	-pre-analytical methodology (thawing, annexin V used)

		relative VO_{2max} Trained; 25.8 ± 3.3 years 68.1 ± 4.5 relative VO_{2max}	Test consisted of cycle ergometer at 80% IAT for 90 min		EMP ↑ 45-min after exercise from rest values in trained only	
(Madden et al., 2010)	5 healthy males	n/a	Dive breathing compressed air (283 kPa; 18 msw) for 60 min bottom time; 1 week later same dive but breathing 100% O_2	EndoPAT and plasma CD105 (FC), CD62E & CD62P (ELISA)	CD105+ MP ↑ post dive breathing air, no significance breathing O_2	-small sample size
(Harrison et al., 2009)	8 recreationally active males	26.9 ± 4.1 years 46.8 ± 4.9 VO_{2peak} 26.0 ± 3.6 BMI	Each subject completed both trials in randomised order. Evening prior to OFTT, either rested at home or prolonged exercise (70RPM for 90-min at 70% VO_{2peak}then 10x1min sprints with 1-min recovery.	Plasma for assays of CD31+/42b- for EMP (FC). Serum for assays of sCD106 and sICAM-1 (sandwich ELISA)	EMP (CD31+/42b-) ↑ postprandially in both groups, no difference between groups. No change in sCD106 or sICAM-1	-pre analytical methodology (frozen, ELISA) -relatively small sample size
(Vince et al., 2009b)	6 healthy males	n/a	Simulated dive of 78 min bottom time at 2.8 ATA breathing a) compressed air or b) 100% O_2 - 1 week later simulated dive breathing air, 1 week later breathing O_2	Plasma used with CD106 (FC)	CD106 ↑ post compressed air dive, no change breathing O_2 CD106 positively correlated with TBARS	-relatively small sample size

(Vince et al., 2009a)	8 healthy males	18-24 years	Breathing hypoxic air (15% O_2, air mix) for 80 min	Plasma for assay of CD106 (FC)	CD106 ↑ post-hypoxic breathing, remained significantly higher 5 hr after breathing began	-relatively small sample size
(Bartzeliotou et al., 2007)	20 healthy males	Median age 43.2 years	"Spartathlon" race – 246 km continuous exercise	Plasma for assays of s-CD62E-, sCD62P-. selectins (ELISA), sICAM-1, sCD106 (multiplex assay), sTM (ELISA)	↑ sCD106, sICAM-1, sCD62E, s-TM post race.	-not most specific EF markers used

ATA = atmosphere; DBP = diastolic blood pressure; FC = flow cytometry; IAT = individual anaerobic threshold; OFTT = oral fat tolerance tests; SBP = systolic blood pressure; sCD106 = soluble vascular cell adhesion molecule; sCD62E = soluble E-selectin; sCD62P = soluble P-selectin; sICAM-1 = soluble intra cellular adhesion molecule; sTM = soluble thrombomodulin; TBARS = thiobarbituric acid reactivesubstances.

2.9 Polycystic ovary syndrome

2.9.1 Overview

Initial descriptions of PCOS date back to the 18th (Vallisneri, 1971) and 19th centuries (Chereau, 1844) respectively. Later, the breakthrough in PCOS research was investigated by Stein and Leventhal (1935), who were the first to report the link between bilateral polycystic ovaries and clinical symptoms resulting in PCOS being known as a multifaceted metabolic disease (Ehrmann, 2005; Lobo & Carmina, 2000; Orio, Palomba, & Colao, 2006) defined by the presence of clinical or biochemical indicators of androgen excess (e.g. elevated testosterone or hirsutism), menstrual irregularity, and polycystic ovaries (Fig 2.4) (Ehrmann, 2005). PCOS gained attention in research following this discovery, and in 1980, an association between PCOS and IR was found (Burghen, Givens & Kitabchi, 1980). Fasting plasma insulin, androstenedione and testosterone were found to be higher in obese PCOS women compared to obese controls without the condition. Additionally, hyperinsulinaemia is well correlated with hyperandrogenism (Burghen, Givens & Kitabchi, 1980).

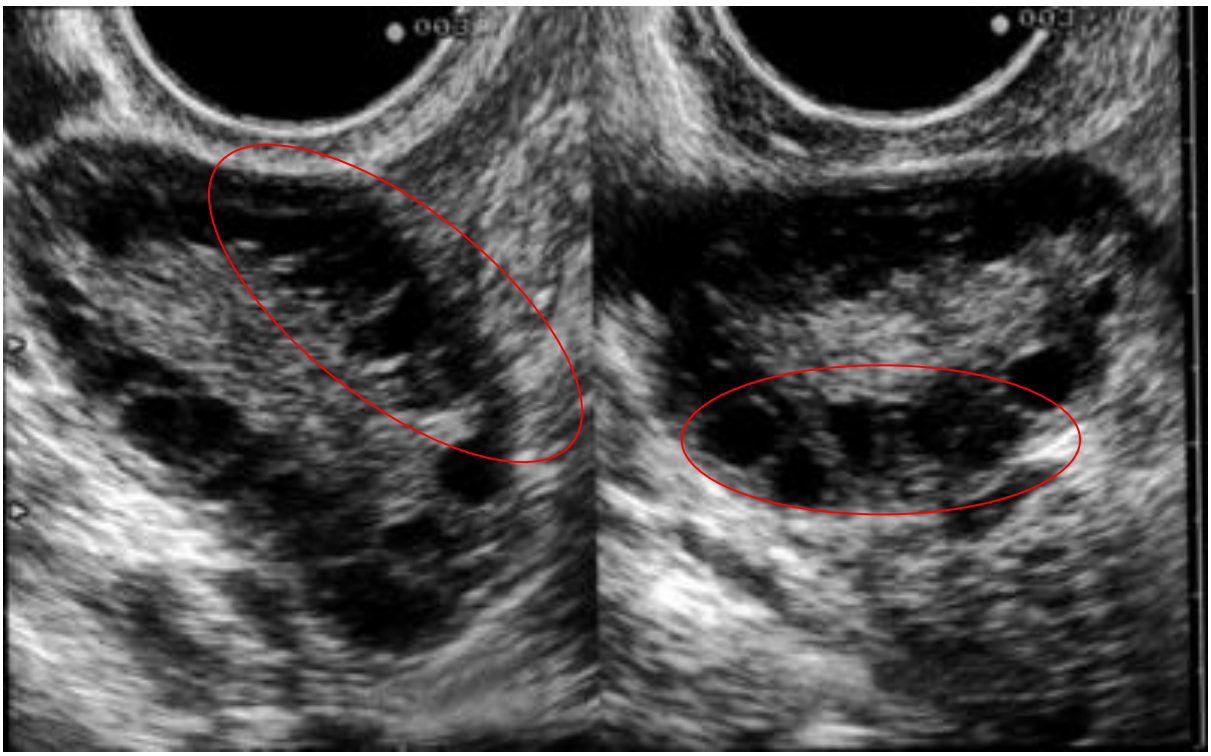


Fig 2.4 Polycystic ovaries (B mode ultrasound, transvaginal route) as assessed on ultrasound in both the left andright ovaries. Red outline highlights the ovarian cysts. Adapted from (Balen et al., 2003).

2.9.2 Diagnosis of PCOS

Initially, the diagnosis of PCOS was provided by the National Institute of Health (NIH) in 1990, which set out the following diagnostic criteria in order of importance: 1) hyperandrogenism and/or hyperandrogenaemia, 2) oligomenorrhoea and/or anovulation and 3) exclusion of other endocrine disorders, such as Cushing's syndrome, hyperprolactinaemia and congenital adrenal hyperplasia (Rotterdam, 2004). Then, in

2003, PCOS experts in a PCOS consensus workshop reassessed this previous criterion, and this was known as the Rotterdam Criteria from 2003 (Rotterdam, 2004). This criteria state that PCOS can be diagnosed after the exclusion of related disorders by the presence of two out of the following features: 1) oligomenorrhoea or anovulation 2) clinical and/or biochemical hyperandrogenism and 3) polycystic ovaries. When looking at the original criteria (NIH, 1990), two new phenotypes have been added, one with ovulatory dysfunction and polycystic ovaries with no hyperandrogenism, and the other one with hyperandrogenism and polycystic ovaries but no ovulatory dysfunction. There have been debates on the correct use of criteria, highlighting strengths and weaknesses of each, but it should be noted that the Rotterdam criteria was used for this thesis in the recruitment of PCOS women in chapter 8.

2.9.3 Prevalence of PCOS

PCOS is known to be the most common endocrine disorder affecting reproductive women (Azziz et al., 2005; Diamanti-Kandarakis, 2008; Dunaif, 1997; Goodarzi et al., 2011; March et al., 2010; Moro et al., 2009; Norman et al., 2007), with a prevalence of 6-10% reported when applying the NIH criteria, but this figure may increase up to as much as 18% using the Rotterdam criteria (Harrison et al., 2011; March et al., 2010; Rotterdam, 2004).

2.9.4 Pathogenesis of PCOS

The primary etiology of PCOS itself remains unclear, with several key factors postulated to contribute to its existence (Diamanti-Kandarakis, Christakou, & Markinakis, 2012). Both environmental and genetic factors have been implicated (Diamanti-Kandarakis, Christakou, & Markinakis, 2012), but the exact mechanism has not yet been studied intensively. Polycystic ovaries develop when the ovaries are stimulated to produce excessive amounts of male hormones (androgens), mainly testosterone, either through the release of excessive luteinising hormone (LH) by the anterior pituitary gland or through high levels of insulin in the blood (hyperinsulinaemia) in women whose ovaries are sensitive to this stimulus (Phipps, 2001). Although androgen excess is considered by some to be the integral cause of PCOS, only 80-85% of women who present with clinical hyperandrogenism have PCOS (Azziz et al., 2009). During regular ovarian follicular development, primordial follicles are recruited into a group of follicles that are growing, and from this group, one antral follicle is selected to go on and ovulate (Goodarzi et al., 2011). This process requires coordinated reproductive, metabolic and intra-ovarian interactions, which can be disrupted in PCOS women. This disruption occurs due to the characteristics seen in PCOS, namely ovarian hyperandrogenism, hyperinsulinaemia from IR, and altered intra-ovarian paracrine signalling, thus affecting follicle growth, leading to polycystic morphology (Goodarzi et al., 2011; Rotterdam, 2004). Existing evidence suggests that a primary abnormality of the ovarian theca cells exists, which are the major source of androgen production and secretion (GillingSmith et al., 1997) thus being impaired and play a key role in the etiology. Importantly, lifestyle is known to be a key part of the expression of PCOS. An increase in weight gain worsens the metabolic and reproductive abnormalities seen in PCOS, evidenced by increased IR and abdominal

obesity, menstrual irregularity and hyperandrogenism in women with the most severe of PCOS forms (Carmina et al., 2005).

2.9.5 Abnormalities associated with PCOS

There is a metabolic-hormonal complexity that is very apparent in PCOS and represents an interesting relationship between hormone pattern and cardiovascular risk profile (Giallauria et al., 2008a; Hoffman & Ehrmann, 2008). Even at an early age, IR (Dunaif et al., 1989; Ehrmann, 2005), impaired glucose metabolism (Legro et al., 1999), hypertension (Chen et al., 2007), low-grade chronic inflammation (Kelly et al., 2001) and dyslipidaemia are more common in PCOS, even after adjustment for obesity (Sathyapalan & Atkin, 2012). It should also be noted that metabolic syndrome (MS) plays a key role and should be considered in the evaluation and treatment of PCOS (Dokras et al., 2005). MS may be defined as the presence of at least 3 of the following 5 conditions: abdominal obesity, defined by a WC of > 88 cm; serum triglycerides (TG) 150 mg/dL or greater; serum high density lipoprotein cholesterol (HDL-C) < 50 mg/dL; SBP/DBP of 130/85 mmHg or greater and serum fasting glucose 110 mg/dL or more ("Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report," 2002). Women with PCOS clearly have an increased risk of both impaired glucose tolerance and T2DM (Sathyapalan & Atkin, 2012), and it has been reported that this risk is 3-7 fold higher overall, and 2-fold higher compared with age and BMI matched women with normal cycles (Randeva et al., 2012). IR is proposed to be a key pathophysiological feature of PCOS, contributing to both reproductive and metabolic disturbances (Randeva et al., 2012). Furthermore, there is a

known increased prevalence of mental health problems associated with PCOS, such as depression, anxiety, binge eating disorders and low quality of life (Himelein & Thatcher, 2006; Kerschner et al., 2009). A systematic review in 2012 expressed these findings, with results indicating that the prevalence of anxiety symptoms is significantly greater in PCOS women (20.4%) compared to controls (3.9%) (Dokras et al., 2012).

2.9.6 Obesity and PCOS

Obesity has increasingly become a worldwide public health concern of epidemic proportions (Esposito et al., 2006). Obesity is associated with IR, glucose intolerance and abnormal concentrations of lipids/lipoproteins which can lead to development of T2DM (Chung et al., 2008). Although obesity prevalence in women with and without PCOS has certainly increased in the last few decades, obesity *per se* may not be intrinsic to PCOS, given that the degree of IR in PCOS is greater than that predicted by the BMI, although it must be recognised that 40–50% of women with PCOS are not obese (Azziz et al., 2009). Even though considerable evidence exists that suggests obesity plays a negative role in the pathophysiology of PCOS (Diamanti-Kandarakis, 2008; Goodarzi et al., 2003; Yildiz, Knochenhauer, & Azziz, 2008) it remains unclear if women with PCOS have a predisposition to obesity, or if obesity is a secondary factor that worsens the condition (Hoeger & Oberfield, 2012). The difficulty with making the relative contribution of obesity in PCOS is that obesity is not a defining feature of the syndrome, due to the fact that PCOS is seen in both normal weight and obese women (Ketel et al., 2008). However, there is tangible evidence that obesity, in particularly abdominal obesity, worsens both the clinical and endocrine features of the syndrome (Esposito et al., 2006; Sam, 2007). When compared with weight-matched healthy

women, women with PCOS have a similar amount of total and trunk fat, but they have a higher quantity of central abdominal fat (Carmina et al., 2007). This central fat is associated with an increase in low-grade chronic inflammation and IR (Carmina et al., 2007) and with a metabolic dysfunction in women with PCOS (Lord et al., 2006). Excess visceral fat (VF) appears predictive not only of the MS, but also of CVD (Grundy, 2002; Sowers, 1998). An increased quantity of VF has been found in PCOS women and with it comes several complications such as EF and premature atherosclerosis (Liu et al., 2005). The latter showed that a correlation exists between VF and early signs of vascular damage and reduced FMD (Liu et al., 2005). Yildirim, Sabir & Kaleli (2003) actually found that non obese women with PCOS had an increased intra-abdominal fat accumulation that may contribute to the development of the associated disorders (Yildirim, Sabir, & Kaleli, 2003). It was therefore the idea of this thesis to test women from a range of BMI categories in order to test across the spectrum of the disorder and to see how exercise would affect PCOS women that were lean and obese.

2.9.7 ED in PCOS

Women with PCOS also present with ED (Carmina et al., 2006; Diamanti-Kandarakis et al., 2005; Paradisi et al., 2001). This is not surprising given that ED is strongly related to IR (Kravariti et al., 2005; Moran et al., 2011; Tarkun et al., 2004) and hyperandrogenism (Paradisi et al., 2001), both defining features of PCOS. Lowenstein et al (2007) measured EF by the EndoPAT-2000 device by recording the finger arterial pulse wave amplitude (PWA) to detect the PAT in 31 women with PCOS and 33 healthy controls without PCOS (Lowenstein et al., 2007). This study found that PCOS

women had a significantly lower PAT hyperaemia ratio than controls did, which implies that EF is adversely affected in women with PCOS. Therefore, the finding from this study that women with PCOS suffer from ED, regardless of the mechanism, suggests that these patients are at an increased risk for early onset of CVD (Orio et al., 2004; Orio, Palomba, & Colao, 2006). The effects of PCOS on brachial artery FMD (BA-FMD) is not consistent between studies, and BA-FMD has been shown to be lower (Meyer, McGrath & Teede, 2005; Tarkun et al., 2004), but also it has been shown that there were no changes compared to controls (Brinkworth et al., 2006; Mather et al., 2000). A recent meta-analysis consisting of 21 published studies (PCOS n= 908; controls n = 566), looked at the assessment of FMD in patients with and without PCOS and found that there was a reduction in FMD in PCOS than in controls, but with high heterogeneity between studies (Sprung et al., 2013b).

2.9.8 Exercise intervention on EF in PCOS

Despite the well-established positive effects of weight loss and exercise on IR, hyperandrogenism and CVD risk markers in overweight women with PCOS (Giallauria et al., 2008a; Moran et al., 2003; Palomba et al., 2010; Thomson et al., 2008; Vigorito et al., 2007) there are limited data on the effect of exercise and/or diet on EF in women with PCOS. Recently, Thomson et al (2012) assessed the effects of exercise and lifestyle intervention on soluble endothelial adhesion molecules (sCD106, sICAM-1 and PAI-1; plasminogen activator inhibitor-1). This study actually used the frozen serum samples from a previous study (Thomson et al., 2010). This study randomly divided PCOS women into 1of 3 groups. One group received a structured diet only (DO, approximately 6000 kJ/day energy restricted high protein meal plan (30% protein, 40%

as CHO and 30% fat), one group received the same diet but with the additional effects of an aerobic exercise (DA, the above diet and 5 walking sessions/week) and diet and combined aerobic-resistance exercise (DC, the above diet and 3 walking and 2 strength training sessions/week). The intensity for aerobic exercise was progressed from 60-65% HR_{max} to 45 min at 75-80% HR_{max}. Results showed a significant improvement for all 3 groups in the previously mentioned soluble adhesion markers. This present study interestingly showed no differences between the groups, but it should be noted that greater reductions in sCD106 appear to have occurred in the exercising groups compared to the diet only group, suggesting greater benefits with exercise, although this was not significant. Additionally, this trend was shown with significantly greater improvements in cardiorespiratory fitness and total body fat mass in the exercise groups compared to the diet only group, suggesting added benefits of exercise in a cohort of at risk participants. Recently, Sprung et al (2013a) conducted a study that was highly novel, aiming to assess EF in women with PCOS after a 16 week exercise intervention. EF was assessed using BA-FMD in women with PCOS. Ten PCOS women underwent supervised 30 min exercise 3 times a week at 30% heart rate reserve (HRR), which progressed weekly based on HR responses (up to 60% HRR). Acting as the control group, fellow women with PCOS received conventional care only, consisting of lifestyle advice from clinical consultations prompting them to lose weight and increase their physical activity levels. Various other assessments were measured during the study, including cardiorespiratory fitness, visceral and abdominal subcutaneous adipose tissue, glycaemic control, and lipid and hormone profiles. When comparing the 2 groups after the 16 week intervention, BA-FMD and cardiorespiratory fitness improved from pre to post exercise intervention in the exercising group only, suggesting that supervised aerobic exercise in women with PCOS can enhance EF. This study suggests that EF may be improved in this population, and considering the multifactorial issues present in

PCOS, it seems fit to promote exercise. In a recent review, Harrison et al (2011) highlighted just how limited exercise interventions are in PCOS, and were able to pinpoint areas of concerns in the study designs of previous studies. A total of 8 manuscripts were identified, 5 of these were randomised controlled trials, and 3 were cohort studies. Compliance was a huge concern in these studies, as it was found that dropout rates were as high as 40-45% across 3 studies (Brown et al., 2009; Randeva et al., 2002; Thomson et al., 2008). The major influence in high dropout were those of longer study duration (20-24 weeks) and greater exercise sessions per week (4 to 7) compared to other studies. This may have important implications, as various benefits have been seen in relatively short duration studies, such as Sprung et al (2013a) who only exercised for 16 weeks, 3 times a week. Furthermore, Vigorito et al (2007) exercised PCOS women for just 12 weeks compared with a control group of PCOS women. Significant improvements in cardiorespiratory fitness, BMI, CRP and insulin sensitivity were witnessed after just 3 months of cycling 30 min x 3 times/week (60-70% VO_{2max}) compared with the control group.

PCOS is a prevalent disorder, underpinned by weight independent IR, coupled with reproductive and CV issues (Harrison et al., 2011). Exercise does hold important benefits for women with PCOS, such as BMI (Giallauria et al., 2008b; Stener-Victorin et al., 2009), cardiorespiratory fitness (Vigorito et al., 2007) and metabolic and hormone profiles (Palomba et al., 2008; Vigorito et al., 2007). Lifestyle modification, including exercise, is the first line therapeutic approach to the treatment of PCOS (Wild et al., 2010), but little data exists still, suggesting that more research is required in this area.

Chapter 3: General Methodologies

3.1 Recruitment and screening of participants

The Department of Sport, Health and Exercise Science research ethics committee at the University of Hull approved all research protocols. All of the participants were recruited through advertisement at the University, through local hospital staff and word of mouth, as well as volunteers from staff and student email services at the University of Hull. For the PCOS experimental chapter (chapter 8), PCOS patients were also recruited through newspaper and e-bulletin advertisements and letters to the patients with the condition who were seen at Hull and East Yorkshire Diabetes, Endocrinology and Metabolism Clinic in the past 5 years. All of the participants recruited for this thesis were aged between 18 and 40 years of age. Any volunteer suffering from an existing medical condition that was defined as a contraindication to exercise or injury was excluded from the test, as was any volunteer with a history of cardiovascular complaints.

At each initial visit, participants were screened via a pre-exercise medical questionnaire to highlight any contraindications to the test protocol and written informed consent was provided following successful completion of the questionnaire. Prior to any participation in exercise for any of the same day studies (chapters 4, 5, 6 and 7), each participant was asked to refrain from any unaccustomed heavy exercise and alcohol during the preceding 48 hr and, to consume only water on the day of testing. During exercise session visits in chapter 8, fluid intake was permitted *ad libitum*. All trials involving blood sampling were performed with the participant in a fasted state from the night before, which began from the last meal the night before or 2200 hr, depending on which came first.

3.1.1 Data storage

All data was stored anonymously by using the participants' initials and a personal identification number that only the author was knowledgeable about. Electronic participant information, including data and documentation was kept on a computer with a University secured network that was password protected by the author. Study documents (paper) were retained in a secure (locked when not in use) place in the University of Hull that had no access by the public. Any remaining plasma samples were stored at -80 ºC Revco Ultima II freezer (Kendro Laboratory Products, Bishops Stortford, UK) in the Department of Sport, Health and Exercise Science at the University of Hull that had no access to the public.

3.2 Anthropometric measurements

3.2.1 Blood pressure

SBP and diastolic blood pressures (DBP) were assessed using an Omron M6 Upper Arm Blood Pressure Monitor (Omron Healthcare UK LTD, Milton Keynes, UK). Participants were stationed on an adjustable chair so that their feet lay flat to the floor, and sat to rest in a quiet room for 5 min. They were then instructed to rest their left arm on the surface of the table with their palms facing upwards. The left arm was positioned as closely as possible to heart height using the adjustable chair if needed. This procedure was then repeated leaving a few min between each measurement until two SBP readings fell within 5 mmHg of each other, and the mean of these results were

used. A maximum number of 3 readings were taken to avoid discomfort for patients. Should the mean SBP reading be recorded above 150 mmHg, then the participant would be excluded from taking part in any exercise in coherence with ethical procedures.

3.2.2 Height

Participants' height was measured in centimetres (cm) to one decimal place. Participants were asked to remove their footwear and stand upright with their backs positioned against a Holtain stadiometer (Holtain Ltd, Crymych, Dyfed). Participants were instructed to place their heels together, and have heels, buttocks, and shoulders pressed against the stadiometer. Arms were hung freely with their palms facing their thighs. The measurement was taken following a deep inhalation while standing tall, looking straight ahead with the head upright and not tilted backwards.

3.2.3 Body mass

Body mass was recorded in kilograms (kg) to one decimal place and was measured using a set of SECA 635 digital scales (SECA, Birmingham, UK). Participants were provided with changing facilities and asked to change into light clothing that they would later be exercising in. All jewellery, belongings from any pockets and footwear were removed prior to measurement, and participants were then informed to step on to the centre of the digital scales and stand erect until a reading was recorded.

3.2.4 Manual waist to hip measurements

Participants in chapters 7 and 8 had their manual waist to hip measurements taken at each assessment visit. Waist circumference (WC) was taken using a SECA 201 measuring tape and was defined as the level of the trunk where the girth was minimal. This is where there was a noticeable indentation of the trunk when viewed from the front. If there was no such obvious indentation, then the measurement was made at the level that was midway between the lowest rib (laterally) and the ilio axilla landmark (Marfell-Jones et al., 2006). The measurement was performed with the required area of skin accessible with permission from the patient. The hip circumference (HC) was made at the level where there was greatest protuberance of the gluteals. The participant was stood with both feet together for this whole process. In order to calculate the waist hip ratio (WHR), the WC was divided by the HC.

3.3 Peak power output (PPO) test

The exercise protocol in chapters 4, 5 and 6 were individually prescribed for each participant in order to control for the workload between experimental conditions. Each participant then worked at a set percentage of their maximum as determined in the PPO test. For chapters 4, 5 and 6 a PPO test was conducted in visit 1 on a mechanically braked cycle ergometer (Lode Sport Excalibur, Netherlands). The PPO test consisted of a 5 min warm-up at 50 Watts (W) followed by a ramp protocol, whereby workload increased at a rate of 30 $W \cdot min^{-1}$ until participants felt they could no longer persist with

the test. Participants were instructed to pedal at a comfortable frequency above a minimum of 60 revolutions per minute (RPM) throughout the test. Participants were then given a warning once this frequency first fell below 60 RPM and the test was terminated when RPM fell below 50. Participants were verbally encouraged throughout the test to provide maximal effort until they could no longer continue, and were told to remain seated at all times. Water was provided *ad libitum* throughout all exercise procedures where possible and was completely dependent upon the participants' individual preference. Additionally, during the repeated sprint cycling protocol, participants were also asked to rate themselves on their perceived readiness ratings (PRR) (Karu et al., 2000) prior to each sprint using a scale from 5 to 1 (where 5 relates to completely ready to begin, and 1 is not at all ready to begin).

3.4 Sub maximal exercise test

For experimental chapter 7, a sub-maximal exercise test was administered in order to prescribe the correct exercise intensity for subsequent sessions. Once appropriate screening had taken place and eligibility for the exercise programme had been confirmed, participants then performed a ramped sub-maximal exercise test ($VO_{2submax}$). The test was performed on a Woodway ELG55 motorised treadmill (Woodway, Weil an rhein, Germany) set at 0.1% gradient throughout the test, which began at a speed of 4.5 $km \cdot hr^{-1}$ for 3.5 min. Gas collection was made using an Oxycon Pro Metabolic System (Jaegger, Hoechberg, Germany) throughout for monitoring and analysis of VO_2. Participants were asked to wear a facemask that covered the mouth and nose, and a rotary flow sensor was placed in the mouthpiece for gas collection. The Oxycon Pro was calibrated using a 3 ml Hans Rudolph volume calibrating syringe (Hans Rudolph

model 5530, Kansas, USA). A rating of perceived exertion (RPE) was recorded each min by asking participants how they felt using a 0-10 scale (Foster et al., 2001), with 0 correlating to a feeling of rest, while 10 is maximal effort. Each participant wore a Polar Heart Rate Monitor (Polar Electro, OY, Finland) to monitor HR throughout the testing procedure, and a safety harness was worn for the test procedure in order to ensure the safety of each participant from tripping/falling, particularly as there were some who were not accustomed to treadmill exercise. A crash mat was placed behind the treadmill at all times while participants exercised. Readings of RPE, HR and VO_2/kg were manually recorded each min. Participants were instructed to provide maximal effort and persist with the test until they felt they could no longer continue with exercising, or they reached 85% of their age predicted maximum HR (using Karvonen's principle of 220-age; Hansen et al., 2012) in which case the treadmill was stopped using the data display module that ensured a gradual ceasing of the treadmill belt. Gas collection was then immediately stopped and the facemask and mouthpiece removed for the participants' comfort and well-being. Participants were then instructed to perform a cool-down period of 5 min walking at a self-selected comfortable speed on the treadmill (usually the same as the warm-up speed) and once completed, participants were withdrawn from the safety harness and monitored intensively during recovery, where they remained under supervision until heart rate returned to within 120% of basal levels. Participants were then free to leave after organising the visits for the start of their individual exercise programme.

3.4.1 Maximal exercise test

For experimental chapter 8, the exact test procedure as section 3.4 was followed, except participants were allowed to continue with the test until volitional exhaustion, and so participants were verbally encouraged to provide maximal effort in the test. Safety measures were in place as in section 3.4, and participants were taught how to straddle the treadmill before they began the exercise in order to ensure a safe method of ceasing exercise, especially considering the participants were somewhat unaccustomed to exercise.

3.5 Expired gas analysis

Respiratory gas exchange measurements were analysed breath-by-breath using an Oxycon Pro Metabolic System (Jaegger, Hoechberg, Germany) throughout testing for monitoring and analysis of VO_2. The system was calibrated prior to each use according to the manufacturer's specifications. Initially this involved standard gas (17.05% O_2 and 4.98% CO_2, Cranlea, UK). The flow rate was calibrated using a 3 L syringe (Hans-Rudolph). The VO_2 data was 30 s stationary time-averaged, and the highest 30 s average reading in the incremental phase was regarded as the VO_{2max} (Midgley et al., 2009). The 30 s stationary time average method has been shown to provide a sound concession between removing noise and maintaining the underlying trend in relatively rapidly changing VO_2 data (Midgley et al., 2009). Sub maximal data from 3.4 was linearly extrapolated in order to establish the VO_{2max} from this data set.

3.6 Blood collection

3.6.1 Capillary blood samples

Capillary blood samples were collected using a standard finger prick technique (Roche Accu-Chek Safe-T-Pro Lancets, Roche Diagnostics, North America). A clean finger prick was used for each separate collection. Blood for the analysis of pH, bicarbonate (HCO_3^-), base excess, H^+, and lactate was collected in 100 µl balanced heparin blood gas capillary tubes and analysed immediately where possible, otherwise was placed on ice until analysed (ABL800, Radiometer, UK). Blood for the analysis of haematocrit was collected in duplicate into glass capillary tubes and analysed using a Micro Haematocrit Reader (Hawksley, UK).

3.6.2 Venous blood samples

Venous blood samples were drawn from the antecubital vein via a standard venepuncture procedure into Vacuette blood tubes (Vacuette®, Greiner BIO-one, UK). Samples were collected by different persons depending on the study, but within each study samples were collected by the same independent trained consultant. Tri-sodium citrate tubes were centrifuged immediately at 12,000 g for 10 min where possible; otherwise they were placed on ice and analysed as soon as possible, but were never left on ice for longer than 30 min. Analysis of blood tubes will be discussed in the next sections, but once the required plasma had been removed and analysed, the remaining

PFP from the tri-sodium citrate tubes were aliquoted into cryovials and stored at -80 °C for later analysis.

3.7 EMP

Citrated blood was analysed by obtaining platelet rich plasma through centrifugation (180 g, 10 min) using a Heraeus Labofuge 400R Centrifuge (Kendro Laboratory products, Bishops Stortford, UK), which was then removed carefully into a 1.5 ml polypropylene tube. Platelets were removed by further centrifugation (12,000 g, 10 min) using a HeraeusBiofuge Pico Centrifuge (Kendro Laboratory products, Bishops Stortford, UK). From this, samples of PFP (25 µl) were incubated with 4 µl of either IgG1 CD105:FITC conjugate (AbDSerotec, UK) or IgG1 CD106:FITC conjugate (AbDSerotec, UK) in the dark at room temperature for 30 min. Quantification was achieved by adding filtered phosphate buffered saline (150 µl PBS; 0.01 M phosphate buffer, 0.0027 M KCl, 0.137 M NaCl, pH 7.4 and autoclaved; Sigma) and counting beads (25 µl, Caltag Laboratories) immediately prior to analysis by FC (BDFACSCalibur®). Considering that MP are determined between a size range of 0.1-1 µm MP, the counting beads used are a mixture of fluorescent beads with diameter in the range of 0.5 and 0.9 µm and platelet size ranging from 0.9 to 3 µm, which allows the ability to set the cytometer software so that MP can be studied within a fixed range for a more reproducible MP count. A MP region was previously determined using megamix beads (Biocytex, France) according to an established protocol and used consistently throughout the thesis (Lacroix et al., 2010) (Fig 3.1), and 25,000 events were counted for MP analysis, with positive MP being defined as an increase in mean fluorescence intensity over the isotope matched negative control, and were quantified in relation to

counting beads according to manufacturers' instructions. Considering that a known volume of counting beads was combined with the same known volume of PFP, the number of MP/µl (the absolute count) was obtained by relating the number of MP counted with the total number of fluorescent bead events (Fig 3.2). The MP number was then multiplied by the number of total fluorospheres per unit of volume, as per the following formula;

$$\text{MP concentration (MP/µl)} = \frac{\text{Number of cells counted}}{\text{Total number of beads counted}} \times \text{Number of Beads per µl}$$

3.7.1 Sample analysis of EMP

Fig 3.1 represents the gating strategy used from the flow cytometer used for quantification of CD105+ MP and CD106+ MP. FSC and SSC criteria were set for the assessment of + MP. The MP gates were set using megamix beads of 0.5 and 0.9 µm.

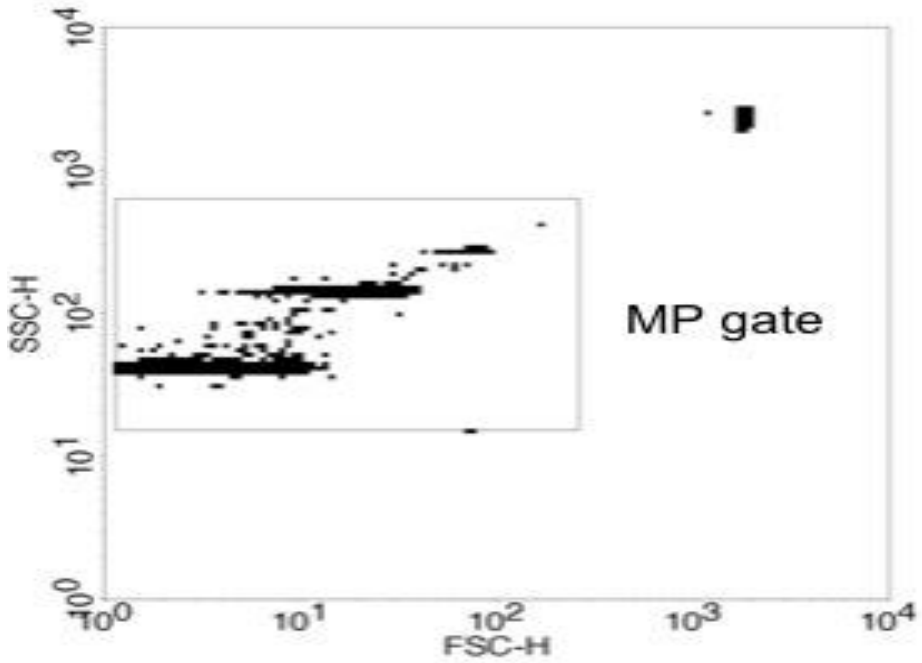


Fig 3.1 Representation of the flow cytometry MP gating used to determine MP numbers throughout the entire thesis showing forward scatter (FSC) and side scatter (SSC) plots.

Fig 3.2 display a typical representation of the detection of EMP. For illustrative purposes, the MP gate is shown as a rectangular gate, and this illustration is a typical CD105 staining of MP showing positive events in the lower right quadrant, negative events in the lower left quadrant, and counting beads in the upper right quadrant.

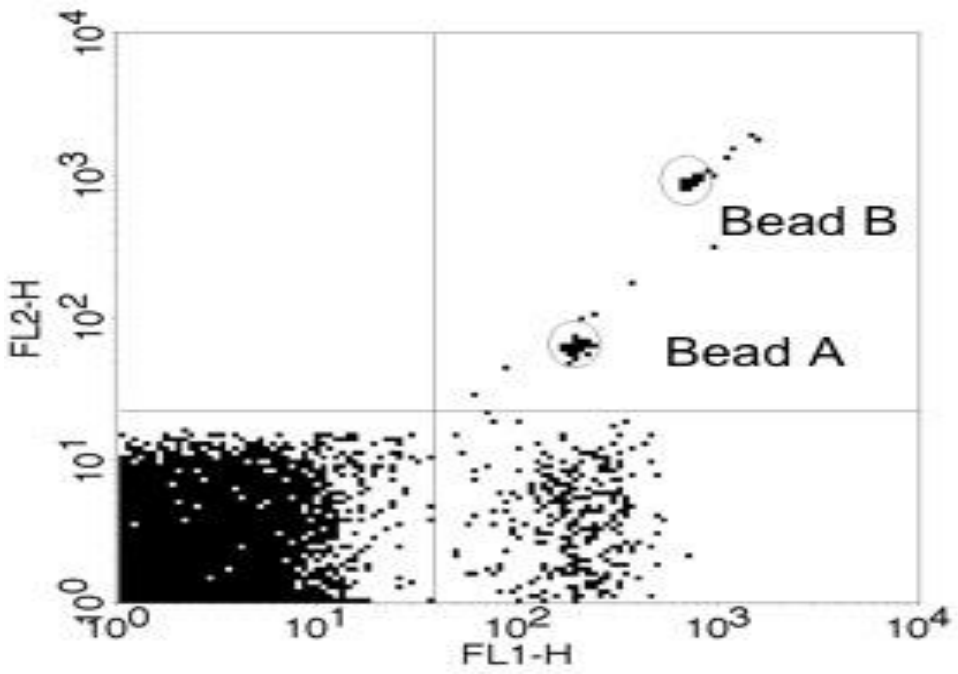


Fig 3.2 Typical representation of flow cytometry quantification of EMP.

3.8 EF using the EndoPAT-2000

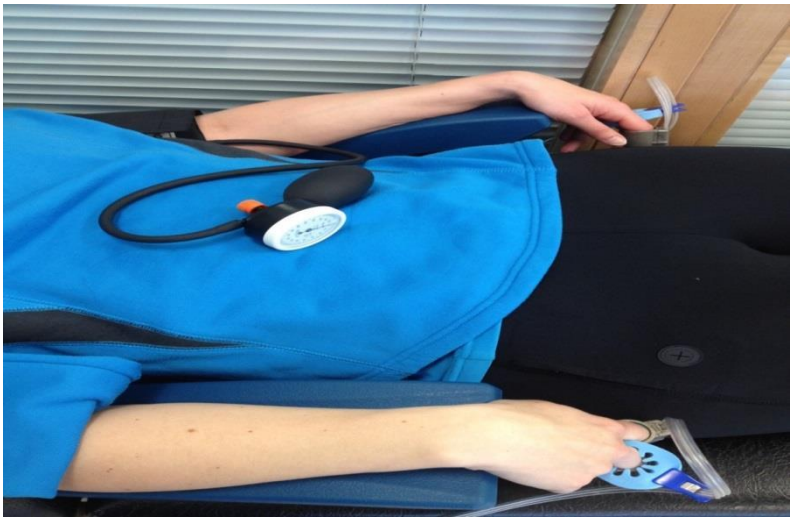
Additionally to EMP quantification, EF was assessed during the sedentary study (chapter 7) using a non-invasive reactive hyperaemia technique through an EndoPAT-2000 device (Fig 3.4). Participants were measured at rest in a fasting state between the hours of 0730 am and 0800 am prior to exercise, and then again immediately after 60 min of exercise. A trained professional with vast experience using this device was

employed to carry out the procedure, and the procedure was performed in a quiet, thermo-neutral laboratory. All mobile phones and pagers were silenced, and restrictive clothing or jewellery was removed as it may have interfered with blood flow. The patient's fingers were inspected prior to experimentation for any injuries or deformities that could have affected the procedure. Fingernails were trimmed if they extended more than 5 mm or 1/5 of an inch beyond the tip of the finger in use as they may have interfered with the internal membranes of the PAT probes. The patient was asked to lay supine on a bed for a period of 15 min at complete rest with the two arm supporters placed along each of the patient's sides. SBP and DBP was then recorded in this state using the control arm (the arm not to be occluded during the study) using a BP cuff. A BP cuff was then applied snugly to the arm to be occluded but was not inflated at this time (Fig 3.5).

Fig 3.3 EndoPAT-2000 machine

The EndoPAT-2000 software was then launched and all personal details entered for that current patient, including age, gender, height, weight, SBP and DBP. Two pneumatic finger probes, which are thimble-shaped sensors, were used for each test. One was attached to the index finger of the right arm, and another probe was attached to the index finger of the contralateral arm. It was confirmed with the patient that he or she could feel the very end of the probes, and then the inflate button was activated. A foam anchor ring was placed at the base of the adjacent middle finger, ensuring that the probe and the foam ring were not in contact in order to limit noise in the analysis. The arms of the patients were positioned so that the forearms were supported on the arm supports and the fingers dangled freely off the edge of the support. The patient was instructed not to move the fingers and to relax throughout the testing procedure. The test was initiated and a 5 min baseline recording period was performed. The blood pressure cuff was then manually inflated at a rapid rate to a supra-systolic pressure of 60 mmHg above the patient's SBP or to 200 mmHg, whichever was higher, and a stopwatch was started. After 5 min the cuff was released abruptly as quickly as possible and a 5 min post occlusion recording period was taken. The test was then stopped causing the probes to deflate automatically. The probes were then discarded after use and brand new probes used for each test.

A



B

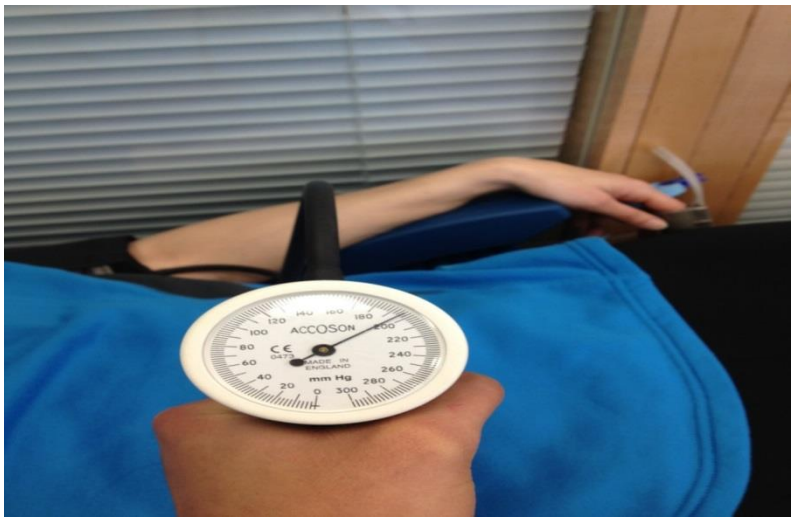


Fig. 3.4 Representation of the EndoPAT procedure used in experimental trials (chapter 7). A displays the setup of the system including the finger probes on each hand, whilst B shows the blood pressure reading and the extent of the pressure required to occlude the brachial artery.

Chapter 4: Investigation into the effects of repeated supra maximal cycling on the EMP response in healthy males

4.1 Introduction

It is known that exercise induces several types of physiological stress, such as increased heat production, reactive oxygen species and shear stress (Marsh & Coombes, 2005), and that oxidative stress activates the endothelium (Lehoux, Castier, & Tedgui, 2006; Ungvari, Wolin, & Csiszar, 2006). The study of EMP after exercise in healthy individuals is scarce, with a limited number of investigations being conducted in this area. An increase in levels of EMP, specifically CD106+ MP was shown following a simulated (hyperbaric chamber) dive after breathing air at depth in 6 healthy male participants (Vince, et al., 2009b); suggesting this may be a sign of endothelial activation. Bartzeliotou et al (2007) investigated circulating levels of adhesion molecules, including CD62E and soluble CD106, induced by a "Spartathlon" ultra-distance foot race. CD62E was quantified in plasma with a biochip array analyser and then an ELISA technique. Soluble CD106 was determined in plasma using a multiplex assay kit. sCD106 was significantly greater at the end of this 246 km race, with levels returning to normal 48 hr after the end of the race (Bartzeliotou et al., 2007). In a recent study (Sossdorf et al., 2011) it was shown that a moderate bout of cycling exercise was enough to elicit an increase in the quantity of cell-derived EMP (CD62E) concentration in 16 healthy male individuals. Conversely, Chaar et al (2011) were unable to see any significant changes in soluble adhesion molecules or MP, which included CD106+ MP and sCD106. Chaar et al (2011) tested 7 healthy males and the exercise consisted of 3 progressive and maximal ramp exercise tests with a constant pedalling speed of 70 RPM that were interspersed with 10 min of recovery.

In addition to the limited research in the effects of exercise upon EMP concentration, there is also limited data on the impact of $NaHCO_3$ ingestion on the stress response

following exercise. $NaHCO_3$ is an effective buffering agent for enhancing performance (McNaughton, Siegler, & Midgley, 2008), and can improve acid base recovery following exercise of an anaerobic nature (Siegler et al., 2008). $NaHCO_3$ is proposed to increase the body's natural bicarbonate reserve, acting as a buffer due to its ability to accept a proton to form carbonic acid ($H^+ + HCO_3^- \leftrightarrow H_2CO_3$). In turn, this mechanism increases the extracellular reserve enhancing H^+ efflux from the cells (McNaughton, Siegler & Midgeley, 2008). In addition, it is possible that the extra buffering of H^+ may result in a reduced production of free radicals (Kellum, Song, & Li, 2004), and thus reduce exercise induced oxidative stress, a possible stimulus for EMP release (Vince et al., 2009b). It has been shown previously in cultured cells and cardiac myocytes that acidosis is a major trigger of apoptosis (Thatte et al., 2004). A decrease in endothelial cell pH is part of the physiological response to exercise (Morikawa et al., 1994), and avoiding acidosis through the use of a buffering agent may attenuate MP release, and therefore endothelial damage/activation. Based on the evidence given here, we hypothesised that the plasma concentration of MP may be influenced by a strenuous cycling protocol. Thus the aim of this chapter was to assess damage/activation of the vascular endothelium as a result of a high intensity cycling exercise through quantification of EMP in healthy male participants. An additional aim of the chapter was to investigate the effects that ingesting $NaHCO_3$ prior to strenuous exercise may have, if any, upon the numbers of circulating EMP and whether the acidosis response to high intensity exercise may be attenuated in healthy human participants.

4.2 Methods

4.2.1 Participant characteristics

Seven healthy, non-smoking male participants (mean ± SD, height, body mass, age, absolute PPO, relative PPO and physical activity: 182 ± 0.06 cm, 81.3 ± 8.4 kg, 22.1 ± 3.2 years, 300 ± 22 W, 3.73 ± 0.50 $W \cdot kg^{-1}$, 3.9 ± 1.0 hr $week^{-1}$) volunteered to take part in this study. All ethical approval and pre-test procedures were made in accordance with the information provided in the general methodologies in section 3.1.

4.2.2 Experimental design

Participants reported to the laboratory on three separate occasions, each separated by one week. Visit 1 consisted of a PPO test (described in detail in section 3.3), which preceded a familiarisation to the intermittent cycling protocol. The PPO attained was used determine the workload for the intermittent exercise protocol during the experimental trials. The intermittent protocol began with a 5 min warm up at 50 W, which was followed by 10 x 15 s sprints at 120% PPO determined in visit 1, which were separated by 45 s of active recovery at 50 W. During the experimental trials, all participants were vocally encouraged to achieve maximal power output. PRR was also recorded prior to each sprint.

The two experimental visits were organised in a randomised and double blinded manner, which consisted of either $NaHCO_3$ (experimental) or placebo trials. $NaHCO_3$ was administered at a dose of 0.3 $g \cdot kg \cdot BW^{-1}$, contained in approximately 15-20 gelatine capsules that were taken with water. The placebo was administered at a dose of 0.045 $g \cdot kg \cdot BW^{-1}$ of NaCl made up with flour to provide the same amount of pills. Pills were consumed 60 min prior to exercise (Siegler et al., 2010). All testing was performed at the same time of day, with the pills being consumed at 0830 am, in order to control for any circadian variations that may affect results, which has been shown previously in the expression of CD106+ MP (Madden et al., 2008) and exercise performance (Drust et al., 2005).

Capillary blood samples were taken for the measurement of acid base characteristics (pH, H^+, HCO_3^- and base excess) immediately before and 60 min after ingestion of the capsules (both pre exercise) and analysed as described in section 3.6.1. Venous blood samples for subsequent EMP quantification were made as described in section 3.6.2. Blood draws were made immediately before exercise (Rest), immediately post exercise (Immediate), 90 min post exercise (90 min), and 180 min post exercise (180 min).

The expression of CD105+ MP and CD106+ MP were characterised by FC (sections 3.7 and 3.7.1 for MP quantification and sample analysis).

4.2.3 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 19.0 (SPSS Inc, Chicago, IL). Central tendency and dispersion of the sample data are represented as the mean ± SD. Any changes in biochemical markers across condition and time were analysed using linear mixed models. Post hoc tests with Sidak adjusted p values were used to locate significant paired differences where a significant F ratio was observed. The change in acid base status from pre to post ingestion within the two experimental conditions, and comparisons across conditions was investigated using paired samples t-tests with Sidak adjusted p values to control for family wise type I error rate. All of the data are presented as means and standard deviation of the mean ± SD unless specified. Two-tailed statistical significance was accepted at $p < 0.05$.

4.3 Results

4.3.1 Acid base homeostasis

Capillary blood acid base variables are presented in Table 4.1. There were no significant differences in pH. HCO_3^-, base excess or H^+ ions between the placebo and experimental trials prior to the ingestion of pills. Significant changes occurred in all acid base characteristics after the supplementation with $NaHCO_3$. pH increased significantly from 7.40 to 7.46 after $NaHCO_3$ ingestion in the experimental group only ($p < 0.001$). HCO_3^-

was significantly higher post ingestion in the experimental group only, rising from 25.58 to 29.30 mmol/L ($p < 0.05$). Base excess rose from 1.62 to 5.68 mEq/L in a significant manner ($p < 0.001$), again in the experimental group only, with the placebo group remaining unchanged. Finally, H^+ ions decreased significantly from 39.50 to 34.93 in those who ingested $NaHCO_3$. Those who consumed $NaHCO_3$ had significantly different values in each of the four acid base variables measured compared with placebo group post ingestion.

Table 4.1 Acid base characteristics (pH, HCO_3^-, base excess, and H^+) measured pre and post ingestion of $NaHCO_3$ (means ± SD).

Variable	Pre ingestion	Post ingestion
pH		
PLACEBO	7.40 ± 0.01	7.41 ± 0.04
EXPERIMENTAL	7.40 ± 0.02	7.46 ± 0.02[ab]
HCO_3^- (mmol/L)		
PLACEBO	25.36 ± 2.54	25.29 ± 1.18
EXPERIMENTAL	25.58 ± 0.58	29.30 ± 1.34[ab]
Base excess (mEq/L)		
PLACEBO	1.24 ± 0.47	1.43 ± 0.75
EXPERIMENTAL	1.62 ± 0.56	5.68 ± 1.66[ab]
$[H^+]$		
PLACEBO	39.41 ± 1.12	39.31 ± 3.67
EXPERIMENTAL	39.50 ± 1.39	34.93 ± 2.03[ab]

[a] significantly different to pre ingestion ($p < 0.05$), [b] significantly different to placebo ($p < 0.05$).

4.3.2 PRR

Results of PRR suggest that regardless of experimental condition, as the exercise progressed, participants perceived each subsequent sprint more difficult than the previous sprint. Fig 4.1 displays the trend for PRR scores as the number of sprints increased. PRR showed significant pair wise comparisons across almost all time points

in both conditions, with a significant overall main effect for time found ($F = 28.503$, $p < 0.001$). There was however no significant main effect found between the two experimental groups for condition ($F = 0.604$, $p = 0.443$).

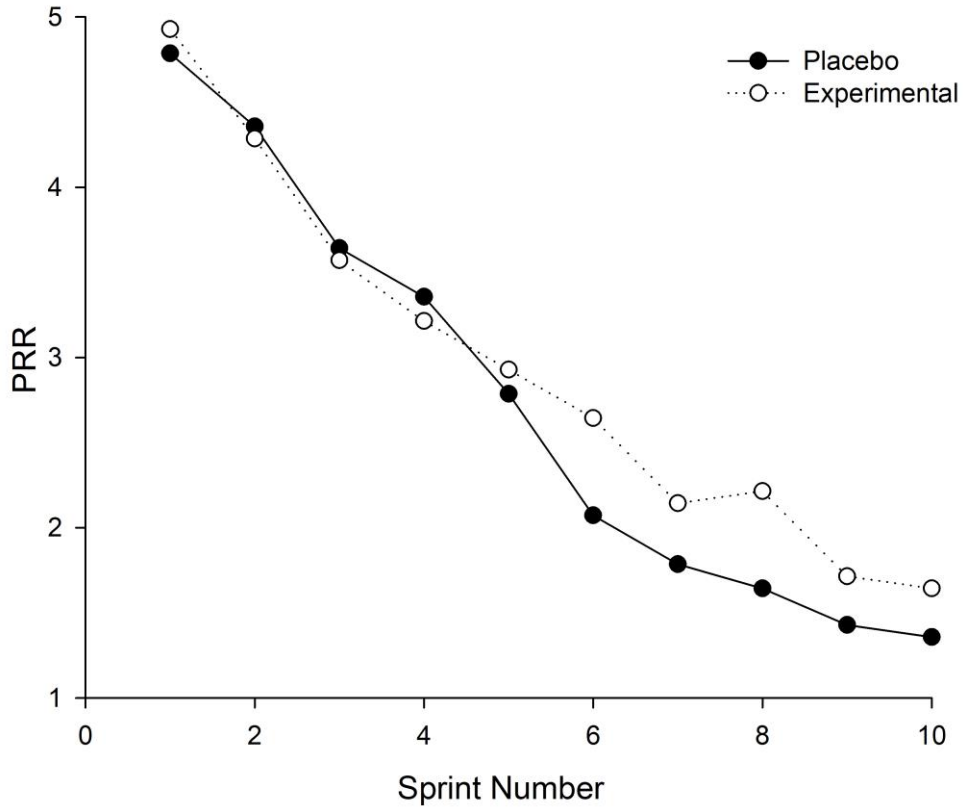


Fig 4.1 Time dependent profile for the PRR scores in each experimental group expressed from sprint number 1 to 10 during the placebo (●) and experimental (○) trials (n = 7). Error bars have been excluded for clarity that would have represented the SD of the mean PRR.

4.3.3 CD105+ MP

CD105+ MP at rest and during the 180 min post exercise recovery for the two groups is displayed in Fig 4.2. The mean quantity of CD105+ MP increased from rest to a maximum level at 90 min post exercise in both experimental groups. In the placebo group, an increase of 52.7% was observed compared to an increase of 32.1% in the experimental group from rest to 90 min. The interaction effect of condition and time produced no significance ($F = 0.267$, $p = 0.848$). A significant main effect for time was present ($F = 2.969$, $p = 0.046$) suggesting the EMP response for CD105+ MP was significantly higher 90 min after exercise, and levels had returned back to basal levels after 180 min. Post hoc tests indicated that there was a significant increase in CD105+ MP in both conditions immediately after exercise to 90 min, placebo group ($p = 0.018$), and experimental group ($p = 0.040$). Levels of CD105+ MP decreased from the peak value at 90 min to 180 min during recovery in both groups, but this decline was not significant. No main effect significance was found for condition across trials, indicating that the results gained were not due to the condition but the exercise protocol itself.

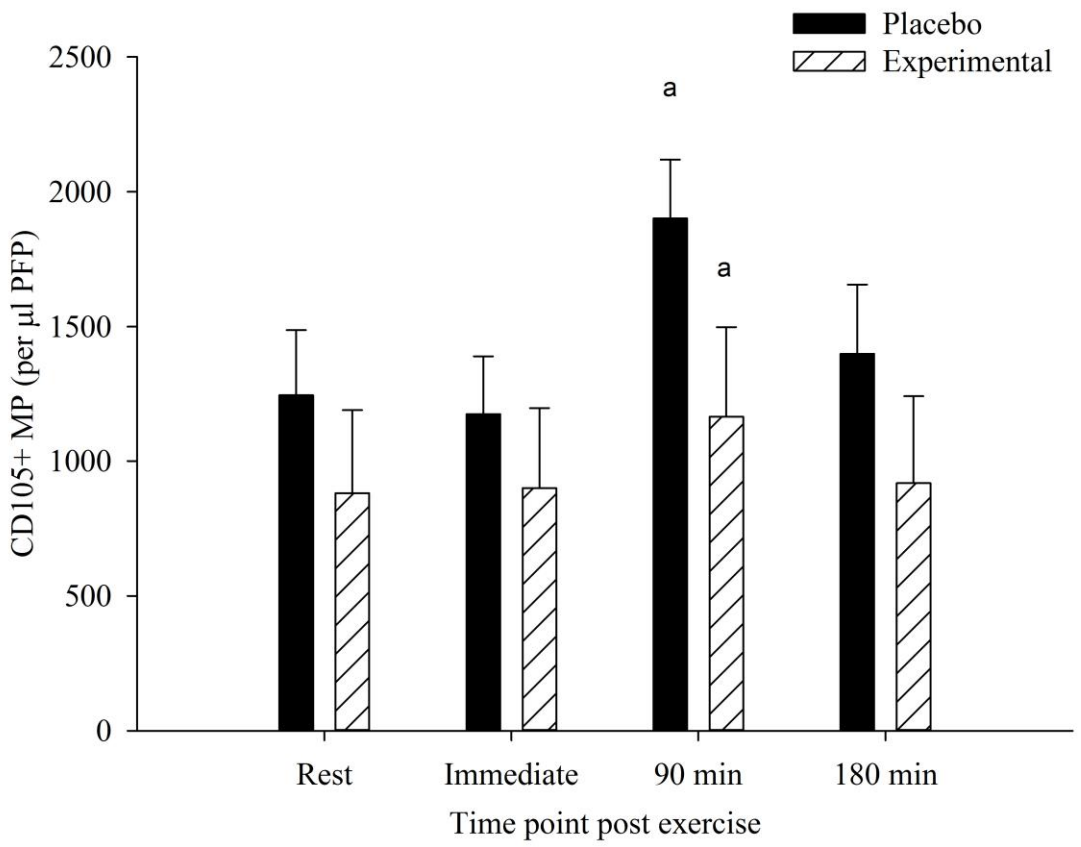


Fig 4.2 CD105+ MP at rest, and immediately, 90 and 180 min post exercise in placebo (dark bars) and experimental (hatched bars) participants. Data represented as the concentration of CD105+ MP per µl PFP (mean ± SEM). [a] significant difference to immediate ($p < 0.05$). Error bars represent mean ± SEM.

4.3.4 CD106+ MP

CD106+ MP data followed a similar trend to that observed for CD105+ MP in that the peak value was attained 90 min post exercise in both experimental groups (Fig 4.2). In the placebo group, an increase of 39.2% was observed from rest to the peak at 90 min, compared with 64.3% in the experimental arm for the same time points. The interaction effect of condition and time produced no significance ($F= 32.144$, $p = 0.925$). However, a significant main effect for time was present ($F = 7.861$, $p = 0.011$) indicating that the response to exercise was significantly increased at 90 min, and levels returned towards basal values after 180 min. The results of the post hoc test showed that placebo and experimental groups displayed significance from immediately post exercise to 90 min ($p = 0.045$, $p = 0.009$ respectively). No main effect significance was found for condition across trials, indicating that the results gained were not due to the condition but the exercise protocol itself.

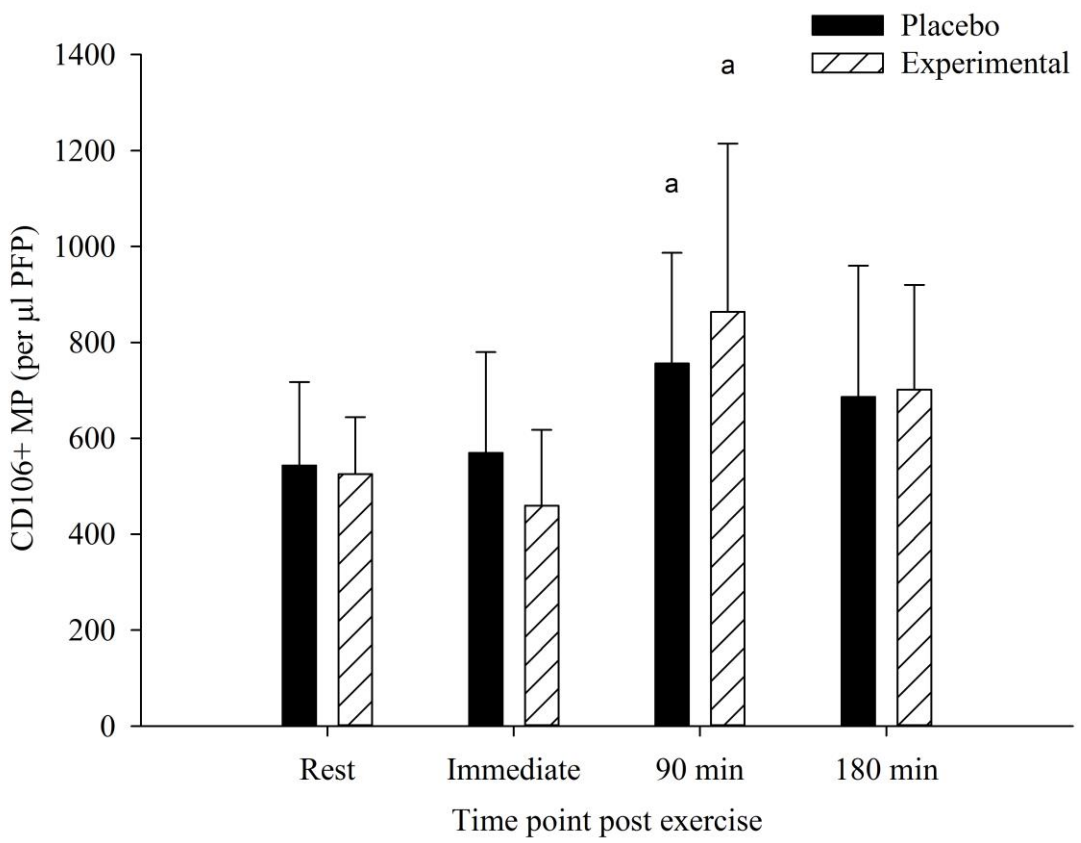


Fig 4.3 CD106+ MP at rest, and immediately, 90 and 180 min post exercise in placebo (dark bars) and experimental (hatched bars) participants. Data represented as the concentration of CD106+ MP per µl PFP (mean ± SEM). [a] significant difference to immediate ($p < 0.05$). Error bars represent mean ± SEM.

4.3.5 Overall + MP response to exercise

As no significant differences were observed between the experimental conditions at each of the time points (placebo and experimental), data has been grouped thus combining values from all participants (n = 14) regardless of their experimental condition in order to demonstrate the trend over time that both markers of EF displayed. Fig 4.4 shows that there was significance for both markers across several time-points, indicating a significant change across time in EF. Levels of CD105+ MP were significantly increased from resting values of 1077 CD105+ MP per µl PFP to a peak value of 1499 CD105+ MP per µl PFP at 90 min ($p = 0.010$). The number of CD105+ MP remained elevated from resting values to 180 min (1159 CD105+ MP per µl PFP) post exercise ($p = 0.043$). CD105+ MP also increased from immediately post exercise from a value of 1048 CD105+ MP per µl PFP to 90 min post exercise ($p = 0.019$). CD106+ MP also peaked at 90 min (865 CD106+ MP per µl PFP) and this was significantly greater than at immediately post exercise (491 CD106+ MP per µl PFP; $p = 0.020$). There was also a significant increase at 180 min post exercise from immediately post exercise as levels of CD106+ MP rose from 491 to 694 CD106+ MP per µl PFP ($p = 0.015$).

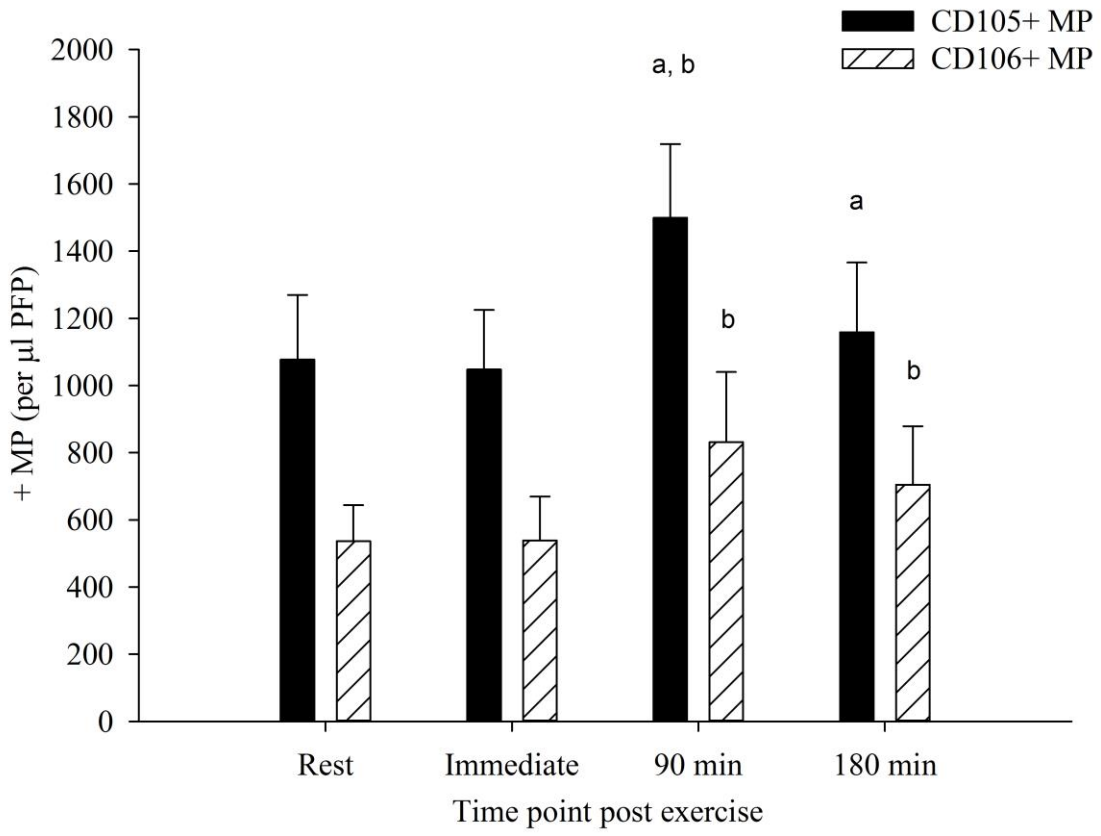


Fig 4.4 CD105+ MP (dark bars) and CD106+ MP (hatched bars) at rest, and immediately, 90 and 180 min post exercise (n = 14). [a] significant difference to rest, [b] significant difference to immediate ($p < 0.05$). Error bars represent mean ± SEM.

4.4 Discussion

The main finding from this chapter is that a high intensity repeated sprint exercise stimulates a rise in EMP, which then shows a decline towards resting levels within 180 min of ceasing exercise. Another finding from the experiment is that pre ingestion of $NaHCO_3$ to maintain acid base balance closer to homeostatic levels did not attenuate the release of EMP from the endothelium following exercise.

To date, there has not been vast research conducted to describe the effect of physical exercise on the overall cell-derived MP release from endothelial cells in healthy humans. Results here show that this particular bout of acute exercise was capable of increasing the concentration of EMP in comparison to resting levels. Additionally, the levels of EMP were returning to resting values after 180 min of recovery. In comparison to another study of this kind, Chaar et al. (2011) were unable to detect any changes in MP production from endothelial cells as characterised by no detection at all in levels of CD106+ MP (Chaar et al., 2011). Additionally to this, plasma levels of sCD106 were unchanged as a result of exercise. These findings may be due to pre analytical methodology, including using previously frozen plasma (Lacroix et al., 2010) or the exclusivity of enumerating MP that were also positive for annexin V staining, as not all MP express PS. Chaar et al. (2011) utilised a cycling protocol involving three separate progressive, maximal ramp exercise tests in healthy males, which would be expected to elicit sufficient damage to the endothelium due to its high intensity nature (Chaar et al., 2011). Therefore this leads to the presumption that the methodology was responsible for this.

There was an observed rise in EMP following this exercise that may be postulated to be as a result of the nature of the exercise. The reason for such a rise in these markers may be due to the sensitive nature of the endothelium to oxidative stress and shear rate (Lehoux, Castier, & Tedgui, 2006; Ungvari, Wolin, & Csiszar, 2006). Cell adhesion molecules are expressed by the endothelium and soluble isoforms of these molecules are found in the plasma, thought to be as a result of shedding from cell surfaces (Marsh & Coombes, 2005) and although there was no precise measures of shear stress in this study, it is a suggestion that has been evident since in further work (Sossdorf et al., 2011; Stachenfeld, Yeckel, & Taylor, 2010), but precise measures of shear stress during

and post exercise are required in order to determine effects on MP quantification. Given the supra maximal nature of the exercise in this study, it is reasonable to assume there was a significant increase in shear rate, suggested to be a key reason in the shedding of these molecules (Shephard et al., 2000). Oxidative stress may also be a determining factor in increased MP release from cells, and has previously been shown with EMP release in response to hypoxia (Vince et al., 2009a;b) as well as heat shock protein (HSP) response to a single bout of high intensity anaerobic exercise (Peart et al., 2011) suggesting a more global circulatory stress involving regulatory triggers could contribute to increased MP release.

Nielsen and Lyberg (2004) measured plasma concentrations of soluble adhesion molecules (sCD62E, sCD106) in a total of 43 participants (35 males, 8 females) pre exercise and immediately post exercise (Nielsen & Lyberg, 2004). This study included data from 3 separate marathons and half-marathons in Oslo, Norway. Levels of sCD106 and sCD62E rose significantly immediately after exercise from resting values in both the marathon and half-marathon races. The full marathon race in 1999 took an average time of 3.45 hr to complete, while the mean finishing time for the 2000 race was 3.40 hr. The half-marathon race was completed in 1.41 hr for men and 1.53 hr for women. Unfortunately, no post exercise data following a recovery period was available in the study by Nielsen and Lyberg (2004) that may have indicated if the endothelium was able to recover as in accordance with the results from the current chapter. However, in the results of the present chapter, the rise in EMP was not observed until 90 min post exercise, and values immediately post exercise were remarkably similar to resting levels.

Future protocols could include additional time points to further characterise the time course of MP concentration after an acute stress. It could be assumed that from this work, and others previously mentioned (Jensen, Bangsbo, & Hellsten, 2004; Sossdorf et al., 2011; Wahl et al., 2011) that high intensities are required to activate endothelial cells, and there is an intensity dependent increase in MP, suggesting that in order to significantly stress the endothelium of healthy individuals, strenuous activity is required to see such a rise. This intensity dependent increase is thought to be as a result of shedding of the cell adhesion molecules via adrenergic mechanisms (Rehman et al., 1997), but it may also be expected following a bout of exercise of this intensity, and so could be as a result of an increase in shear stress. As the time interval from cessation of the exercise protocol to the observed increase in EMP was relatively short, it may be postulated that increased levels of EMP are as a result of increases in shear stress due to increased HR. Endothelial cells are constantly shedding MP at a nominal rate (Freyssinet, 2003) and a sudden increase in shear stress may result in a one off cleaving of MP that are in a process of being released from the endothelium, resulting in temporary increase in circulating EMP. Sossdorf et al. (2011) provided a strong suggestion that shear stress may play a role in EMP release in a recent study (Sossdorf et al., 2011). This group found that cardiopulmonary exertion was significantly higher in the 'trained' group throughout a 90 min cycling exercise at a fixed percentage (85%) of participants' IAT. HR and BP were monitored at regular intervals throughout the exercise and immediately after, suggesting a greater shear stress was the reason for a transient increase in numbers of EMP (CD62E) at 45 min in the 'trained' group versus the 'untrained' group. This finding may also suggest biological differences in the effectiveness of the stress induced mediators between 'trained' and 'untrained' participants. There were similarities between the current study and the aforementioned in terms of a time delay from cessation of exercise in order to see an increase in EMP,

even in such a moderately intense protocol. Further to this, participants from this chapter were fit and healthy individuals partaking in regular physical activity per week, and were accustomed to high intensity exercise. Such characteristics could link to the trained group in the study by Sossdorf et al. (2011), although aerobic capacity was not measured in the present chapter, and if this is indeed the case, then it may be reasonable to assume that a similar time course was present in both studies. Although the exact timings of the sample collection were slightly different, it appears that trained individuals hit a peak of endothelial stress and subsequent recovery within a similar timeframe.

A further aim of this chapter was to determine if the stress response placed upon the endothelium as a result of exercise, by means of changes in pH, may be attenuated, and therefore MP release reduced by the inclusion of a $NaHCO_3$ buffer. This has previously been shown to be successful in other markers of exercise related stress, such as HSP72 (Peart et al., 2011). Recently, Wahl et al (2011) investigated the effects of exercise induced acidosis on VEGF levels following 3 experimental trials. In the first 2 weeks, 11 healthy male participants performed 4 sets of 30 s all out cycle sprints separated by 5 min rest, in a randomised order of bicarbonate and placebo trials. The third trial was a constant load exercise for 60 min at 50% PPO in the third week (Wahl et al., 2011). VEGF stimulates angiogenesis, thus resulting in increased oxygen delivery to tissues; however over-expression can be detrimental (Wahl et al., 2011). This group found that following ingestion of $NaHCO_3$ (0.3 $g.kg^{-1}$ BW suspended in 0.02 ml H_2O kg^{-1} BW over 90 min period), there was no attenuation to the VEGF response following highly strenuous exercise. The endothelium is exposed to a more acidic environment during physical exercise (Morikawa et al., 1994) and the rate of VEGF production has been shown to be increased at acidic pH in an endothelial cell culture model system

(Burbridge et al., 1999) suggesting a possible oxidative stress response. However, *in vitro* studies in bovine aortic endothelial cells have actually found that acidosis inhibits endothelial cell apoptosis and inhibits angiogenesis despite increased VEGF mRNA expression (D'Arcangelo et al., 2000) whilst Fukumura et al (2001) also demonstrated a more acidic environment increased VEGF expression (Fukumura et al., 2001). However, this study demonstrated no attenuation of EMP release, which may suggest that MP release is not influenced by changes in blood pH in healthy human subjects.

It was hypothesised that changes in the circulating levels of EMP following strenuous activity would reflect the changes in the state of the endothelium. Taking this into account, it is reasonable to assume that the levels at which EMP were highest would represent the time at which the endothelial cell stress occurred, which was 90 min post exercise. Detecting the time point at which the highest amount of endothelial damage occurs is something to consider in future work, as it may be that the EMP quantity was rising/falling at 90 min thus greater endothelial damage may occur at a different time point post exercise. The effects of exercise on the vasculature are not particularly well characterised, and here attention has been drawn to MP as potential novel markers that are able to offer an insight into the state of the endothelium of healthy human participants during strenuous physical activity.

In conclusion, a strenuous bout of exercise is accompanied by a rise in EMP within the circulation. The endothelium of healthy individuals is highly stressed after such exercise, but shows signs of rapid recovery within 180 min of ceasing exercise. Additionally, EMP release was shown to be unaffected by changes in blood pH by means of $NaHCO_3$ ingestion prior to exercise, suggesting that shear stress may be a

contributing factor resulting in the increased circulating EMP due to its exhaustive nature. Shear stress factors were not investigated in this current chapter, but future work is necessary to elucidate any relationship between shear stress and EMP release. Finally, this chapter strengthens the notion that CD105+ MP and CD106+ MP appear novel biomarkers as to the state of the endothelium, specifically as shown here in healthy human participants.

Chapter 5: Investigation into the effects of repeated supra maximal cycling on the EMP response in healthy females

5.1 Introduction

The previous experimental chapter verified the novel finding that CD105+ MP and CD106+ MP appear effective markers of EF in healthy males during a high intensity exercise bout, and that there appears to be a trend in the pattern of EMP quantity observed during recovery. This increase was not attenuated by $NaHCO_3$ intake prior to exercise, and so it is thought that altering acid base variables does not impact EMP levels. Therefore, the influence of $NaHCO_3$ was not measured in this instance. There was also a suggestion that the levels of EMP may be related to performance measures such as HR and BP and warranted further experimentation.

The effects of acute exercise on EF in females are very uncommon, and to the author's knowledge, no other group have investigated the effects of an acute bout of strenuous exercise on EMP levels in females. A recent study did however assess PMP release after anaerobic exercise in a cohort of young, healthy individuals which consisted of a combination of 9 females and 9 males (Maruyama, Kadono & Morishita, 2012). The Bruce protocol was used on a treadmill as previously described in detail (Jae et al., 2007). Briefly, expired gas and exercise HR was measured during the last min of each 3 min stage using a 12 lead ECG. Participant's ceased exercise once they reached 85% of their age predicted heart rate during this protocol. It was stated that PMP were assessed through ELISA, but thorough details of the procedure was lacking in this study, making conclusions very difficult to draw. Levels of PMP were significantly increased from baseline at immediately post exercise and also at 60 min post exercise, although these values were not analysed separately between males and females, making interpretation very difficult. Although this study looked at PMP levels and not EMP levels, it suggests that acute exercise may impair EF in young healthy females, though it would have been

useful to see additional blood samples during recovery in order to monitor EF, as well as separate males from females. Interestingly, levels of PMP were declining very slightly from immediately post exercise to 60 min post exercise, but this change was not significant. Further to this, the units of PMP were unclear in this study as they were reported in an unfamiliar context (U/ml) making the methodology difficult to interpret.

There are a few studies that have looked at EF in acute exercise using other assessment methods. Hwang et al (2012) recently assessed FMD in a cohort that included 39 young healthy females and 35 young healthy males following acute treadmill exercise using the a standard Bruce protocol (Hwang et al., 2012). Results showed that post exercise FMD was significantly impaired when compared with baseline values in healthy, young females. Interestingly, the males studied did not show any significant change, confirming that there was a greater impairment in EF in females than in males in this study, alleviating to the suggestion that ED is greater in females than males following acute exercise. Unfortunately, there was no data available during a post exercise recovery period, which would have been useful in order to assess any potential return to baseline in EF. One limitation to the study was the effect of ethnicity of the participants, given that all those involved were of Korean ethnicity, and it has been reported that there may be ethnic differences between Asians and Caucasians (Hwang et al., 2012 McCrohon, Woo & Celermajer, 2000; Woo et al., 1997). This study does however appear to suggest that acute high intensity exercise impairs EF in healthy females as measured by FMD. High intensity is, by definition, the equivalent to $\geq$ 9 metabolic equivalents (METS) (Norton, Norton, & Sadgrove, 2010), and females were working at a mean of 12.4 METS in this study. Further data was provided that informed us that participants worked at a mean of 90.41% of their maximum age predicted HR, allowing us to determine that this was a strenuous activity. However, later analysis confirmed

that no association between FMD and exercise intensity was present, indicating that the burden of exercise did not influence results (Hwang et al., 2012).

Silvestro et al (2002) were actually able to demonstrate that both FMD and levels of sICAM-1 were affected by maximal exercise in a way that represented ED (Silvestro et al., 2002). However, this group only included 1 female (30 males), with their specific results not detailed, making conclusions of ED in females after acute exercise very difficult in this instance. This study included 31 patients with intermittent claudication and a control group of 10 participants matched to the patients for age and sex. The patients were randomised to group 1 (exercise until claudication pain becomes intolerable, maximal exercise) or group 2 (exercise until the onset of pain in affected leg, submaximal exercise). Control participants exercised until they reached their maximal HR endpoints. Treadmill exercise started with an initial stage of 3 $km \cdot h^{-1}$ and a 3% grade with subsequent 3% grade increases every 2 min up to a maximum of 15%. FMD and sICAM-1 was recorded at baseline, and immediately after exercise. Results showed that maximal exercise reduced FMD significantly and increased levels of sICAM-1, but no changes were seen in the submaximal exercise group or the control group. This study shows that acute exercise of an intense nature is enough to elicit endothelial damage in intermittent claudication, although the population was made up almost entirely of males.

In contrast to these findings, there were increases in FMD after an acute exercise bout in 13 postmenopausal women and fourteen premenopausal women (Harvey et al., 2005). Results here showed that 45 min of acute, moderate intensity (60% VO_{2max}) treadmill exercise bout increased FMD up to 90 min post exercise in postmenopausal women only. This study shows that an acute bout moderate intensity exercise can improve

endothelial FMD in sedentary normotensive postmenopausal women, a finding concurrent with Padilla et al (2006) who used FMD in healthy young adults (5 males, 3 females). FMD results should also be interpreted with caution as it has been shown previously that FMD is directly affected by the baseline brachial artery diameter (Hwang et al., 2012; Pyke & Tschakovsky, 2005). Exercise causes an increase in blood flow and this in turn causes vasodilatation, which then directly impacts the magnitude of FMD (Pyke & Tschakovsky, 2005). One mechanism that has been postulated to explain this increase in EF is that the increased blood flow from exercise may have produced an adequate shear stress mechanism to release NO, which could then have enhanced EF (Jungersten et al., 1997). These contrasting findings show that caution is required when assessing methods, and stress the importance of a better understanding of this particular area and the implications that findings may have, particularly the impact and implications that EMP may have as markers of ED.

The peripheral vasculature is exposed to various physical stimuli during exercise, including BP, HR and compressive forces, all of which contribute to shear stress changes (Dick et al., 2009; Niebauer & Cooke, 1996). It is important to be aware of the influence that shear stress may have on the release on EMP. Sossdorf et al (2011) showed that in healthy males, SBP and HR were significantly increased during and immediately after moderate exercise compared to baseline values (Sossdorf et al., 2011). All participants performed a single bout of cycling for 90 min at 80% of their IAT. EMP (CD62E) was identified through FC according to the binding of 5 µl of FITC-labelled annexin V and CD62E+ MP. The increases in hemodynamic values were interestingly associated with a rise in EMP (CD62E) levels, occurring 45 min post exercise in participants deemed highly trained (VO_{2max}> 65 $ml/min^{-1}{\cdot}kg^{-1}$) only. This group produced much higher values of SBP and HR compared to the ‘untrained’ group

(VO_{2max} < 65 $ml/min^{-1}\cdot kg^{-1}$), and considering that EMP levels were only increased in this group, the notion of shear stress and an intensity dependent influence on MP release seems logical. Recently, work has been produced showing that high shear stress applied *in vitro* of platelet rich plasma samples increased shedding of PMP in 24 healthy, sedentary, male volunteers after strenuous exercise (Chen, Chen, & Wang, 2010). This data further strengthens the hypothesis that shear stress is a major trigger of MP release, although data here was sourced from PMP origin. There is evidence of both *in vivo* and *in vitro* aspects of MP release and its association with shear stress. In a study by Vince et al (2009b), it was proposed that oxidative stress and shear stress may play a key role in MP release as CD106+ MP was increased following a SCUBA dive breathing in compressed air in 6 healthy males. However, despite these findings, data is still limited in the effects of exercise and the measure of EMP, particularly in a cohort of healthy females.

Assessing if there is a difference between EMP levels in males and females is an intriguing area, and a question that remains relatively unanswered. This question is important given the possible stimulus of gender itself and influencing factors such as menstrual cycle and hormones (Toth et al., 2007). Recently, it was examined if there were any gender specific and menstrual cycle dependent differences in circulating levels of MP (Toth et al., 2007). Twenty-seven healthy women in different phases of their menstrual cycles, and 18 healthy males were assessed. Both PMP (CD61) and EMP (CD62E) were analysed by FC, and levels of each were elevated in women compared to men. Additionally, this effect was solely due to women in the luteal phase of their cycle, when compared to the follicular phase in both types of MP (Toth et al., 2007). Notably, this group also measured CD144+ exposing MP, which did not show any gender specific or menstrual cycle dependent differences. The study by Toth et al (2007) leads

to the suggestion that EMP release may indeed be dependent upon gender, and although more data is required to answer this, it is an intriguing thought. This concept was further strengthened by finding from Jayachandran et al (2009) who showed that healthy women display menstrual cycle specific differences in both PMP and EMP, which differ significantly from age matched males. The aims of this chapter were to observe the EMP response to acute, strenuous exercise in healthy females, and also attempt to look at performance variables during acute exercise in this cohort. An additional aim was to speculatively determine the time at which optimal ED may occur by the addition of an extra blood draw.

5.2 Methods

5.2.1 Participant characteristics

Ten healthy, non-smoking female participants (mean ± SD, height, body mass, age, absolute PPO, relative PPO and physical activity: 162.34 ± 1.72 cm, 59.18 ± 4.60 kg, 24.2 ± 4.76 years, 229.1 ± 18.94 W, 3.91 ± 0.56 $W \cdot kg^{-1}$, 6.55 ± 2.44 hr $week^{-1}$) volunteered for this study. All ethical approval and pre-test procedures were made in accordance with the information provided in the general methodologies section 3.1.

5.2.2 Experimental design

Participants reported to the laboratory on two separate occasions, each separated by one week. Visit 1 consisted of a PPO test (described in detail in section 3.3), which

preceded a familiarisation of 2 sprints out of the intermittent cycling protocol. The PPO attained was used to determine the workload for the intermittent exercise protocol during the experimental trial which took place in visit 2. The intermittent protocol was the same as that described in chapter 4 (section 4.2.2). Additionally, HR was measured and recorded 10 s prior to each sprint ($HR_{recovery}$) and again immediately following each sprint where a peak HR was recorded (HR_{peak}). SBP and DBP were recorded using an Omron M6 Upper Arm Blood Pressure Monitor prior to the intermittent exercise protocol (pre), immediately after exercise (post) prior to the venous blood sample being taken and subsequently 10 min after exercise had ceased (10 min). The peak RPM achieved per sprint was also recorded, as well as the RPE using the 0-10 scale (Foster et al., 2001) as well as the PRR (Karu et al., 2000) prior to each cycle sprint. All testing was performed at the same time of day (participants arriving between 0830 and 0900) in order to control for any circadian variations that may affect results as mentioned in chapter 4, and to match the previous chapter where possible.

Capillary blood samples were taken for the measurement of acid base characteristics (pH, H^+, lactate, hematocrit (Hctc) and base excess) at rest, immediately post exercise (post) then subsequently at the same time points as venous blood sampling (45 min, 90 min, and 180 min) and were analysed as described in section 3.6.1. Venous blood samples for subsequent MP quantification were made as described in section 3.6.2. Blood draws were made immediately before exercise (Rest), immediately post exercise (Immediate), 45 min post exercise (45 min), 90 min post exercise (90 min), and 180 min post exercise (180 min).

The concentration of CD105+ MP and CD106+ MP were measured by FC. Preparation of blood for MP quantification and analysis can be found in section 3.7.

5.2.3 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 19.0. Central tendency and dispersion of the sample data are represented as the mean ± SD. Any changes in biochemical markers across time were analysed using a paired samples t-test, and data is expressed as concentrations of MP per µl of PFP. Maximum HR percentages were calculated using the well-established Karvonen formula of 220-age (Camarda et al., 2008). The change in acid base status and hemodynamic values from rest over time were investigated using paired samples t-tests with Sidak adjusted p values to control for family wise type I error rate. All of the data are presented as standard deviation of the mean (± SD) unless stated. Two tailed statistical significance was accepted at $p < 0.05$.

5.3 Results

5.3.1 Acid base homeostasis

The change in acid base variables as a result of exercise are displayed in Table 5.1 pH decreased significantly from resting values (pH = 7.41) to immediately post exercise (pH = 7.23; $p < 0.001$), at 45 min post exercise ($p = 0.018$) and at 90 min post exercise ($p = 0.002$). pH was also significantly greater when compared with immediately post exercise values at 45 min ($p < 0.001$), 90 min ($p < 0.001$) and 180 min post exercise ($p < 0.001$). Blood lactate values showed a significant increase from resting values (lactate = 1.32 mmol/L) to a peak immediately post exercise (lactate = 11.93 mmol/L; $p <$

0.001). Lactate remained significantly higher compared to rest at 45 min ($p < 0.001$) and 90 min post exercise ($p = 0.021$). Lactate was significantly lower and showing a trend of returning to baseline levels when compared with the peak immediately after exercise at 45, 90 and 180 min post exercise ($p < 0.001$). When compared to 45 min post exercise, lactate was significantly lower at 90 min ($p = 0.004$) and 180 min post exercise ($p < 0.001$). Levels of H^+ were significantly higher compared to resting values ($H^+ = 38.34$) and at a peak value immediately after exercise ($H^+ = 60.6$; $p < 0.001$). H^+ remained higher 45 min ($p = 0.012$) and 90 min post exercise ($p = 0.019$). When compared to immediately after exercise, H^+ showed a trend of declining to baseline levels and were significantly lower at 45 min ($p < 0.001$), 90 min ($p = 0.023$) and 180 min ($p = 0.011$). Base excess displayed significant decreases immediately after exercise ($p < 0.001$), 45 min ($p = 0.003$), 90 min (0.004) and 180 min post exercise ($p = 0.005$). Base excess began to rise back to baseline levels when compared to the peak value immediately post exercise at 45, 90 and 180 min post exercise ($p < 0.001$). Hctc was only significantly lower to resting values at 45 min post exercise ($p = 0.02$).

Table 5.1 Acid base characteristics (pH, lactate, H^+, Hctc, and base excess) measured at rest, immediately, 45, 90 and 180 min post exercise (means ± SD).

Variable	Rest	Immediate	45 min	90 min	180 min
pH	7.41 ± 0.01	7.23 ± 0.04[a]	7.35 ± 0.03[ab]	7.39 ±0.01[ab]	7.40 ± 0.02[b]
Lactate (mmol/L)	1.32 ± 0.46	11.93 ± 1.52[a]	5.26 ±1.24[ab]	2.06 ± 0.53[abc]	1.64 ± 0.37[bc]
H+	38.34 ± 1.99	60.6 ± 5.89[a]	47.57 ± 7.12[ab]	40.5 ± 1.69[ab]	40.19 ± 1.41[b]
Hctc (%)	39.74 ± 3.13	41.2 ± 3.59	38.5 ± 4.44[a]	39.77 ± 4.60	40.09 ± 4.97
Base excess (mEq/L)	1.28 ± 0.57	-13.14 ± 2.52[a]	-5.12 ± 2.75[ab]	-1.28 ± 1.64[ab]	0.44 ± 0.68[abc]

[a] significantly different to rest ($p < 0.05$), [b] significantly different to immediately post ($p < 0.01$), [c] significantly different to 45 min ($p < 0.05$).

5.3.2 Performance data

5.3.3 SBP, DBP, HR and % HRmax

Table 5.2 shows the SBP, DBP, HR and % maximum HR at rest and during exercise, then for the recovery period after exercise. Data shows that SBP rose significantly immediately after exercise ($p < 0.001$) compared with resting values, and then decreased significantly ($p < 0.001$) during a recovery period of 10 min post exercise bout. SBP at 10 min post exercise had returned back to resting levels. The peak SBP

value was a mean of 139 mmHg, achieved immediately post exercise. Notably, DBP did not significantly alter at all as a result of exercise and returned to a level very similar to resting values. As expected, HR rose significantly from resting values to the peak obtained during exercise and immediately post exercise ($p < 0.001$). The peak HR recorded during exercise elevated to a mean of 176 $b \cdot min^{-1}$, which was the equivalent of 90% of maximum HR using the Karvonen formula (Camarda et al., 2008).

Table 5.2 SBP, DBP, HR and percentage of maximum HR recorded at rest, during, immediately after exercise, and during post exercise recovery in participants (means ± SD).

	Rest	Peak during exercise	Immediate	10 min
SBP (mmHg)	121.1 ± 10.21	n/a	161.0 ± 8.98[a]	126.8 ± 6.86[c]
DBP (mmHg)	77.1 ± 6.71	n/a	77.7 ± 6.68	77.6 ± 5.97
HR ($b \cdot min^{-1}$)	73.1 ± 3.98	176.2 ± 8.46[a]	163.7 ± 10.47[ad]	85.8 ± 7.18[bcd]
Maximum HR (%)	37.38 ± 2.73	90.02 ± 4.51[a]	83.65 ± 5.64[ad]	43.84 ± 3.76[bcd]

[a] significantly different to rest ($p < 0.001$), [b] significantly different to rest ($p < 0.05$), [c] significantly different to immediate ($p < 0.001$), [d] significantly different to peak during exercise ($p < 0.001$). n/a denotes no test performed for that specific time point.

5.3.4 RPE, PRR and RPM and HR during exercise

PRR and RPE both showed linear trends as they decreased and increased respectively across the sprints with significant pair wise comparisons across almost all sprints (Fig 5.1). There was a significant main effect for time found for PRR ($F = 12.968$, $p < 0.001$) and RPE ($F = 34.128$, $p < 0.001$). This suggests that as the sprints went on, participants perceived each subsequent sprint to be physically more demanding. A final sprint RPE score of 8.4 ± 1.17 shows just how physically exerting participants found the exercise bout. Equally, a PRR score in the final sprint of just 2.35 ± 0.75 shows just how demanding this exercise bout was perceived to be.

RPM decreased steadily as the sprints progressed as expected and there were several significant pair wise comparisons across sprints (Fig 5.1). There was a significant main effect for time found ($F = 3.982$, $p < 0.001$). Notably, there was no sign of any improvement from sprint 9 to sprint 10 (decrease from 134.3 ± 11.78 to 132.6 ± 12.33) even though participants were aware that was the final sprint, which strengthens the high intensity nature of the exercise.

HR reached a peak value after sprint 7 (175 $b \cdot min^{-1}$), which was a 10% increase from that recorded after sprint number 1 (Fig 5.1). For the remaining few sprints, HR remained very close to this peak value but with a slight decrease. Additionally, $HR_{recovery}$ showed a significant main effect for time ($F = 39.574$, $p < 0.001$), indicating an increase in $HR_{recovery}$ as sprint number increased. The $HR_{recovery}$ never increased significantly beyond the level it reached after sprint 3, and nor did it fall significantly thereafter**.**

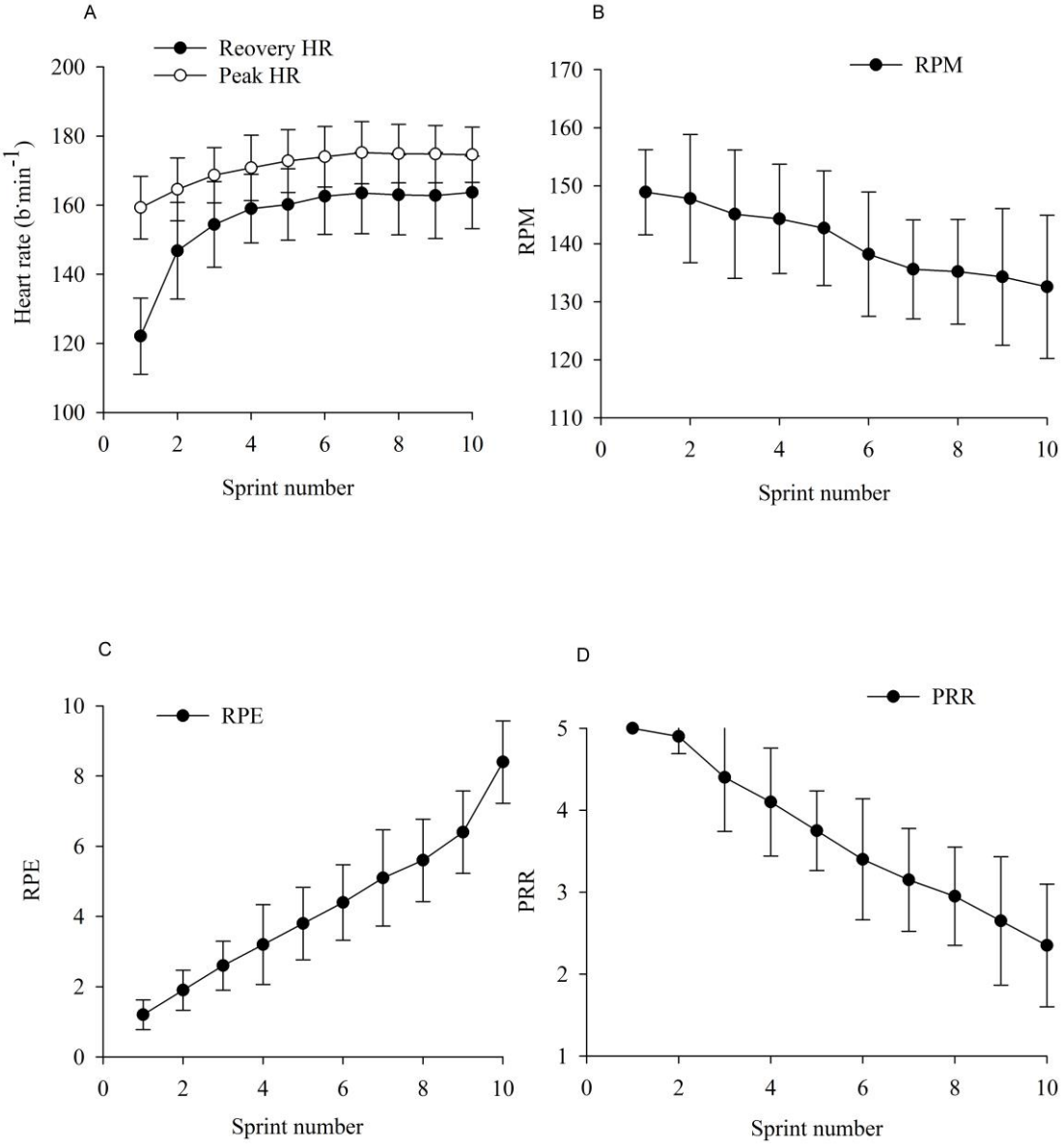


Fig. 5.1 Performance measures during exercise. A displays HR (both recovery and peak) as sprints progressed. B shows the peak RPM achieved for each sprint, C indicates the RPE scores with increasing sprints, and D displays the PRR of participants. Significance marks have been left out for better clarity due to the great amount of significant data between data points. Error bars represent mean ± SD.

5.3.5 CD105+ MP

Levels of CD105+ MP are expressed at rest and during a 180 min post exercise recovery period are represented in Fig 5.2. CD 105+ MP were significantly different from immediately post exercise to 90 min post exercise ($p = 0.042$), characterised by a mean increase from 791 CD105+ MP per µl PFP immediately post exercise to 1574 CD105+ MP per µl PFP at 90 min post exercise, a mean increase of 92.3%. CD105+ MP also decreased from the peak value down to levels that appear close to resting levels at 180 min post exercise, however no significance was found.

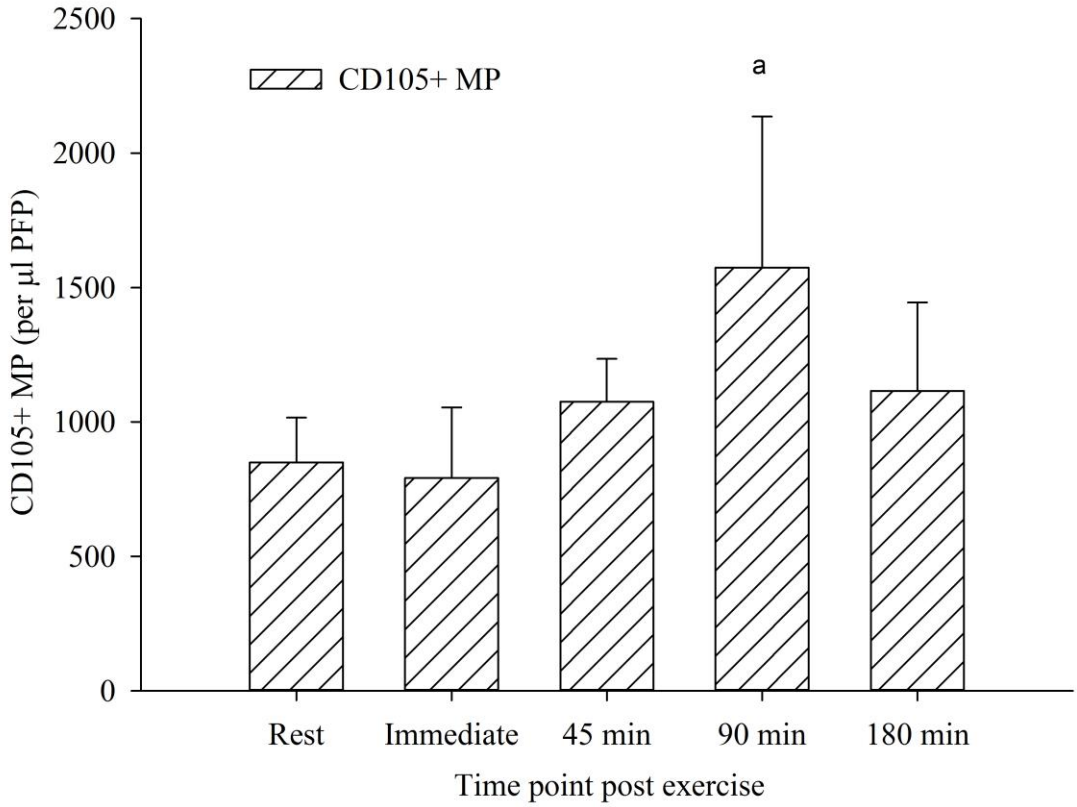


Fig. 5.2 CD105+ MP at rest and immediately, 45, 90 and 180 min post exercise in participants. Data represented as the concentration of CD105+ MP per µl PFP (mean ± SEM). [a] significantly greater than immediately post exercise ($p = 0.042$). Error bars represent mean ± SEM.

5.3.6 CD106+ MP

Levels of CD106+ MP are expressed at rest and during a 180 min post exercise recovery period are represented in Fig 5.3. CD106+ MP were increased from 1959 CD106+ MP per µl PFP at rest, to 2880 CD106+ MP per µl PFP at 90 min post exercise, an increase in 104.5%. Despite this, there was no significant difference in CD106+ MP. CD106+ MP also decreased from the peak value down to levels that appear close to resting levels at 180 min post exercise, however no significance was found.

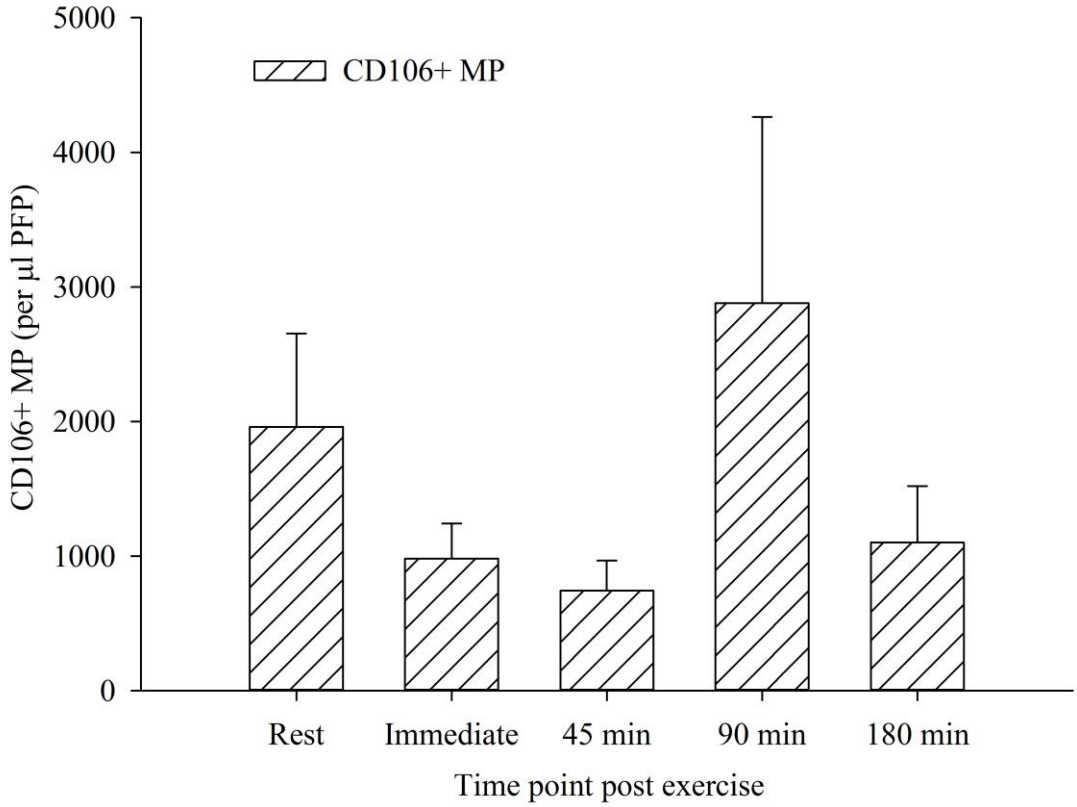


Fig. 5.3 CD106+ MP at rest and immediately, 45, 90 and 180 min post exercise in participants. Data represented as the concentration of CD106+ MP per µl PFP (mean ± SEM). Error bars represent mean ± SEM.

5.4 Discussion

This chapter provides evidence that exercise of a highly strenuous nature is sufficient to elicit ED in healthy females, and that signs of a rapid recovery of the endothelium is evident. The study further strengthens the evidence for the use of such markers as a measure of EF.

In comparison to the findings in chapter 4, it is visible from Fig 5.2 and Fig 5.3 that the trend of EMP quantity follows an almost identical pattern for both markers used, more of which is detailed in chapter 9. Females showed a peak at 90 min for both markers, and a trend for a return to basal levels at 180 min, which is the same as the healthy males study. However, the only significant value in this study was for CD105+ MP from immediately post exercise to the peak at 90 min. As discussed earlier, Toth et al (2007) are a group who assessed if there were any gender specific differences in the amount of MP in healthy males and females. EMP (CD62E+) were elevated in women compared with men. It is also intriguing from this study that circulating MP were maximal during the luteal phase of the menstrual cycle, because this may suggest an unknown influence of ovulation and luteal function on the release of MP (Toth et al., 2007). This may also reflect the low overall physical activity in females compared to males, which eventually leads to impaired EF (Hwang et al., 2012). It would have been useful and of clinical value to have recorded the menstrual phase of the individuals in this chapter.

Another aim of this study was to determine the pattern of ED post exercise by adding in an extra blood draw at 45 min post exercise, as it would be assumed that the higher the level of EMP the greater the ED. In the male study in chapter 4, the peak value was found to be at 90 min post exercise, but it was unclear if levels of EMP were actually falling, or still rising. In the present chapter, the greatest amount of ED was indicated to occur at 90 min post exercise for both markers of EF. The addition of 45 min sampling showed that CD105+ MP were higher than at baseline, but CD106+ MP was actually lower than baseline levels, allowing to draw a speculative assumption that that the greatest amount of ED occurs between 45 min and 180 min post exercise. This finding has possible impact and relevance in prescribing exercise programmes and in recovery, suggesting that the endothelium expresses greatest detriments approximately 1 to 3 hr post intense exercise, but should recover or show signs of recovery in both healthy male and female individuals within 3 hr of strenuous activity. MP production and clearance has been studied in animal models, but very few studies have investigated human clearance (Rank et al., 2011). Augustine et al (2014) recently investigated a large cohort of 119 patients referred for stress echocardiography. After FC analysis, EMP (CD31+ CD41-) were elevated immediately after a stress echocardiogram and decreased to baseline levels after 1 hr. These findings show that EMP released during cardiac stress are significantly reduced within 1 hr, meaning that EMP are cleared from the circulation during this time. Although the stress induced and marker of EF differed, these results owe to those in the present chapter in that there is an obvious decrease, and therefore clearance, in MP following exercise stress within hours.

It is known that certain subtypes of MP, more commonly PMP, increase after strenuous exercise in healthy individuals (Maruyama, Kadono & Morishita, 2012; Sossdorf et al., 2011), suggesting that MP release is intensity dependent and that MP have a physiological function to remove stress induced cellular by products (Augustine et al., 2014; Dignat-George & Boulanger, 2011). There were similarities between a study that looked at MP release after anaerobic exercise and the present chapter (Maruyama, Kadono & Morishita, 2012). Drawing comparisons is difficult however, not only because Maruyama, Kadono & Morishita (2012) measured PMP, but they also used an ELISA technique, which differs to that used in this chapter. This group studied 18 healthy volunteers which was a combination of 9 male and 9 female participants, with a mean age difference of just 3 years to the current chapter. When referring back to the results of this chapter, the exercise intensity adhered to in Maruyama, Kadono & Morishita (2012) study are lower, as a mean maximal HR of 90% was met in the present study compared to 85% in the aforementioned study. This group also measured SBP post exercise, and this figure was significantly higher immediately after exercise, possibly accounting for the elevations in PMP. PMP levels were significantly increased after exercise, and were prolonged for up to an hr after exercise. The fact that this experiment combined male and female data means it is not clear if gender specific differences affect MP release after acute exercise. The fact that SBP significantly increased after exercise in the current study may also account for the increase in EMP that was seen post exercise. The significant increases in HR both during exercise, and immediately post exercise, may also contribute to the rises in EMP that were witnessed.

In conclusion, a strenuous bout of acute exercise is enough to elicit a significant rise in EMP in a group of healthy females. This population is also capable of showing recovery

of the endothelium towards resting levels within 3 hr. These findings also lead towards shear stress as an important factor in the release of EMP (Sossdorf et al., 2011; Vince et al., 2009b) as shown by the high levels of exercise intensity and significant increases in HR and SBP after exercise, although these were not tested sufficiently to correlate with EMP quantity. This chapter further strengthens the use of CD105+ MP and CD106+ MP as markers of EF in a population of healthy females, adding to the concept of use in healthy males from the previous chapter.

Chapter 6: Investigation into the effect of a long duration intermittent cycling protocol on the EMP release in healthy males

6.1 Introduction

The previous two chapters studied the effects of highly strenuous, acute exercise on EMP release and the use of CD105 and CD106 as novel biomarkers to characterise the endothelial response to exercise in healthy individuals. The findings showed that in both males and females, EF appears to be most impaired at 90 min post exercise, and that the endothelium has the ability to repair itself within a relatively short time frame of 3 hr. Little is known about the acute effects of prolonged, strenuous exercise on the vascular endothelium as this area has not been well studied (Dawson et al., 2008). However, there have been recent studies that have attempted to look at a more prolonged bout of acute exercise on the endothelium in healthy individuals using an array of assessment methods (Bartzeliotou et al., 2007; Jee & Jin, 2012; Ramirez-Velez, Ramirez & Aguilar, 2010; Smith et al., 2013).

Ramirez-Velez, Ramirez & Aguilar (2010) tested the femoral artery (FA)-FMD, posthyperemic FA diameter (FA-P) and baeline FA diameter (FA-B) changes of 9 healthy recreational male runners participating in a half marathon (21,097 m distance, completed in a range of 96-121 min). FMD was recorded prior to the race and at 1, 24, 48 hr and 6 days post race. One hr after the race, EF was impaired significantly when compared to resting levels, but returned back to baseline levels thereafter as measured by changes in FA-FMD. Dawson et al (2008) confirmed these findings following the London Marathon (distance 42.2 km) in 15 healthy, non-elite males. FA-FMD was reduced post marathon, whereas BA-FMD was not impaired, suggesting a depressed EF as a result of prolonged exercise. Further to this, Smith et al (2013) performed a case study on 3 marathon runners participating in a full marathon (26.2 miles) and the

runners were selected due to their differences in training history and fitness levels. One runner had a history of marathon running (participant A), one was relatively new to running but was following a standard marathon training schedule (participant B), and one ran very minimally but participated regularly in HIIT (participant C). BA-FMD was used to assess EF at baseline, within 90 min of crossing the finishing line and at 24 and 48 hr following the race. All participants showed impaired EF immediately following the race compared with baseline values. All of the participants showed full restoration of baseline FMD values by 48 hr post marathon race. There was however no statistical analyses performed during this study, and so it is not possible to discuss the significance of the data due to the limited sample size. Furthermore, it was not clear what impact factors such as age, diet and body composition may have on the findings of this case study because of the limited number of participants. The results of this case study did however show that a single bout of prolonged, strenuous exercise resulted in impairment of EF in the participants tested in agreement with other studies (Dawson et al., 2008; Ramirez-Velez, Ramirez & Aguilar, 2010; Sossdorf et al., 2011).

There have been recent studies looking at MP release after moderate endurance exercise of 90 min in duration. Sossdorf et al (2010) studied 16 males who underwent 90 min of exercise on a cycle ergometer with a constant power of 80% of their individual IAT). Blood samples for the measurement of EMP (CD62E+) as analysed by FC were made pre, post, 45 min and 2 hr post exercise. CD62E+ MP levels remained unchanged as a result of exercise. This group (Sossdorf et al., 2011) then studied 16 healthy males who followed the exact protocol as above (Sossdorf et al., 2010) but divided participants up into 8 trained (VO_{2max} > 65 $ml \cdot min^{-1} \cdot kg^{-1}$) and 8 untrained ($VO_{2max} \leq 50$ $ml \cdot min^{-1} \cdot kg^{-1}$). The exercise protocol resulted in a significant increase in CD62E+ MP in the trained group 45 min after exercise had ceased, but not in the untrained group. This group

suggested that the rise in EMP seen in the trained group may have been due to shear stress factors, and this is supported by the data of Chen, Chen & Wang (2010) who suggested that shear stress is a major trigger of MP release during physical exercise. Sossdorf et al (2011) showed that there was a significantly higher SBP in the trained group compared with the untrained group, at each of the measured time points during exercise itself. Although the concentration of EMP was not correlated with SBP, it may be suggested that the significant rise in EMP in the trained group may be due to the significant rise in SBP compared with untrained individuals. This study may also provide differences in the kinetics and dynamics of EMP release and clearance, as evidenced from the almost continuous, steady increase in EMP in untrained participants, reaching maximum levels at 2 hr post exercise (the final blood draw taken). However, in the trained group, the peak value was reached at an earlier time point of 45 min post exercise, and levels of EMP were falling at 2 hr post exercise.

The purpose of this chapter was to investigate the effects of a longer duration, intermittent exercise protocol on levels of CD105+ MP and CD106+ MP in healthy male participants and to investigate the possible relationship between changes in shear stress and EMP release.

6.2 Methods

6.2.1 Participant characteristics

Fourteen healthy, non-smoking, physically active male participants were recruited for this study (mean ± SD, height, body mass, age, absolute PPO and relative PPO: 179.5 ± 5.5 cm, 76.5 ± 6.8 kg, 23.2 ± 2.9 years, 315 ± 36 W and 4.15 ± 0.59 $W \cdot kg^{-1}$). None of the participants had been training excessively in the past two months, nor had they been supplementing their diet with any ergogenic aids prior to testing and ethical approval and informed consent was gained (as detailed in section 3.1).

6.2.2. Experimental design

Participants reported to the laboratory on two separate occasions, each separated by one week. Visit 1 considered of a PPO test (described in section 3.3), which preceded a familiarisation to the intermittent cycling protocol. The following visit consisted of a 90 min intermittent cycling protocol which was made up of 10 x 9 min blocks. The 9 min blocks began with 306 s at 40% PPO, followed by 60% PPO for 204 s, and then an 8 s period with no load before participants were asked to sprint for 14 s at 120% PPO (in the same sprint intensity used in chapters 4 and 5), before an 8 s recovery at no load until the next block started again. HR was recorded prior to each sprint ($HR_{recovery}$) and after each sprint (HR_{peak}), and RPE and PRR were recorded prior to each sprint.

6.2.3 Blood sampling

Capillary blood samples were taken at rest and at 18 min intervals during the exercise for the measurement of acid base variables (pH and lactate) and analysed as described in section 3.6.1 of chapter 3.

Venous blood samples for subsequent EMP quantification were made as described in section 3.7, and were taken at rest, immediately post (immediate), 45 min, 90 min and 180 min post exercise and were analysed as described in section 3.7 and 3.7.1 of chapter 3. CD105+ MP and CD106+ MP were measured by FC as detailed in section 3.8.

6.2.4 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 19.0. Any changes in biochemical markers across time were analysed using a paired samples t-test, and data is expressed as the quantity of MP per µl PFP. The change in acid-base status and haemodynamic values from rest over time were investigated using paired samples t-tests with Sidak-adjusted p values to control for family-wise type I error rate. All of the data are presented as means (± SD) unless otherwise stated. Two-tailed statistical significance was accepted at $p < 0.05$.

6.3 Results

6.3.1 Acid-base homeostasis

Exercise significantly decreased pH and increased blood lactate compared to resting levels (Table 6.1). pH rose to a peak value at 72 min and remained elevated 90 min into exercise, which were both significantly greater than at rest ($p < 0.001$). Blood lactate levels reached a peak value of 7.98 mmol/L (at 54 min) compared to a resting value of 1.26 mmol/L ($p < 0.001$). Lactate levels then declined significantly from this peak value to the end of the exercise ($p = 0.015$).

Table 6.1 Acid base characteristics at rest and subsequently at 18 min intervals throughout the exercise (means ± SD).

Variable	Rest	18 min	36 min	54 min	72 min	90 min
pH	7.41 ± 0.01	7.34 ± 0.02[a]	7.35 ± 0.02[ab]	7.36 ± 0.04[ab]	7.38 ± 0.05[abcd]	7.38 ± 0.04[abc]
Lactate (mmol/L)	1.26 ± 0.46	7.15 ± 0.83[a]	7.64 ± 0.77[ab]	7.98 ± 1.54[abc]	7.77 ± 1.43[ab]	7.41 ± 1.05[abde]

[a] significantly different to rest ($p < 0.001$).[b] significantly different to 18 min ($p < 0.001$). [c] significantly different to 36 min ($p < 0.001$). [d] significantly different to 54 min ($p < 0.05$).[e] significantly different to 72 min ($p = 0.020$).

6.3.2 HR data

HR data is shown in Fig 6.1. $HR_{recovery}$ showed a consistent trend across the duration of exercise whereby $HR_{recovery}$ reached a peak value at sprint 4 (161 $b \cdot min^{-1}$) and thereafter this value was maintained within a very small range (159-161 $b \cdot min^{-1}$) (Fig 6.1). There was a significant rise in $HR_{recovery}$ from sprint 1 to sprint 4 ($p = 0.007$), and this value was still significantly greater at sprint 10 compared to the first sprint ($p = 0.018$). HR_{peak} followed a steady increase as the exercise progressed for the first 3 sprints (rising from 170 to 174 $b \cdot min^{-1}$) until values hit a plateau, with HR remaining at 174 $b \cdot min^{-1}$ from sprint 3 to sprint 8. HR_{peak} was greatest at sprint 9 (176 $b \cdot min^{-1}$), which was significantly greater than sprint 1 ($p < 0.001$).

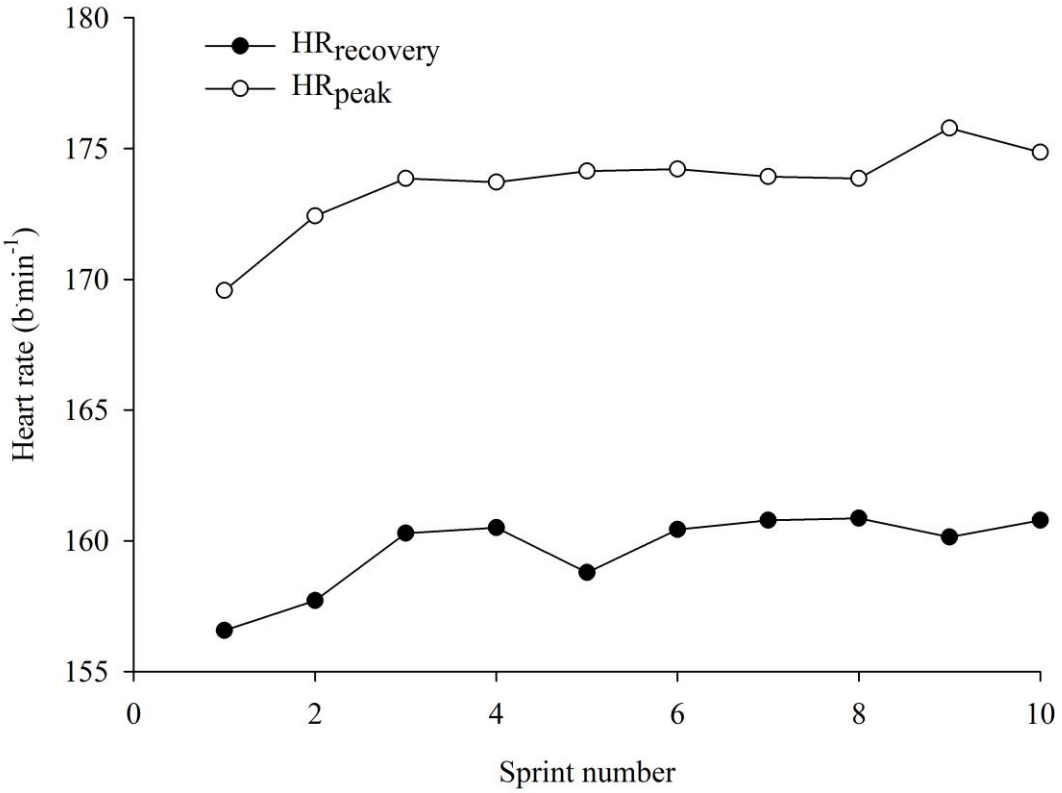


Fig. 6.1 $HR_{recovery}$ (black circles) and HR_{peak} (white circles) across sprints 1-10. Significance marks and error bars have been left out for better clarity due to the large amount of significant data between data points.

6.3.3 PRR and RPE and during exercise

PRR and RPE both showed linear trends as they decreased and increased respectively across the sprints with significant pair wise comparisons across almost all sprints (Fig 6.2). There was a significant main effect for time found for PRR ($F = 132.25$, $p < 0.0005$) and RPE ($F = 434.82$, $p < 0.0005$). This suggests that as the sprints went on, participants perceived each subsequent sprint to be physically more demanding. A final sprint RPE score of 9.71 ± 0.61 demonstrates just how physically exerting participants

found the exercise bout. Equally, a PRR score in the final sprint of just 1.0 ± 0 shows just how demanding this exercise bout was perceived to be.

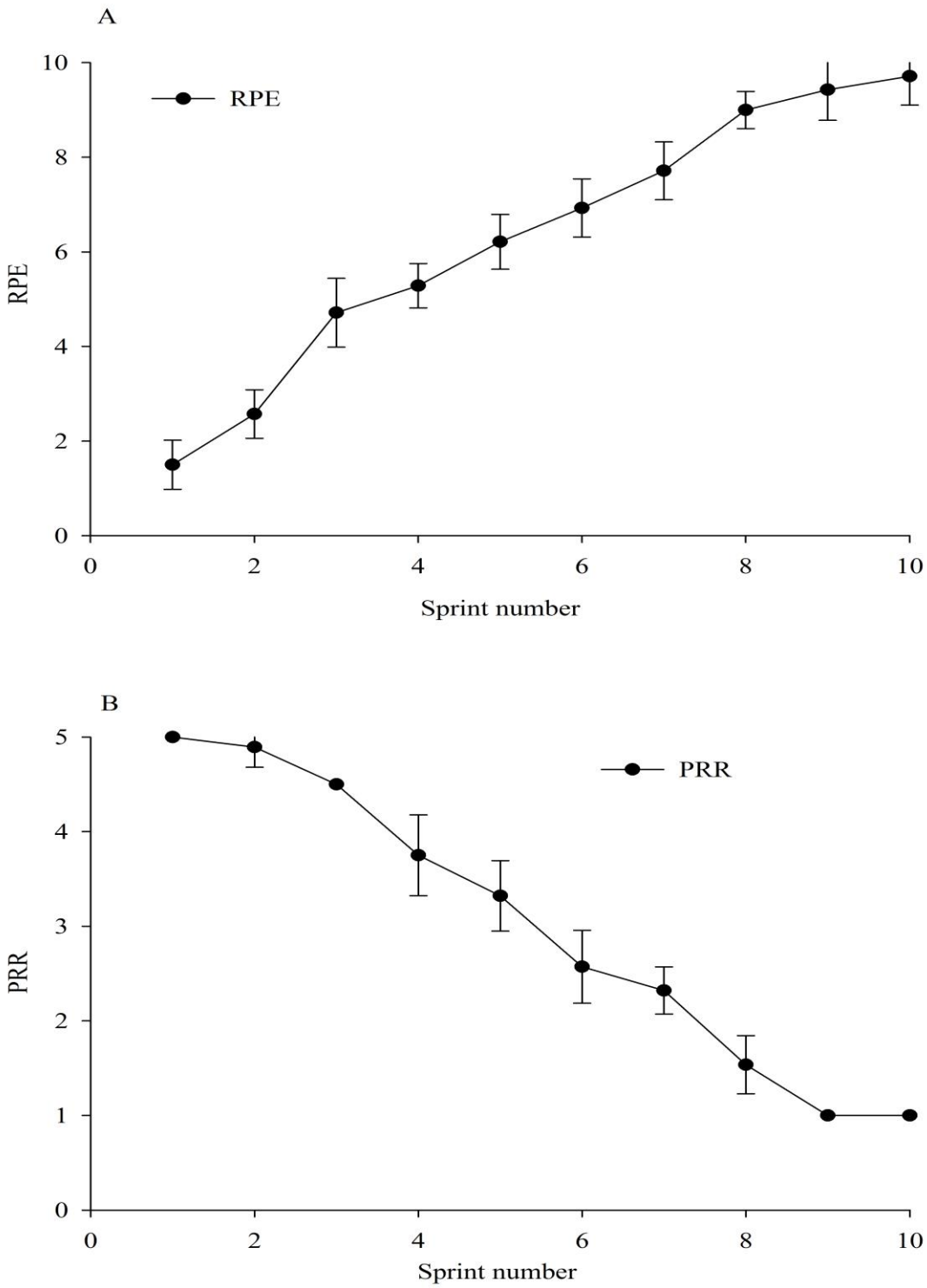


Fig 6.2 Performance measures during exercise. A displays RPE as sprints progressed and B shows the PRR achieved for each sprint. Significance marks have been left out for better clarity due to the large amount of significant data between data points.

6.3.4 CD105+ MP

CD105+ MP at rest and during the 180 min post exercise recovery is displayed in Fig 6.2. The mean quantity of CD105+ MP increased significantly from rest to a maximum level at 90 min post exercise, which was a 66% increase ($p = 0.019$). Levels of CD105+ MP were also significantly higher at 45 min post exercise compared to rest ($p = 0.013$) and immediately post exercise ($p = 0.019$). During recovery, CD105+ MP decreased from 45 min to 180 min ($p = 0.006$) as well as from 90 min to 180 min post exercise ($p = 0.009$), indicating a restoration of the endothelium. In fact, the concentration of CD105+ MP had decreased from resting values to those measured at 180 min post exercise ($p = 0.04$).

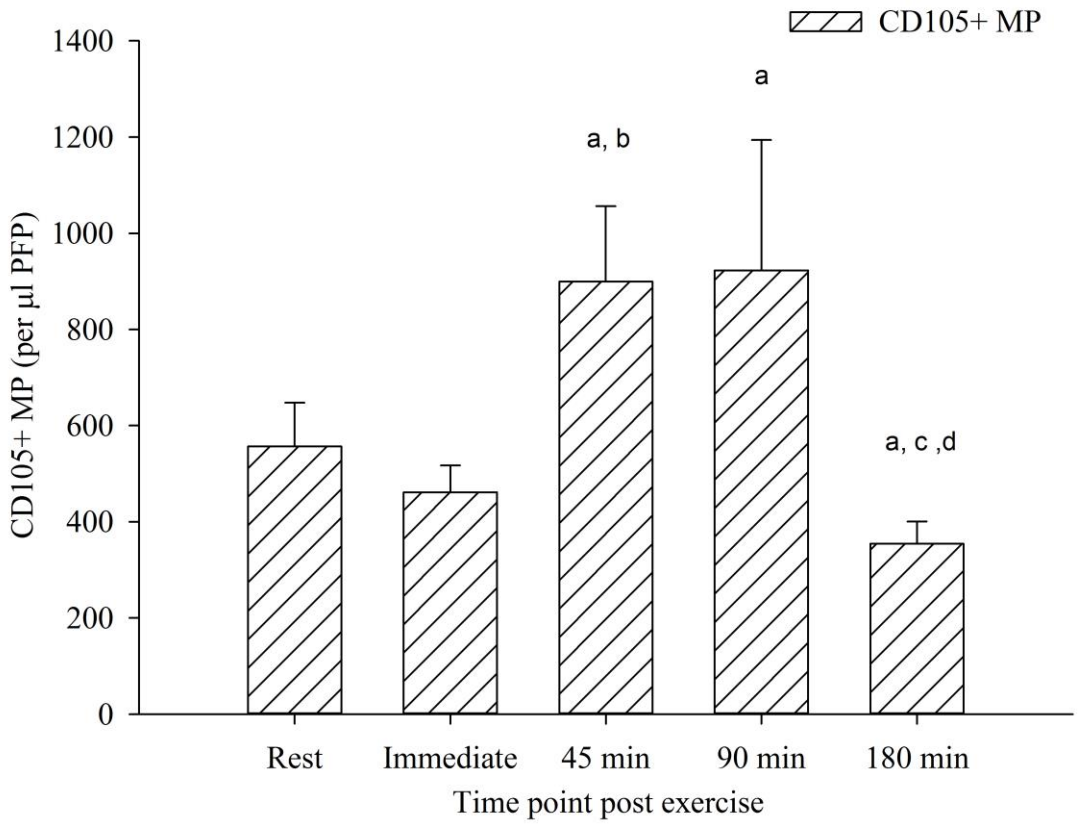


Fig 6.3 CD105+ MP at rest, and immediately, 45, 90 and 180 min post exercise in participants (n =14). Data represented as the concentration of CD105+ MP per µl PFP (mean ± SEM). [a] significantly different to rest. [b] significantly different to immediately post exercise. [c] significantly different to 45 min post exercise. [d] significantly different to 90 min post exercise. Error bars represent mean ± SEM.

6.3.5 CD106+ MP

CD106+ MP at rest and during the 180 min post exercise recovery is shown in Fig 6.3. The highest values of CD106+ MP were actually at rest, with a value of 3762 CD106+ MP per µl of PFP. This value was significantly greater than immediately post exercise ($p = 0.044$), and 180 min post exercise ($p = 0.015$). Ignoring the value at rest, the peak value post exercise was at 45 min (3317 CD106+ MP per µl PFP). The levels of

CD106+ MP declined significantly from 45 min to 180 min post exercise ($p = 0.022$), and also from 90 min to 180 min ($p = 0.04$).

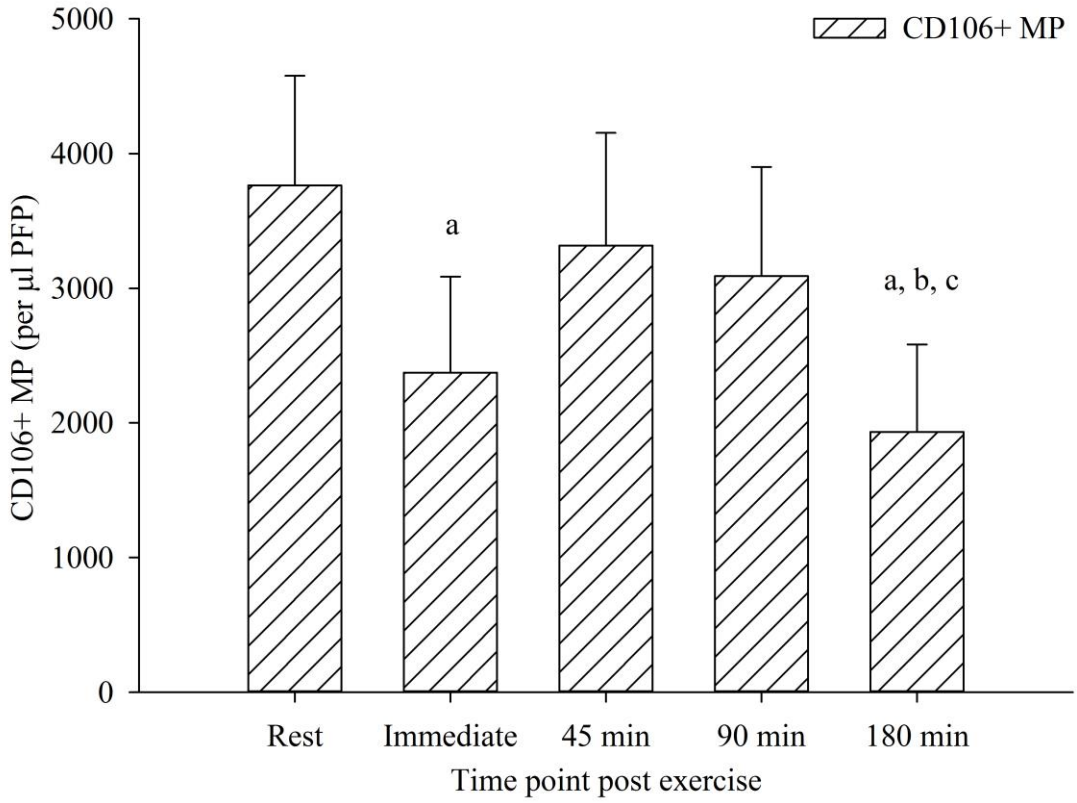


Fig. 6.4 CD106+ MP at rest, and immediately, 45, 90 and 180 min post exercise in participants (n =14). Data represented as the concentration of CD106+ MP per µl PFP (mean ± SEM). [a] significantly different to rest. [b] significantly different to 45 min post exercise. [c] significantly different to 90 min post exercise. Error bars represent mean ± SEM.

6.4 Discussion

The main finding from this chapter is that a single bout of sustained high intensity aerobic exercise is sufficient to elicit ED in healthy male participants and besides the exhaustive nature of this exercise, their endothelium seems to be able to recover effectively within 3 hr of ceasing exercise. Additionally, this chapter further strengthens the use of CD105+ MP and CD106+ MP as markers of EF. Previous studies (Bartzeliotou et al., 2007; Jee & Jin, 2012; Ramirez-Velez, Ramirez & Aguilar, 2010; Smith et al., 2013; Sossdorf et al., 2011) have shown that a single bout of sustained high intensity aerobic exercise resulted in impairment of EF in healthy participants, with baseline values also restored within a relatively short time frame in those that measured into recovery (Bartzeliotou et al., 2007; Ramirez-Velez, Ramirez & Aguilar, 2010; Smith et al., 2013), following findings from the current chapter.

Considering that EMP are considered markers of ED (Horstman et al., 2004), then the highest concentration of CD105+ MP and CD106+ MP within the circulation could speculatively be regarded as the time at which EF is at its most poor, within the testing period. This point occurred at 90 min post exercise for CD105+ MP, and when looking at post exercise values for CD106+ MP, this value occurred 45 min post exercise. Further to this, it may be suggested that the lowest concentration of these markers may represent the time at which EF is most optimal. In both markers of EF, this point occurred at 180 min post exercise, therefore representing the peak EF. These results indicate that EF was impaired after exercise up to 90 min post exercise, and that the endothelium was able to be restored thereafter. This finding is also consistent with the previous two experimental chapters, as the exercise in these two chapters produced the

greatest detriment in EF 90 min post exercise within the time frame measured. Sossdorf et al (2011) also demonstrated that CD62E+ MP were highest 45 min after exercise of moderate intensity (80% IAT) in a 'trained' group of healthy male participants, suggesting that the highest level of ED occurred within this time period after exercise as determined by this biomarker. Participants in the current chapter were not assessed in terms of their VO_{2max} scores, but the fact that they were all able to successfully complete this demanding exercise bout is suggestive of a group of trained athletes accustomed to sustained high intensity aerobic exercise. Additionally, the HR_{peak} and $HR_{recovery}$ measured during exercise in the current chapter appear higher than those recorded in the study by Sossdorf et al (2011) for both trained and untrained groups, albeit measured at slightly different time points during exercise. Future work may be useful in studying the release kinetics of MP during exercise, but would require a continuous set of blood draws and the difficulties of practically assessing this would be high. Sossdorf et al (2011) are only able to speculate the difference in kinetics of MP release between their two groups (trained and untrained). It may be suggested however that this speculated difference is due to the significant differences between the two groups in terms of their performance measures, as the trained group had significantly higher maximum power, IAT and relative heart volume, meaning it is difficult to compare groups, particular based on only four blood draws.

Jee and Jin (2012) investigated the EF response to a 308 km ultra-marathon race in 24 male marathon runners. This marathon places huge physical demand on the athletes participating, and the distance covered is significantly greater compared to the current chapter and other studies previously mentioned (Ramirez-Velez, Ramirez & Aguilar, 2010; Smith et al., 2013). Jee and Jin (2012) assessed EF by identifying changes in serum markers through an ELISA technique 2 hr before, 100 km into, 200 km into, and

immediately after (at 308 km) the ultra-marathon. Serum analysis of creatine kinase (CK), CRP, TNF-α, sCD106 and sCD62E was carried out at the aforementioned time points. This group showed that sCD106 and sCD62E were significantly increased, thus suggesting EF was impaired, when compared with resting levels at each of the measured time points. Levels of CK, CRP and TNF-α all showed significant increases in the same manner as the soluble adhesion molecules measured. Unfortunately, blood was only taken during the exercise itself and immediately post, and there was no recovery data available in order to see any possible restoration of the endothelium. The significantly greater increases in sCD106 and sCD62E at 100 km and again at subsequent time points suggest a relationship between exercise intensity and EF, a finding suggested in the current chapter as well as previous research (Chen, Chen & Wang, 2010; Nielsen & Lyberg, 2004; Sossdorf et al., 2011). Also, in the study by Jee & Jin (2012), a significant relationship was found between running speed and sCD106, whereby increased levels of sCD106 were strongly correlated to running speed, further supporting a positive relationship between vascular function and exercise intensity.

The current chapter witnessed significant changes in HR (Fig 6.1), lactate and pH (Table 6.1), suggesting that this protocol can be considered an exhaustive exercise protocol in a group of physically active, healthy persons. This was further evidenced by a significantly increased RPE score and significantly reduced PRR score (Fig 6.2) from the onset of exercise, and one that corresponded to maximal effort in the final part of exercise (Foster et al., 2001). This further supports that a relationship may exist between exercise intensity/shear stress factors and EF (Jee & Jin, 2012; Sossdorf et al., 2011), adding to the suggestion that these mechanisms contribute to EMP release (Vince et al., 2009b). Very recently, Jenkins et al (2013) were able to show *in vivo* that disturbed blood flow acutely induces endothelial activation and apoptosis, as reflected by a

release of activated (CD62E) and apoptotic (CD31+/CD42b-) EMP measured by FC. Ten healthy male participants were studied and cuff inflation occurred on the one arm, while the other arm was used as a control arm for a period of 20 min. This study provides data of an *in vivo* experiment of disturbed blood flow inducing endothelial damage in healthy humans. Due to the complex design and implementation issues that would be required, the present chapter was unable to measure shear stress in detail, but future work should investigate this further by incorporating shear stress measurements during exercise modes and a recovery period in order to quantify and form relationships with EMP and shear stress in humans more closely. This paper also reflects upon a mechanism that may be inducing this endothelial response. Jenkins et al (2013) suggest that disturbed blood flow confers an injurious stimulus, as would be seen during exercise, and it would therefore be reasonable to expect that circulating EMP could preferentially originate from classically atheroprone parts of the vasculature (e.g. branch points, curvatures, bifurcations), as these regions are characterised by disturbances in flow (Jenkins et al., 2013). Exhaustive, intense exercise induces various significant physiological changes such as an increased heat production, HR, leukocyte count and shear stress (Suzuki et al., 1999). Additionally, increased blood flow is an observation common with increased exercise (Bartzeliotou et al., 2007), existing due to higher oxygen demand in the working muscles and a subsequent increase in cardiac output (Jee and Jin, 2012). Increased exercise intensity causes an increased resistance or laminar shear force by the blood on the blood vessel walls which could be enough to elicit activation of endothelial cell markers (Marsh and Coombe, 2005; Stewart et al., 2004), as seen in the present chapter.

This chapter was able to demonstrate that a prolonged bout of strenuous cycling exercise was sufficient enough to observe some endothelial damage in healthy male

participants as evidenced by a significant change in CD105+ MP. This chapter also showed that although the exercise was exhaustive in nature, the endothelium in these participants recovered effectively within the time frame analysed. There is further suggestion that shear stress factors appear to be important in the release of EMP and warrants further detailed investigation as a result of exercise (Jenkins et al., 2013).

Chapter 7: EF response to acute exercise in healthy, sedentary individuals measured using EMP and EndoPAT-2000

7.1 Introduction

A healthy endothelium generally exerts a vasodilator response on vascular smooth muscle (Axtell, Gomari, & Cooke, 2010). As already discussed in previous chapters, a number of methods exist for assessing EF, including EMP (Horstman et al., 2004) and FMD (Barac, Campia & Panza 2007). Emerging evidence has supported the assessment of digital PAT as a measure of EF using the EndoPAT-2000 device, which is a new approach to assessing EF in a rapid, non-invasive manner (Axtell, Gomari & Cooke, 2010; Hamburg & Benjamin, 2009). The device works by recording endothelium-mediated changes in the digital pulse waveform known as the PAT signal, which are measured by a pair of plethysmographic probes situated on the index finger of each hand (Axtell, Gomari & Cooke, 2010).

Previous studies investigating the relations of EndoPAT-2000 and clinical risk factors have been limited to small, selected samples (Hamburg & Benjamin, 2009). Furthermore, studies that have assessed both EndoPAT-2000 and EMP are very limited, and this remains a particular novel area of research (Bruyndonckx et al., 2013). A very recent study looked at assessing serum concentrations of sCD54, sCD106, sCD62E and sCD62P by ELISA. This group also measured EF by EndoPAT-2000 in in 25 women with PCOS alone, or PCOS and non-alcoholic fatty liver disease (13 with Non-alcoholic fatty liver disease; NAFLD, 12 with PCOS only) (Dawson et al., 2014). All of the aforementioned variables were the same in both groups, and the results of this study may suggest that there is no difference in the measure of EF when using EndoPAT or serum markers of EF (CD54, CD106, CD62E and CD62P), although statistical significance between the assessment methods weren't tested specifically.

RH-PAT has previously been shown to correlate with other measures of EF. BA-FMD was correlated with RH-PAT in male and female patients suffering with chest pain (Kuvin et al., 2003). Eighty-nine patients in total were assessed (54 men and 35 women) by EndoPAT-2000 and compared with the findings from data using BA-FMD in the 89 subjects. A linear relationship was present between the PAT hyperaemia ratio and FMD, supporting the concept that analysis of PWA with PAT during reactive hyperaemia may be used to study EF. EndoPAT is used due to its non-invasive manner, and has been shown to be a suitable tool to assess ED (Flammer et al., 2012). The EndoPAT was developed because of the limitations in the FMD, which include being operator dependent (Vogel, Corretti & Plotnick, 2000), and that it is only measured in one arm (Moerland et al., 2012). Results from a recent Framingham Heart Study (Hamburg et al., 2008b) provided positive, reliable data when using the EndoPAT-2000 device. Almost 2,000 individuals were assessed and the EndoPAT-2000 was shown to have an inverse association with several cardiovascular risk factors, such as BMI, total/HDL cholesterol, diabetes and smoking, with a positive association found with age (Hamburg et al., 2008b). Despite these positive findings, contrasting results do exist (Moerland et al., 2012). A recent study investigated acute changes in EF using EndoPAT-2000 in 6 renally impaired patients and 16 male and female patients with T2DM (Moerland et al., 2012). Results showed that EF in renally impaired and T2DM patients was not different when compared to healthy volunteers using the EndoPAT (Moerland et al., 2012). Furthermore, the EndoPAT-2000 failed to detect the effect of robust interventions on EF in healthy volunteers, such as glucose loading (75 g glucose in 300 ml consumed within 4 min) and smoking (6 males were smokers ranging from 1-20 cigarettes per day, and participants smoked a cigarette within 4 min of baseline measures), when measured at 30 and 90 min after these respectively. These findings

therefore suggest that EndoPAT-2000 may not be suitable to assess any change in EF in clinical pharmacological studies.

There is a keen interest in studying peripheral cutaneous blood flow responses to exercise due to the unique physiology governing this stressor (Rozanski et al., 2001). Core body temperature is known to increase during exercise, and so the central nervous system selectively decreases its tonicity to the peripheral cutaneous vascular beds, then prompting peripheral vasodilation (Yarbrough & Bradham, 1998). In turn, the increase in finger pulsatile blood volume is an expected response to exercise (Rozanski et al., 2001). The effects of exercise on the EndoPAT score has not been studied extensively (Onkelinx et al., 2012), but recently Kurose et al (2012) looked at the improvement in EF after a six month exercise programme. Forty-three obese patients underwent 30 min on a cycle ergometer or treadmill 3 times a week for 6 months. EndoPAT-2000 was used to assess EF at baseline and at 6 months follow up after the exercise programme. Significant improvements were observed in the RH-PAT index after the programme, and analysis revealed that changes in IR were independently correlated with changes in the RH-PAT index and was the only independent factor influencing improvement in EF. In terms of acute exercise, Chenzbraun et al (2001) investigated the effects of exercise on vascular responses evaluated by PAT in 30 participants with coronary artery disease (CAD) and 30 healthy participants. Altered peripheral vascular responses were detected during and 3 min after a Bruce exercise protocol (Chenzbraun et al., 2001). Interestingly, compared to baseline, the EndoPAT-2000 scores at peak exercise decreased in the participants with CAD, but increased in the controls, showing a different pattern in those with risk factors for atherosclerosis.

The aim of this chapter was to profile the effect of acute exercise on the endothelial stress response in healthy, but otherwise sedentary individuals in comparison to the trained participants used previously, and to investigate the effects of exercise and the correlation between the use of EMP and EndoPAT-2000 for determining EF in both male and female participants.

7.2 Methods

7.2.1 Subject characteristics

Ten healthy, non-smoking participants volunteered for the study, 4 female (mean ± SD, height, body mass, age, VO_{2max}: 174 ± 6.9 cm, 72.9 ± 13.1 kg, 30 ± 8.9 years, 31.74 ± 4.6 $ml \cdot kg^{-1} \cdot min^{-1}$). All ethical approval and pre-test procedures were made in accordance with the information that was provided in general methodologies in section 3.1. An additional inclusion criterion was that all participants were sedentary (no history of regular exercise in the past three months) prior to testing commenced.

7.2.2 Experimental design

Participants reported to the laboratory on two separate occasions, each separated by one week. Visit 1 consisted of an initial screen (anthropometric data and eligibility to the study) and a sub-maximal exercise test as described in section 3.4 in order to determine the exercise intensity for visit 2.

Visit 2 was carried out following an overnight fast, and began between 0730 and 0800 am. The protocol began with the collection of venous blood samples (section 3.6.2) for the measurement of EMP (section 3.7), and then the patient was asked to lay supine on a bed for 15 min completely at rest. EF was then measured using the EndoPAT-2000 device in a manner as described in section 3.8. Due to certain constraints, this device was used at baseline and immediately post exercise only.

7.2.3 Blood sampling

The expression of CD105+ MP and CD106+ MP were measured by FC after venous blood samples were taken at rest, post and 60 min post exercise, and analysed as described in section 3.7.1.

7.2.4 Exercise protocol

The participants began their exercise once the baseline measurements as detailed above had been completed. The participants began with a warm up at 3.5 $km \cdot hr^{-1}$ for 5 min, and then the treadmill speed was increased until a steady state exercise capacity had been reached coinciding with 60% of their individual VO_{2max} scores. Gas measurements were collected after the warm up period for 10 min (section 3.6), and then again at 30 min in to the exercise protocol for a further 10 min in order to check the intensity remained correct. If the intensity was outside of a 10% range either side of the desired intensity then the speed of the treadmill was altered accordingly. HR was measured

throughout and RPE was measured every 10 min. Immediately after the exercise, participants were asked to leave the treadmill and return to lay supine on the bed where the subsequent analyses of EF were made.

7.2.5 Statistical Analysis

All were performed using IBM SPSS Statistics 19.0. All of the data are presented as means and standard deviation of the mean ± SD unless stated. The change in variables and biochemical variables across time were analysed using paired samples t-test. Relationships between EMP and RHI were analysed using Pearson Correlation Coefficients. Two-tailed statistical significance was accepted at $p < 0.05$.

7.3 Results

7.3.1 Baseline characteristics

Table 7.1 shows the baseline characteristics of participants in this experiment. The mean RHI value was 2.43, which shows that the levels were of a preserved EF (a level of > 1.6) (Bonetti et al., 2004). Only one participant showed a value at baseline to be below this value (RHI = 1.49).

Table 7.1 Baseline characteristics of participants showing the mean body mass, SBP, DBP, VO_{2max}, BMI, RHI, fRHI and AI. Data represented as means ± SD (n = 10).

Baseline variable	Value
Body mass (kg)	72.9 ± 13.1
SBP (mmHg)	127 ± 11.5
DBP (mmHg)	71.4 ± 9.3
VO_{2max}(ml/min/kg^{-1})	31.7 ± 4.6
BMI (kg/m^2)	24.0 ± 3.5
RHI	2.4 ± 0.8
Framingham RHI (fRHI)	1.05 ± 0.4
Augmentation Index (AI)	3.2 ± 25.2

Data represented as mean ± SD.

7.3.2 Effects of exercise on blood marker measurements

Table 7.2 shows that SBP and DBP were not significantly altered as a result of exercise. RHI rose from 2.43 to 2.57 post exercise, but this was not a significant rise ($p = 0.35$). AI was significantly reduced from a baseline value of 3.2 to a post exercise value of -10.5 ($p = 0.02$). fRHI was also decreased from 1.05 to 0.75 post exercise, but this was not significantly different ($p = 0.051$).

Table 7.2 Characteristics of participants showing the mean variables measured from pre exercise to post, and 60 min after exercise (n = 10).

	Pre	Post	60 min	P-value (pre to post)	P-value (pre to 60 min)	P-value (post to 60 min)
RHI	2.43 ± 0.76	2.57 ± 0.88	n/a	0.35	n/a	n/a
SBP (mmHg)	127 ± 11.5	127.4 ± 10.9	n/a	0.46	n/a	n/a
DBP (mmHg)	71.4 ± 9.3	73.6 ± 5.9	n/a	0.27	n/a	n/a
AI	3.2 ± 25.2	-10.5 ± 17.2	n/a	0.02[a]	n/a	n/a
fRHI	1.05 ± 0.4	0.75 ± 0.47	n/a	0.051	n/a	n/a

[a] significantly different at $p < 0.05$. n/a represents when the variable was not measured at specified time point.

7.3.3 CD105+ MP

Levels of CD105+ MP showed a slight increase from rest to immediately post exercise, rising from 2299 to 2607 CD105+ MP per µl PFP respectively, but this was not significant ($p = 0.840$) (Fig 7.1). The levels of CD105+ MP then decreased 60 min post exercise to a value of 1419 CD105+ MP per µl PFP, but again this change was not significant from rest ($p = 0.612$) or immediately post exercise ($p = 0.348$).

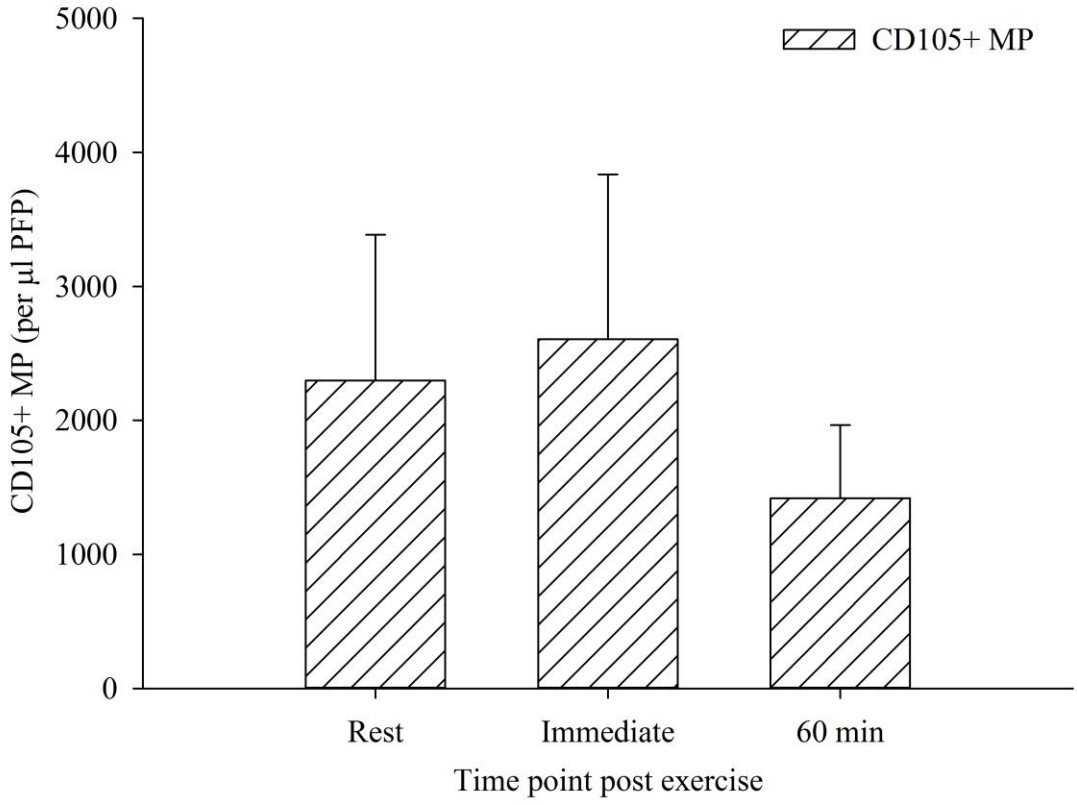


Fig 7.1 CD105+ MP in healthy, sedentary participants at rest, immediately and 60 min post acute exercise. Data represented as the concentration of CD105+ MP per µl PFP (means ± SEM). Error bars represent mean ± SEM.

7.3.4 CD106+ MP

CD106+ MP was reduced from baseline values to post exercise, with levels decreasing from 2514 to 1368 CD106+ MP per µl PFP ($p = 0.09$) (Fig 7.2). The levels of CD106+ MP were lower at 60 min post exercise compared to resting values, decreasing from 2514 to 1294 CD106+ MP per µl PFP respectively ($p = 0.073$).

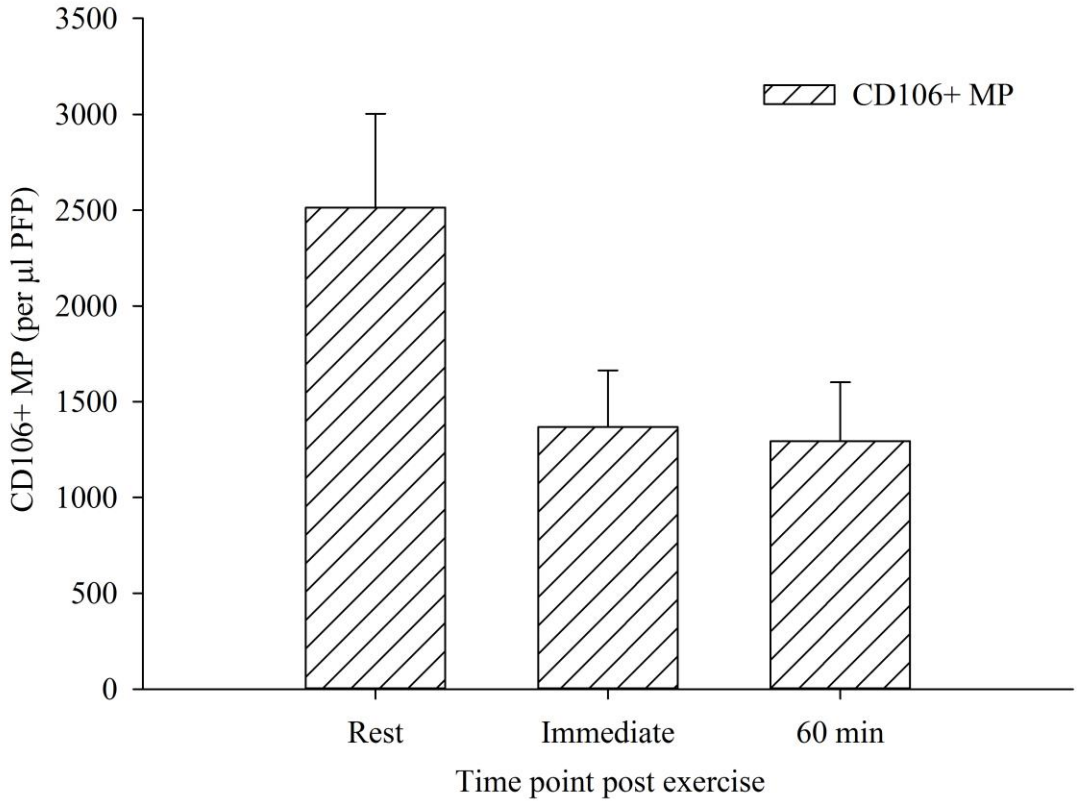


Fig 7.2 CD106+ MP in healthy, sedentary participants at rest, immediately and 60 min post acute exercise. Data represented as the concentration of CD106+ MP per µl PFP (means ± SEM). Error bars represent mean ± SEM.

7.3.5 Correlation of EndoPAT-2000 data with EMP

7.3.6 CD105+ MP

The higher levels of CD105+ MP correlated with lower scores of RHI, r = -0.327 which can be considered a medium effect, but was not significant (p = 0.171) (Fig 7.3). Elsewhere, CD105+ MP were not correlated to fRHI (r = -0.0293, p = 0.211) or AI (r = 0.160, p = 0.502).

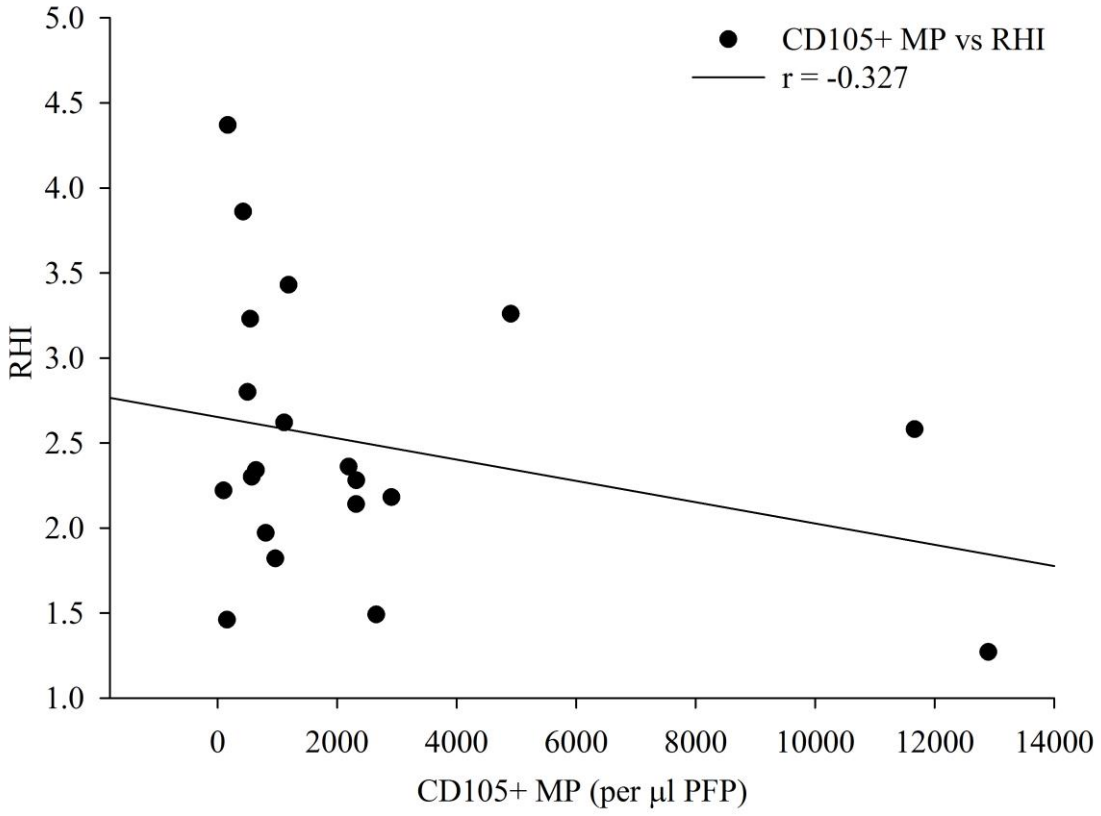


Fig 7.3 RHI correlated with CD105+ MP (r = -0.327).

7.3.7 CD106+ MP

The levels of CD106+ MP were not significantly correlated with the lower RHI scores (r = -0.087, p = 0.717) (Fig 7.4). CD106+ MP showed no correlation with fRHI (r = -0.012, p = 0.959) or AI (r = 0.026, p = 0.914).

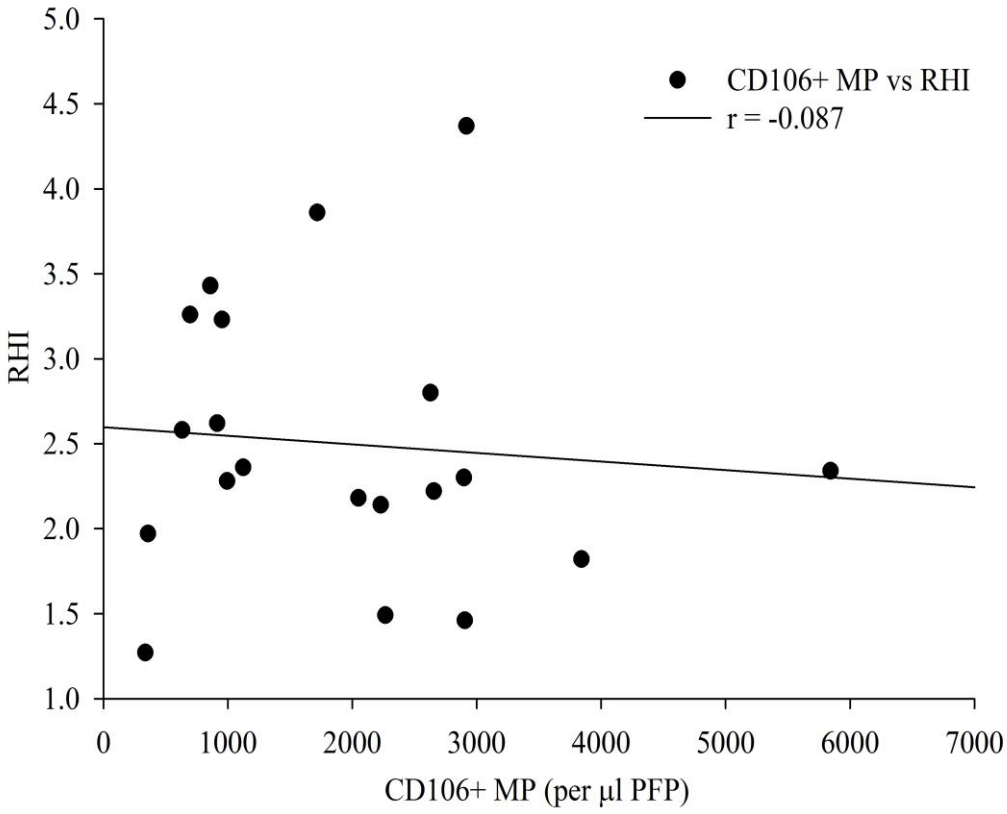


Fig 7.4 RHI in all participants correlated with CD106+ MP (r = -0.087).

7.4 Discussion

The major cause of morbidity and mortality worldwide is atherosclerotic vascular disease, leading to a number of disorders, such as stroke, myocardial infarction, heart failure, renal insufficiency or intermittent claudication (Pasternak et al., 2004; Mozaffarian, Wilson & Kannel, 2008). ED is one of the earliest events in the

pathophysiological process that leads to these atherosclerotic disorders (Davies, 2007). Further to this, ED then contributes to the progression of disease by contributing to inflammation and thrombosis (Libby, Ridker, & Hansson, 2009). Measurement of ED is therefore hugely invaluable, and can be detected in seemingly healthy individuals that are at risk of developing CVD (Celermajer et al., 1992). As reported, detection of ED can be predictive of major adverse cardiovascular events as well as mortality, and this chapter evaluates the use of two different methods of assessing ED.

The main findings from this chapter are that EMP were not elevated by an acute bout of exercise of moderate intensity exercise for 60 min in duration. This chapter also reiterates the finding that EMP seem to recover within a relatively short time frame in healthy individuals, even those who are sedentary and not trained participants as seen in previous chapters. This was shown by a decrease from baseline values compared with 60 min post exercise in both CD105+ MP and CD106+ MP.

In contrast to chapters 4 and 5, this intensity of acute exercise was unable to elevate levels of EMP, and actually decreased markers of EF in sedentary participants. The exercise intensity was much lower in this chapter, with participants exercising at just 60% of their VO_{2max}, in contrast to the previous chapters, whereby exercise was highly exhaustive, with intensity corresponding to 120% PPO in parts and very strenuous in nature. This again suggests that EMP release may be linked to exercise intensity, a finding shown previously after strenuous exercise was able to increase MP in the circulation of healthy individuals (Rehman et al., 1997; Sossdorf et al., 2011). However, in comparison to the findings from the current chapter, exercise does not always increase levels of MP (Guiraud et al., 2013). This group studied 19 males with stable

CHD, and in a random order, they underwent a high intensity interval and a moderate intensity continuous exercise session (28.7 min at 70% PPO) on a cycle ergometer. The high intensity interval training session involved two 10 min sets composed of repeated bouts of 15 s at 100% of PPO interspersed by 15 s passive recovery intervals. EMP (CD31+, also known as PECAM-1, and/or CD62E+) and PMP (CD42b+) were measured by FC 10 min before exercise, and 20 min, 24 hr and 72 hr after exercise. EMP and PMP levels were unchanged after exercises suggesting this particular protocol was not sufficient enough to elicit ED. The participants studied in the Guiraud et al (2013) study were CHD patients, and it is known that patients with CHD are characterised by ED (Liao et al., 2014), suggesting a potentially abnormal response to exercise compared with healthy individuals. Additionally, the time points chosen to measure EMP may not be the most robust in order to measure the effects of an acute bout of exercise on ED, and this study used different markers to measure EF, making comparisons difficult.

This study was able to show that the endothelium was unaffected by a very modest bout of exercise in healthy, otherwise sedentary individuals, as evidenced by no change in RHI measured by EndoPAT-2000. Additionally, no significant changes in concentrations of CD105+ MP and CD106+ MP were found as a result of acute exercise. Although this study was unable to show a significant correlation between EMP levels and RHI, there was a trend for participants with higher EMP levels to have lower RHI scores, as indicated by a medium effect of CD105+ MP and RHI (Fig 7.3). This relationship observed links with a recent *in vivo* study by van lerssel et al (2012) who assessed both EndoPAT-2000 and EMP (CD31+/CD42b-) in 5 healthy volunteers (3 females) free of any cardiovascular risk factors. EMP were defined as particles that were CD31 positive and CD42b negative, and were taken on 3 separate days. Day A

was conducted after an overnight fast, day B was carried out after receiving an NaCl 0.9% infusion during 1 hr, and day C replaced the NaCl with pure lipid emulsion infusion. The study showed an inverse relationship existed between EMP and RHI ($r = -0.758$, $p = 0.011$) when combining data from day A and day B, suggesting a higher cardiovascular risk profile for those individuals with higher numbers of EMP and a lower RHI score, and underscores the valid use of EMP as a marker of EF. One issue that surrounds studies looking at EMP as an assessment for EF is that a number of pre analytical and analytical variables have been described, all of which appear to confound exact EMP quantification (van lerssel et al., 2010). Within the International Society of Thrombosis and Haemostasis, there has been substantial effort in attempting to standardise these issues, but at present, a widely adopted method for a uniform FC method is still lacking (van lerssel et al., 2010). Variables that differ across studies include storage time of whole blood and plasma, and centrifugation speed, which are known to influence the MP measurement (Shah et al., 2008; van lerssel et al., 2010), as well as differences in quantification and different MP associated biomarkers studied. Whilst this can sometimes make comparison between studies difficult, all of these variables have been controlled during this thesis and we have adopted a standard protocol for MP quantification. An MP region was established using megamix beads (Biocytex, France) according to the current International Society on Thrombosis and Haemostasis Scientific and Standardization Subcommittee protocol (Lacroix et al., 2010). This standard protocol has been well used by fellow research colleagues for MP quantification (Chrismas et al., 2010; Madden et al., 2010; Vince et al., 2009a;b).

This current chapter included both males and female participants of varying age and assessed EF by two different techniques. This study demonstrates that it was possible to quantify CD105+ MP and CD106+ MP as potential markers of EF in sedentary

participants, and shows that EndoPAT-2000 seems to correspond to these markers, although further work is needed in a larger population to fully determine the relationship between these two markers of EF. Given the lack of stress to the endothelium as a result of exercise, it would be reasonable to presume that the exercise intensity was not strenuous enough, and further work is needed to determine the EMP response in healthy, but otherwise sedentary individuals. This chapter also strengthens the notion that a healthy person's endothelium has the ability to repair itself within a relatively short time frame after an acute bout of exercise, albeit not strenuous in nature and in sedentary individuals.

Chapter 8: The effects of an 8 week moderate intensity exercise programme on EF in women with and without PCOS

8.1 Introduction

Chapters 4, 5 and 6 have investigated EF in healthy individuals in acute bouts of highly strenuous exercise. The results from these chapters have included both male and female participants, showing that the endothelium was highly affected by acute exercise of these intensities, but showed signs of recovery within a relatively short time frame in healthy participants.

PCOS is one of the most common endocrine disorder in women of reproductive age (Sam, 2007), and can account for up to 18% of the population depending on the diagnostic criteria used (Harrison et al., 2011; March et al., 2010; Wild et al., 2010). PCOS represents a significant burden on the healthcare system due to its exhaustive list of related complications (Harrison et al., 2011). For example, PCOS women are known to have increased intrinsic IR when compared with non PCOS women, independent of obesity (Sam, 2007; Teede, Hutchison & Zoungas 2007). PCOS is also known to increase the prevalence and risk of cardiovascular disorders, including hypertension and dyslipidaemia (Norman et al., 2007; Wild et al., 2010), owing to long term health effects and the development of CVD. Furthermore, it is noted that between 40 to 60% of PCOS women are either overweight (BMI > 25 kg/m^2) or obese (BMI > 30 kg/m^2) with increased central adiposity (Kiddy et al., 1990).

One abnormality that presents in PCOS women is ED (Diamanti-Kandarakis et al., 2006; Orio et al., 2005), which is unsurprising considering the suggested link between ED and the aforementioned diseases (Kravariti et al., 2005; Paradisi et al., 2001). ED has been assessed using various methods in women of this population, such as through FMD (El-Kannishy et al., 2010; Sorensen et al., 2006; Sprung et al., 2013a,c; Sprung et

al., 2014) and EndoPAT-2000 (Dawson et al., 2014). Endothelial adhesion markers in PCOS women have also been studied in this population (Dawson et al., 2014; Diamanti-Kandarakis et al., 2006; Thomson et al., 2012). Diamanti-Kandarakis et al (2006) studied 25 women with PCOS and 25 women without PCOS and were investigating ED by measuring endothelial activation markers, as well as BA-FMD. Endothelin-1 (ET-1) was measured by enzymatic immunoassay, and high sensitivity CRP (hsCRP) by ELISA. Soluble adhesion molecules were analysed (sICAM-1 and sCD106) by ELISA using plasma samples. FMD values were significantly lower in the PCOS group, as well as ET-1 levels. Also, the PCOS group had higher levels of hsCRP, sICAM-1 and sCD106 compared to the control group, suggesting that PCOS women do present with an abnormal endothelial status, and endothelial adhesion markers can predict this altered state.

Incorporating exercise as a treatment for PCOS may be favourable when you consider the benefits that exercise has in other conditions that are associated with PCOS (Moran et al., 2006). Lifestyle modification has been endorsed by the Androgen Excess and PCOS Society as a primary choice in the prevention of CVD (Wild et al., 2010). There are well-established benefits of exercise and weight loss in women with PCOS on factors such as reproductive function (Palomba et al., 2008), and improvements in CVD risk markers such as obesity and cardiorespiratory fitness (Vigorito et al., 2007), but there is limited data on the effect of exercise on EF in women with PCOS (Thomson et al., 2012). Exercise training improves EF in individuals presenting with similar risk factors to PCOS women, such as patients with T2DM (Maiorana et al., 2001) and hypertension (Higashi et al., 1999).

PCOS presents a useful model in which to study the underlying mechanisms of ED and the possible effects of exercise on this condition. Given that PCOS is associated with a greater CVD risk, and reduced endothelial functioning increases this risk (Thomson et al., 2012) it is important to investigate the clinical importance of lifestyle improvement in PCOS. The purpose of this chapter was to investigate the effects of an 8 week moderate intensity aerobic exercise programme, compared to a control group on EF in women with PCOS.

8.2 Methods

8.2.1 Participant Characteristics

Recruitment of participants was made in accordance with that described in section 3.1. It should be noted that fellow research colleagues from the local hospital recruited the PCOS participants, whereas the control participants were recruited by the author. Ethical approval was approved by Hull and East Yorkshire NHS trust R&D approval, as well as the departmental ethics committee at the University of Hull, and participants gave their written informed consent to take part in the study.

For the PCOS arm, pre-menopausal women aged 18-40 years were eligible if they showed characteristics of PCOS as described by the Rotterdam consensus (Rotterdam, 2004). PCOS was diagnosed as having two out of three of the following: evidence of anovulation/oligomenorrhea, clinical or biochemical hyperandrogenism, and/or polycystic ovaries on ultrasound scanning. All non PCOS women had regular menses

and no evidence of clinical or biochemical hyperandrogenism. Other exclusion criteria included smoking, pregnancy, history of CV, renal, hepatic and thyroid disease, diabetes mellitus, family history of sudden death, and regular exercise three times per week for past three months.

8.2.2 Experimental design

The experimental design of participant entry and completion of the study is shown in Fig 8.1. This figure shows the procedure from the start of study recruitment and the number of participants lost throughout the study. The study began with 25 women (14 PCOS, 11 controls) who volunteered to take part in the exercise programme. All of these women were successfully screened and eligible for entry into the study, and they then began the 8 week exercise programme. The end of the exercise programme was deemed as 8 successful weeks, and subsequently the final assessment session occurred (post). Only those participants who managed to successfully finish the 8 week exercise programme were included in data analysis. Participants were discontinued from the study if they became pregnant, missed more than 50% (6 sessions) of exercise per month, if the participant had to use any concomitant medications detailed in the exclusion criteria during the study period, or if her consultant physician or general practitioner requested that the participant should be withdrawn from the study in their best interest. Four women were unable to fully complete the exercise programme and were not entered into the final analysis as a result. Reasons for discontinuation of the study were personal problems (2, both PCOS group), pregnancy (1, PCOS group) and lack of attendance (1, control group). The final participants analysed were those that successfully completed the 8 week exercise programme, which consisted of 21 women

(11 PCOS, 10 controls). All data and information was stored and accessed as according to section 3.1.1.

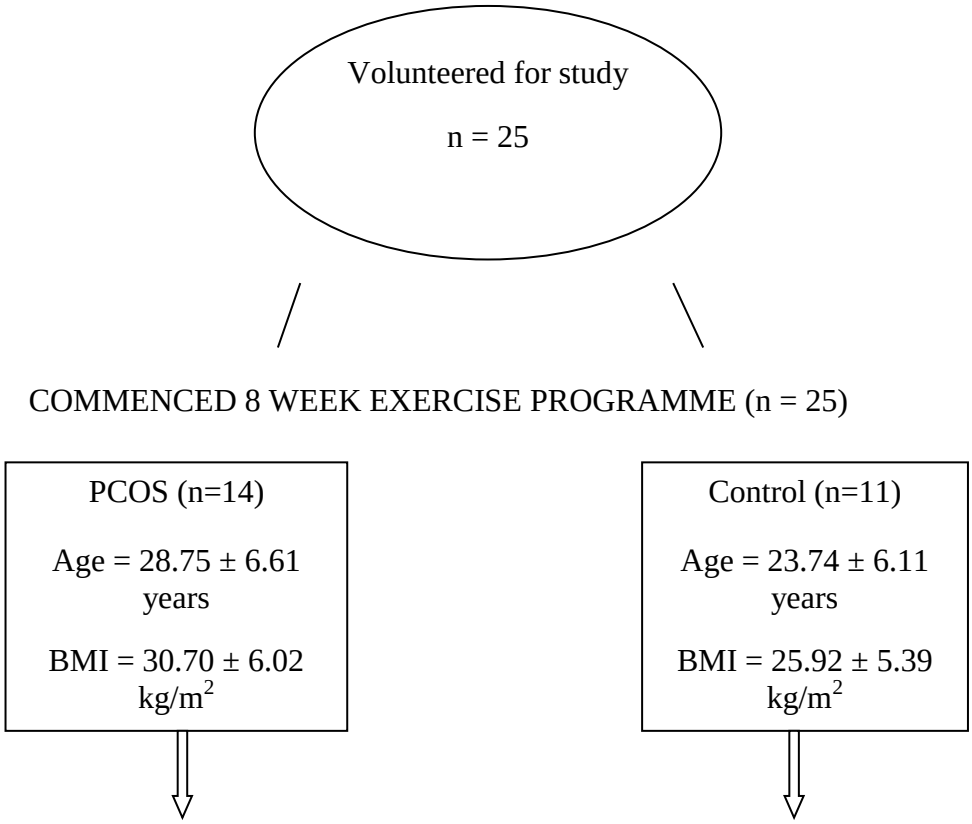


Fig 8.1 Diagram of participant information and study design from initial volunteering of the study to final study completion.

8.2.3 Baseline assessment

Eligible participants for the study attended the Michael White Diabetes Centre, Hull, UK within one week of to the baseline assessment visit (pre), which took place at the University of Hull. At this initial visit, fasting venous blood samples were taken in accordance with the details outlined in section 3.6.2 and analysed immediately for the measurement of CD105+ MP and CD106+ MP (section 3.7). Within one week of this visit, participants arrived at the Department of Sport, Health & Exercise Sciences, University of Hull where they carried out pre exercise questionnaires and consent as detailed in section 3.1. Participants were instructed to abstain from alcohol, caffeine, and exercise 24 hr prior to the testing, and all baseline assessments took place between 0830 and 1000 in order to maintain consistency amongst participants and to reduce the effect of any circadian variation in biomarkers, which have been shown to change over a 24 hr period (Madden et al., 2008).

Once appropriate screening had taken place and eligibility for the exercise programme had been confirmed, anthropometric measures were recorded as detailed in section 3.2. Immediately after this, participants performed a ramped maximal exercise test (VO_{2max}) in order to determine the individuals target workload for the exercise sessions (section 3.4.1). Expired gas measurements were made in accordance with those detailed in section 3.5.

8.2.4 Exercise training programme

Within a week following the baseline assessment, participants began attending 3 supervised exercise sessions per week for a period of 8 weeks. Where possible, each session was 1 hr in duration depending on the schedule and ability to complete the sessions with no complications. The programme began by initially using a Woodway ELG55 motorised treadmill (Woodway, Weil an rhein, Germany), but due to maintenance works in our facility the programme then continued using a HP Cosmos Pulsar Treadmill (H/P/Cosmos), but with no changes to the protocol. Participants performed all sessions on a motorised treadmill working at or as closely as possible to 60% $VO_{2max.}$ VO_2/kg was measured after the warm up, which lasted for 5 min at 4.5 $km{\cdot}hr^{-1}$ and for a period of 10 min in order to confirm the appropriate exercise intensity. The intensity of exercise was then adjusted by altering the speed of the treadmill if this value was not within ± 2.5% of the target oxygen uptake. Following this 10 min gas collection, the face mask was withdrawn with the speed of the treadmill remaining as it was. A further gas collection was made at 40 min to confirm the desired intensity for a 5 min period. If this intensity was out of range then the treadmill speed was once again altered if required. HR and RPE were monitored every 15 min throughout the session. If participants felt that they could not continue with the exercise for reasons such as injury or fatigue, they were able to stop at any time if necessary. Likewise, if it meant dropping the intensity for a period of time in order for them to recover then this was permitted, otherwise the intensity remained at the level pre-determined. Each session ended with a 5 min cool down at 4.5 $km{\cdot}hr^{-1}$ and participants would then be free to leave once HR retuned to within 120% of basal levels. The participants in the study were not asked to alter their diet in any way and were to continue as normal with their calorie consumptions throughout the exercise programme.

8.2.5 Mid-point assessment

Participants performed an exact repeat of the baseline assessment (section 8.2.3) within 3 days after the 4 successful weeks of the exercise programme had been completed. This mid-point assessment also aided in altering the prescribed exercise intensity for the exercise programme sessions thereafter in case the individual managed to improve their fitness over the 4 weeks of initial training. For example, if a participant should improve their VO_{2max} from 26.5 to 28.9 ml/kg/min^{-1}, then their targeted oxygen consumption would change from 15.9 to 17.34 ml/kg/min^{-1}.

8.2.6 Final assessment

This final assessment at the end of the exercise programme for each participant was a repeat of the baseline and mid-point assessments (section 8.2.3).

8.2.7 Venous blood samples

Venous blood samples were collected by the same independent trained consultant at the Michael White Diabetes Centre, Hull, UK. Blood samples were taken no longer than 1 week prior to baseline assessment (pre), then after 4 successful weeks of the exercise programme (mid), and again in the final week no more than a week after ceasing with the exercise programme (post). Venous samples were all taken at or as closely as

possible to 0900 hr following an overnight fast. One 3.5 ml citrated blood tube was immediately placed on ice and transported to the Medical Research Laboratory, Hardy building, University of Hull for analysis. Full details of blood preparation can be found in section 3.6.

Citrated blood was analysed as described in section 3.7. Briefly, an aliquot (4µl) of either IgG1 CD105:FITC conjugate (AbDSerotec, UK) or IgG1 CD106:FITC conjugate (AbDSerotec, UK) were added to 25 µl PFP and the analysed by FC as detailed in section 3.7.1.

8.2.8 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 19.0. Central tendency and dispersion of the sample data are represented as the mean ± SD. Any changes in biochemical markers across condition and time were analysed using linear mixed models. The change in anthropometric measurements across the exercise programme within the two populations were investigated using paired samples t-tests with Sidak adjusted p values to control for family wise type I error rate. Relationships between EMP and variables were analysed using Pearson Correlation Coefficients. All of the data are presented as means and standard deviation of the mean (mean ± SD) unless otherwise specified. Two-tailed statistical significance was accepted at $p < 0.05$.

8.3 Results

8.3.1 Baseline characteristics

Table 8.1 displays the baseline characteristics of control and PCOS women. There was a significant difference between the groups in SBP ($p = 0.027$) which was higher in the PCOS group. VO_{2max} was significantly higher in the control group ($p < 0.001$). WHR ($p = 0.019$) and WC ($p = 0.033$) were significantly higher in the PCOS group when compared to control women. Levels of CD105+ MP were also significantly elevated in women with PCOS ($p = 0.021$).

Table 8.1 Baseline characteristics between control and PCOS women. Body mass, SBP, DBP, VO_{2max}, BMI, WHR, WC, CD105+ MP and CD106+ MP are compared at baseline between the two groups.

Variables	Control baseline (n = 10)	PCOS baseline (n = 11)	P value
Body mass (kg)	71.04 ± 16.42	85.45 ± 18.91	0.080
SBP (mmHg)	123.00 ± 11.44	132.36 ± 11.47	0.027[a]
DBP (mmHg)	77.50 ± 9.30	81.82 ± 11.21	0.290
VO_2max ($ml \cdot min \cdot kg^{-1}$)	36.26± 6.38	26.32 ± 4.63	<0.001[a]
BMI (kg/m^2)	25.92 ± 5.39	31.15 ± 6.30	0.056
WHR	0.79 ± 0.07	0.86 ± 0.06	0.019[a]
WC (cm)	83.01 ± 14.20	98.05 ± 16.35	0.033[a]
CD105+ MP (per µl PFP)	388.2 ± 113.5	2113.9 ± 951.7	0.021[a]
CD106+ MP (per µl PFP)	7165.1 ± 4403.8	7625.6 ± 5427.8	0.942

[a] Significantly different between groups ($p < 0.05$). All data represented as mean ± SD, except for CD105+ MP and CD106+ MP which are represented as mean ± SEM.

8.3.2 Effect of exercise on measured variables

Table 8.2 displays the changes in biomarkers as a result of the exercise programme in control and PCOS women. Body mass was unchanged in control women, but those with PCOS managed to reduce their body mass at mid exercise programme ($p = 0.027$) and post exercise programme ($p = 0.027$) when compared to pre exercise programme. Body mass in PCOS women decreased from baseline from 85.45 kg to 84.45 kg at mid exercise, and then 84.04 kg once the exercise programme was completed. SBP improved pre to mid ($p = 0.031$), pre to post ($p = 0.002$) and mid to post ($p = 0.024$) study in PCOS women. DBP also improved pre to post ($p = 0.020$) and mid to post ($p = 0.005$) study in this group. There were also improvements in VO_{2max} values from pre to post study, with values rising from 26.32 $ml \cdot min \cdot kg^{-1}$ to 29.71 $ml \cdot min \cdot kg^{-1}$ ($p = 0.015$) and also mid to post, from 26.43 $ml \cdot min \cdot kg^{-1}$ to 29.71 $ml \cdot min \cdot kg^{-1}$ ($p = 0.002$) in the PCOS group, while BMI was improved from pre to post ($p = 0.028$) and pre to mid ($p = 0.015$) point in the study. There was a decrease in WC in this population from mid to post exercise intervention, decreasing from 96.97 cm to 96.12 cm ($p = 0.035$). The control population only showed significant improvements in SBP pre to post intervention, decreasing from 123 mmHg to 117.5 mmHg ($p = 0.035$) and VO_{2max} pre to post intervention (36.26 $ml \cdot min \cdot kg^{-1}$ to 39.21 $ml \cdot min \cdot kg^{-1}$ respectively, $p = 0.017$). No other significant change was observed in any other measured variable in this group.

Table 8.2 Clinical characteristics of the control and PCOS women at baseline, mid, and post 8 weeks of aerobic exercise training. Body mass, SBP, DBP, VO_{2max}, BMI, WHR and WC are compared between both groups.

	Control (n = 10)			PCOS (n = 11)		
Variables	**Pre**	**Mid**	**Post**	**Pre**	**Mid**	**Post**
Body mass (kg)	71.04 ± 16.42	70.66 ± 16.19	70.11 ± 15.83	85.45 ± 18.91	84.45 ± 19.02[a]	84.04 ± 19.54[a]
SBP (mmHg)	123.00 ± 11.44	120.50 ± 10.28	117.50 ± 7.65[a]	132.36 ± 11.47	128.73 ± 11.03[a]	124.91± 10.40[ab]
DBP (mmHg)	77.50 ± 9.30	75.00 ± 6.82	73.10 ± 5.43	81.82 ± 11.21	81.36 ± 12.36	76.64 ± 8.82[ab]
VO_2max ($ml \cdot min \cdot kg^{-1}$)	36.26 ± 6.38	37.49 ± 6.96	39.21 ± 5.82[a]	26.32 ± 4.63	26.43 ± 4.09	29.71 ± 5.32[ab]
BMI (kg/m^2)	25.92 ± 5.39	25.78 ± 5.36	25.58 ± 5.18	31.15 ± 6.30	30.79 ± 6.32[a]	30.69 ± 6.48[a]
WHR	0.79 ± 0.07	0.78 ± 0.06	0.79 ± 0.07	0.86 ± 0.06	0.85 ± 0.07	0.85 ± 0.06
WC (cm)	83.01 ± 14.20	82.41 ± 14.19	82.16 ± 14.57	98.05 ± 16.35	96.97 ± 17.90	96.12 ± 14.67[b]

Data are represented as mean ± SD. [a] Significantly different compared to pre ($p < 0.05$). [b] Significantly different compared to mid ($p < 0.05$).

8.3.3 Blood marker measurements

8.3.4 CD105+ MP

The PCOS population were the only group to see any significant changes in blood variables (Fig 8.2), showing significant decreases in CD105+ MP from pre to post exercise intervention ($p = 0.025$). The number of MP decreased from 2114 CD105+ MP per µl PFP to 424 CD105+ MP per µl PFP. The control group conversely showed little change over the exercise programme, and levels of CD105+ MP actually rose from pre (388 CD105+ MP per µl PFP) to post exercise programme (719 CD105+ MP per µl PFP), but this change was not significant ($p = 0.25$).

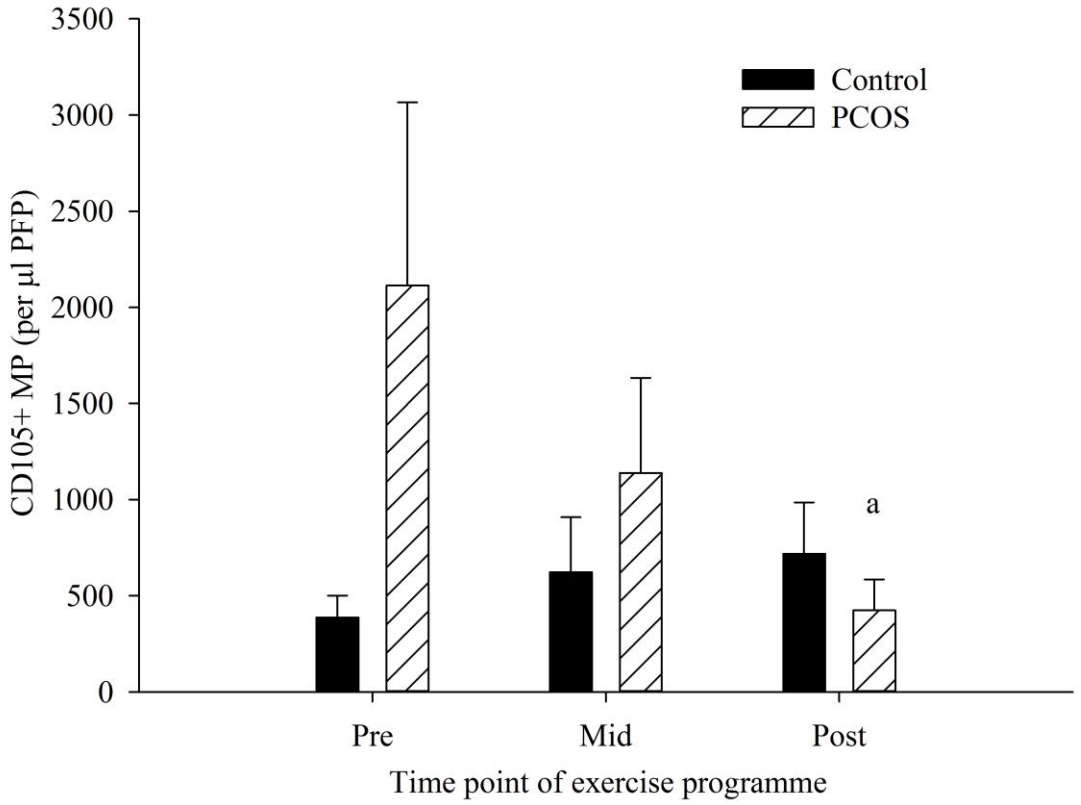


Fig 8.2 CD105+ MP in control (dark bars) and PCOS (hatched bars) participants at pre, mid, and post exercise intervention. Data represented as the concentration of CD105+ MP per µl PFP (means ± SEM). [a] significantly different compared to pre in PCOS group (p = 0.025). Error bars represent mean ± SEM.

8.3.5 CD106+ MP

There were no significant differences in CD106+ MP as a result of exercise at any of the time points measured. The PCOS group showed a very minimal decline from pre to post exercise intervention, with levels of CD106+ MP falling from 7626 to 7210 CD106+ MP per µl PFP (p = 0.95). Similarly, the control groups mean CD106+ MP levels changed from 7165 to 7105 CD106+ MP pre to post exercise intervention (p = 0.99). Interestingly, there was a decrease, albeit it not significant, in the control group

from pre (7165 CD106+ MP per µl PFP) to mid exercise programme (2438.9 CD106+ MP per µl PFP, p = 0.35), with values returning to basal values by the end of the programme. There was also a slight decrease in the PCOS group from pre to mid exercise programme, with CD106+ MP decreasing from 7625 to 5516 CD106+ MP per µl PFP (p = 0.71), with values returning to basal values by the end of the programme.

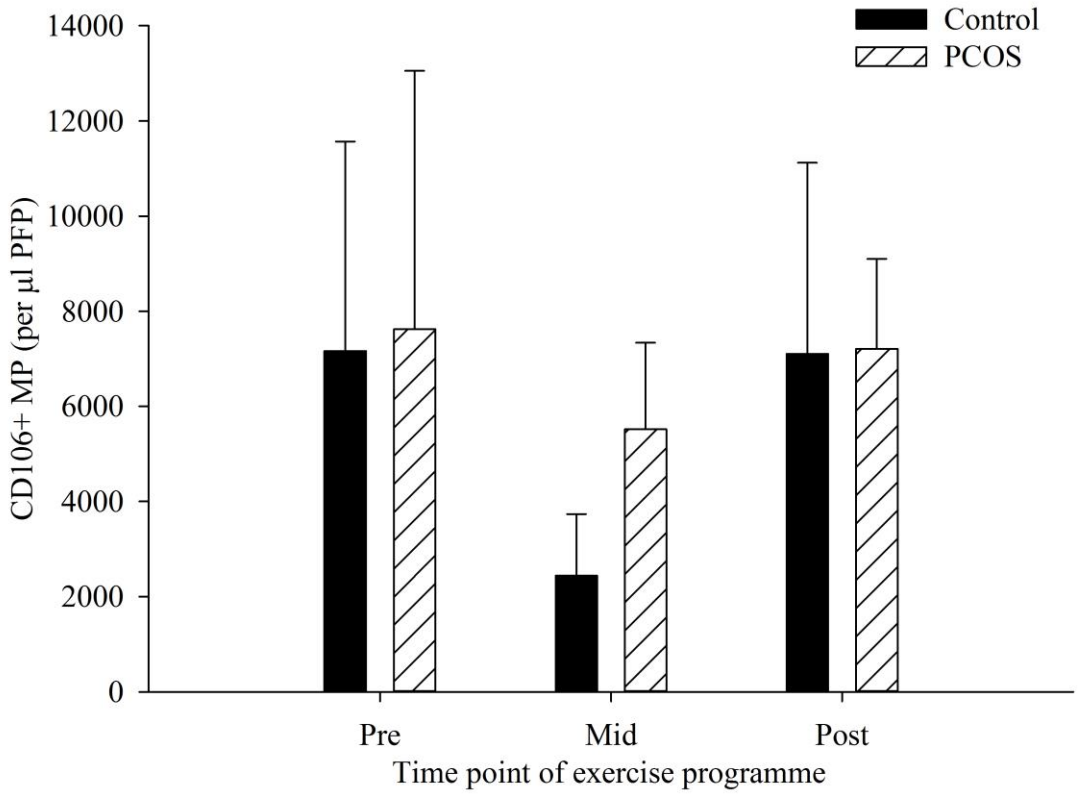


Fig 8.3 CD106+ MP in control (dark bars) and PCOS (hatched bars) participants at pre, mid, and post exercise intervention. Data represented as the concentration of CD106+ MP per µl PFP (means ± SEM). Error bars represent mean ± SEM.

8.3.6 Correlations of anthropometric data with EMP data

Table 8.3 shows the correlations of CD105+ MP and CD106+ MP with the variables measured throughout the exercise programme in all PCOS women and control women. There were no significant correlations with EMP data and any of the measured variables presented in Table 8.3 for either group, suggesting that any changes in EMP were independent of these variables. The strongest correlation occurred between SBP and CD105+ MP in PCOS individuals, with suggestion that the higher the SBP the greater the amount of CD105+ MP, but this was not significant ($p = 0.092$).

Table 8.3 Correlation of CD105+ MP and CD106+MP with variables measured in control and PCOS patients across the exercise programme.

Variable	Control		PCOS	
CD105+MP (per µl PFP)	**r value**	**P value**	**r value**	**P value**
Body mass (kg)	-0.119	0.531	-0.041	0.822
SBP (mmHg)	-0.067	0.724	0.303	0.092
DBP (mmHg)	-0.050	0.794	0.193	0.289
VO_{2max} $(ml \cdot min \cdot kg^{-1})$	0.136	0.481	-0.094	0.613
BMI (kg/m^2)	-0.117	0.537	-0.008	0.966
WHR	0.027	0.891	-0.090	0.648
WC (cm)	0.051	0.789	0.125	0.528
CD106+ MP (per µl PFP)	**r value**	**P value**	**r value**	**P value**
Body mass (kg)	-0.202	0.284	0.020	0.915
SBP (mmHg)	-0.131	0.490	0.176	0.337
DBP (mmHg)	-0.211	0.263	0.022	0.904
VO_{2max} $(ml \cdot min \cdot kg^{-1})$	-0.014	0.944	0.114	0.543
BMI (kg/m^2)	-0.186	0.324	-0.017	0.929
WHR	-0.236	0.218	-0.165	0.402
WC (cm)	-0.249	0.185	-0.074	0.708

r value determined from Pearson's Correlation Coefficient using group means ± SD, except for CD105+ MP and CD106+ MP (mean ± SEM).

8.4 Discussion

The novel finding of this study was that women with PCOS were able to significantly decrease levels of CD105+ MP, indicating that EF can improve as a result of a short term, supervised moderate intensity aerobic exercise programme, independent of dietary restrictions when compared with a control group of women without PCOS. An additional finding of this study was that PCOS women displayed significantly worse EF at baseline when compared to otherwise healthy, non PCOS females (Table 8.1), a finding reiterated by others using a variety of different assessment methods, such as FMD (Diamanti-Kandarakis et al., 2006; Kravariti et al., 2005; Sprung et al., 2014) and endothelial adhesion molecules (Christakou et al., 2011; Diamanti-Kandarakis et al., 2006). Furthermore, women with PCOS significantly improved their body mass, SBP, DBP, aerobic fitness, BMI, and WC by the end of the programme (Table 8.2).

Sprung et al (2013a) found that EF (BA-FMD) was improved in women with PCOS when compared to control women with PCOS who received conventional care following a 16 week aerobic exercise intervention. Ten women with PCOS underwent supervised 30 min exercise 3 times a week at 30% HRR, which progressed weekly based on HR responses (up to 60% HRR). The control group received lifestyle advice from clinical consultations prompting them to lose weight and increase their physical activity. Various assessments were measured during the study, including BA-FMD, cardiorespiratory fitness, visceral and abdominal subcutaneous adipose tissue, glycaemic control, and lipid and hormone profiles. When comparing the 2 groups, BA-FMD and cardiorespiratory fitness improved from pre to post exercise intervention in the exercising group only, suggesting that supervised aerobic exercise in women with

PCOS can enhance EF. Sprung et al (2013a) however did not use women without PCOS as controls. It would also have been useful to see any differences between PCOS women and control women without PCOS to further relate the findings of the current chapter, but nevertheless comparisons may be drawn. Baseline body composition data was similar between the two studies (BMI was 31.1 kg/m^2 in this chapter compared to 31 kg/m^2 in Sprung et al (2013a); WC was 98.05 cm and 100 cm respectively, whilst body mass was 85.45 kg compared to 82.1 kg). That being said, it is very important to state that PCOS is a very complex disorder and has a very wide spectrum of phenotypes (Nardo & Gelbaya, 2008), and it is possible that although baseline body composition data was similar between studies, these studies were based on participants volunteering to participate in regular exercise sessions and therefore participants with more severe phenotypes at the further end of the PCOS spectrum may not have volunteered and therefore been excluded in these studies. Future work should concentrate on investigating the effects of exercise on EF in these more severe phenotypes and the effect of exercise to reduce symptom severity as well as improve EF. Comparative to these findings, Roessler et al (2013) investigated the effects of exercise and group counselling on body composition and aerobic fitness in 17 women with PCOS. WC, BMI, body mass and VO_{2max} were improved in PCOS women following a cross over design of 8 weeks of high intensity aerobic exercise and 8 weeks of group counselling. The exercise programme consisted of 3 sessions per week with 2 days of indoor cycling and 1 day of brisk walking/running. The intensity of exercise was based on HR achieved during a cycling test at baseline, but was interval in nature and periods reached 90% of this HR during walking/running and 100% of the pre-determined HR during cycling.

The exercise in Sprung et al (2013a) was moderate in intensity, although they used %HRR (30% HRR initially, rising to 60% HRR) instead of $\%VO_{2max}$ as in the present chapter (60% VO_{2max}). Initially, exercise was performed 3 times a week, as in the present chapter, but for a period of just 30 min, which was then increased to 5 times per week up to 45 min per session, whereas a fixed protocol was adhered to in this chapter. Nonetheless, Sprung et al (2013a) increased the length of their sessions and frequency of training per week presumably as increases in fitness were observed in each group, similar to our reassessing their mid-point fitness so that improvements in each individual's fitness could be used to re-evaluate the intensity for the second half of the programme. These studies suggest that supervised moderate aerobic exercise of 30-60 min three-five days a week is sufficient to enhance EF and various other measures in women with PCOS. Sprung et al (2013a) showed that the positive improvements in EF as a result of exercise was independent of changes in body weight, liver fat, visceral adipose tissue, or IR. Although the current chapter did observe positive changes in many variables that may be considered CV risk factors, such as WC, body mass and BP, correlations with EMP/EF demonstrated no significant relationships in this instance (Table 8.3). The changes in body mass seen in the PCOS group were very slight from pre to post exercise intervention in the current study (85.45 kg to 84.04 kg) suggesting that improvements in cardiorespiratory fitness without substantial weight loss may reduce the CV risk in women with PCOS, a finding also seen in other studies (Green et al., 2008; Sprung et al., 2013a). Kavanagh et al. (2003) reported that in women referred for cardiac rehabilitation, oxygen consumption was a strong independent predictor of cardiac mortality and with each increase of 1 $ml \cdot kg \cdot min^{-1}$ of initial peak oxygen consumption was associated with a 10% lower cardiac mortality. Therefore, the improvements seen in the present chapter (PCOS 12.9% increase; Control 8.1% increase), as well as that consistent with others (Brown et al., 2009; Giallauria et al.,

2008b; Moro et al., 2009; Randeva et al., 2002; Vigorito et al., 2007) suggest that women with PCOS may be able to decrease their risk of CV complications and mortality by engaging in exercise of the nature discussed.

Assessing EF after exercise interventions in PCOS women by endothelial biomarkers is very limited (Sprung et al., 2013). Recently however, one study (Thomson et al., 2012) looked at the effects of exercise training on EF in PCOS women measuring circulating biomarkers through a multiplex analyser (sCD106, sICAM-1 and PAI-1). This study used 3 different groups, one group was a controlled diet with moderate aerobic exercise (60-80% HR_{max}), one used diet combined with aerobic-resistance training, and the other a diet only without exercise. sCD106, sICAM-1 and PAI-1 all reduced at week 20 in each group compared to pre exercise programme, indicating that EF is improved in PCOS women as a result of different lifestyle interventions, including exercise. There was no differences found between each of the groups in terms of EF improvement, but the most effective treatment groups appear to have been those with exercise included, as there were greater improvements seen in sCD106, weight loss and aerobic fitness in the exercise groups when compared to the diet only group, but there were no significant differences between the groups. The results presented from Thomson et al (2012) are interesting as they show that improvements can be seen in EF without exercise in PCOS women through dietary control, but it must be considered that additional exercise makes improvements more effective. There was no improvements in EF seen in the control group of the present chapter, and one issue from Thomson et al (2012) is that there was no control population of non PCOS women, or of a non-exercise/non diet group in order to study the effects compared with PCOS women, meaning that it is difficult to determine whether these changes were due to the high-protein energy restricted diet and/or weight loss.

Previous studies are in agreement with results of this chapter in that there was a significant impairment in EF in PCOS women at baseline levels compared to control women without PCOS (Carmina et al., 2006; Ilie et al., 2008; Paradisi et al., 2001). El-Kannishy et al (2010) found that EF as assessed by BA-FMD was impaired in both lean and obese women with PCOS. EL-Kannishy et al (2010) assessed 22 obese women with PCOS as well as 14 lean women with PCOS. FMD was significantly decreased in both groups of women with PCOS. Further to this, it was found that FMD was not correlated with BMI or IR, suggesting that the impairment seen in EF would indicate that another risk factor was involved in the development of ED in PCOS women. These findings suggest that women with PCOS who are otherwise free of any other known disease associated with PCOS still show characteristics of ED, and therefore both lean and obese participants could still benefit from an exercise intervention similar to that used in this study to improve EF, even in the absence of other risk factors.

A recent review of PCOS and exercise highlighted the various limitations that are common in exercise interventions in women with PCOS (Harrison et al., 2011). Studies have generally been small in sample size and have had a significant drop out rate (Harrison et al., 2011). The present chapter managed to include 21 participants out of 25 recruited, which unfortunately was not sufficient in number to create sub populations within the data collected, e.g. possible influences of lean, overweight, and obese individuals with and without PCOS. For example, with the current participant population, dividing the groups into the appropriate BMI categories would have left just two obese control women. Lifestyle interventions have also proved difficult to implement and sustain in PCOS and can be a known challenge, with high dropout rates occurring in PCOS lifestyle intervention studies (Brown et al., 2009; Moran et al., 2006; Randeva et al., 2002). In one particular study for example, 45% of the cohort dropped

out of an exercise intervention study altogether (Brown et al., 2009). This paper suggests that the reason for such a high dropout was due to a young age of participants (median age of exercisers and controls 36.5 years and 28.0 years respectively), with accompanying responsibilities for small children, school and work. The present chapter however had a younger cohort (26.5 years) and this dropout was not seen. Brown et al (2009) had study duration of 24 weeks, which may have also contributed to such a high dropout, whereas this study was of a relatively short duration, which may have boosted compliance (8 weeks). It should be noted that compliance to exercise studies in PCOS women has shown the highest dropout rates to be in the longer duration studies (20-24 weeks) and more exercise sessions per week (4-7) when compared to other studies (Brown et al., 2009; Randeva et al., 2002; Thomson et al., 2008). Findings from the limited research available in this area would suggest that highest compliance rates occur in studies of short duration, for example in studies of 12-16 week duration involving three 30 min sessions per week with no dietary component (Giallauria et al., 2008b; Stener-Victorin et al., 2009; Vigorito et al., 2007). The current chapter consisted of 8 weeks, and did not have a high dropout rate at all, with compliance very strong (16% dropout rate). In addition to this, it is important to note that one of the dropouts was actually due to a pregnancy (PCOS group) and so was not a compliance issue. In the authors opinion and based on the verbal feedback provided throughout the study period, this high compliance was mainly due to the supervision and monitoring of the exercise sessions, as well as the duration and intensity of exercise sessions being favourable to participants, and participants recruited were also highly motivated and volunteered to take part. This leads to the suggestion that a home based programme, at least in the early stages of an intervention, may not be the best route to take, and that closely monitored, supervised exercise interventions appear to have a positive effect. A further issue with existing literature is that studies tend to use young women who were

predominantly obese (Harrison et al., 2011). This was one of the reasons for the inclusion of lean women as well as obese in this study, and also women up to an age of 40 years old as we considered that PCOS should be evaluated in a broad range of women across age and BMI in order to evaluate the effects of exercise as a therapeutic agent. It is also important to note that considering participants volunteered for the study, there may have been women with far more severe symptoms/phenotypes of PCOS that were not present within the study.

This study confirms that moderate intensity exercise (60% VO_{2max}) could be recommended for this population due to the improvements seen in various cardiorespiratory and cardiometabolic risk factors. The data here also suggests that this type of moderate intensity exercise is sufficient over a relatively short time frame to see improvements in anthropometric measurements and EF. This is in agreement with other studies who have also found improvements in women with PCOS as a result of moderate intensity exercise (Palombo et al., 2010; Thomson et al., 2012) but future work could also investigate the use of incorporating high intensity interval training or resistance training into an exercise/diet programme in PCOS women which has previously shown favourable results in body composition and aerobic fitness (Roessler et al., 2013).

There are several pharmaceutical interventions for PCOS, which primarily focus on addressing reproductive dysfunction and IR (Harrison et al., 2011). However, despite these available treatments, there is no common consensus as to the ideal pharmacological intervention in PCOS, partly due to the wide spectrum of severity of the disease. With this in mind, lifestyle modification remains the first line management to improve cardiovascular and reproductive risk factors in PCOS women (Harrison et

al., 2011; Moran et al., 2009). This chapter goes some way to strengthen the idea that exercise should remain one of the first line treatments for women with PCOS and there are several benefits that can occur as a result of moderate intensity exercise, such as reduced WC, body mass, SBP, DBP, as well as improved aerobic fitness in addition to improvements seen in EF. This data indicates that women with PCOS exhibit poorer EF compared with control women at baseline as indicated by increased EMP (CD105+ MP and CD106+ MP), and that EF can be significantly improved following the prescription of moderate aerobic exercise training, independent of any dietary restrictions. These findings argue for the prescription of this form of exercise training as a preventative strategy for ED in women with PCOS, as well as an effective treatment to improve various measures of body composition. Future work should address some of the limitations described above, such as the inclusion of longer duration exercise programmes, the use of home based programmes/group based programmes, more markers of EF such as CD62E, CD31 and CD144. Additionally, measuring visceral adiposity would be useful to correlate with any changes seen in EF as a result of exercise.

Chapter 9: Combination of data from previous chapters

9.1 Introduction

As seen in previous experimental chapters, there have been various differences between the design and implementation of study design, ranging from a wide population of participants studied, to the exercise protocol incorporated. It is therefore important to be aware of any differences in EF that may exist in the participants that were covered throughout this thesis, considering that healthy males, healthy females, healthy but otherwise sedentary individuals, and women with and without PCOS were included.

The aim of this final, short experimental chapter therefore is to provide analysis that combines the data from the previous chapters in order to determine any notable differences between resting levels of CD105+ MP and CD106+ MP across the range of participants included in this thesis, and to see a possible trend in the concentration of CD105+ MP and CD106+ MP as a result of exercise.

9.2 Methods

The results from each of the previous experimental chapters (chapters 4-8) were included in the subsequent analysis. The results from chapter 4 are of all participants who were involved regardless of experimental group (C4; n = 14), and the control (C8; Con, n = 10) and PCOS (C8; PCOS, n = 11) groups from chapter 8 have been included, as well as healthy females from chapter 5 (C5; n = 10), healthy males long duration from chapter 6 (C6; n = 14), and sedentary individuals from chapter 7 (C7; n = 10). In order to determine the effects of gender of concentrations of + MP in healthy (trained) individuals and healthy (free from any known disease) the males from chapters 4, 6 and 7 were included, and compared with female data from chapters 5, 7 and 8.

9.2.1 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 19.0. Central tendency and dispersion of the sample data are represented as the mean ± SD. Any differences across groups were analysed with an independent t-test with Sidak adjusted p values to control for family wise type I error rate. Data is expressed as concentrations of MP per µl of PFP. All of the data are presented as standard error of the mean (± SEM) unless stated. Two tailed statistical significance was accepted at $p < 0.05$.

9.3 Results

9.3.1 CD105+ MP baseline data

Fig 9.1 shows the resting/pre exercise data for CD105+ MP across each of the experimental chapters using experimental and control groups. Fig 9.1 shows clearly that the two groups with the greatest concentrations of CD105+ MP at rest/pre exercise were the sedentary participants from chapter 7, and PCOS females from chapter 8. The PCOS females had a significantly higher resting CD105+ MP concentration than the healthy males from chapter 6 ($p = 0.04$), and also showed a higher CD105+ MP concentration than the control females from the same chapter in which they were investigated (chapter 8, $p = 0.021$). Despite PCOS CD105+ MP concentrations (2113 ± 952 CD105+ MP per µl PFP) being almost twice as high as the healthy males in chapter 4 (1078 ± 192 CD105+ MP per µl PFP) and almost three times as great as the healthy females in chapter 5 (849 ± 166 CD105+ MP per µl PFP) respectively, these values were not

significantly greater (p = 0.21, p = 0.19 respectively). The participants from the sedentary chapter (chapter 7) had a significantly elevated concentration of CD105+ MP compared to the healthy males in chapter 6 (p = 0.031) and also the control females from chapter 8 (p = 0.013). Females that were acting as controls during the PCOS study (chapter 8) had a lower concentration of CD105+ MP compared to healthy males from chapter 4 (p = 0.011).

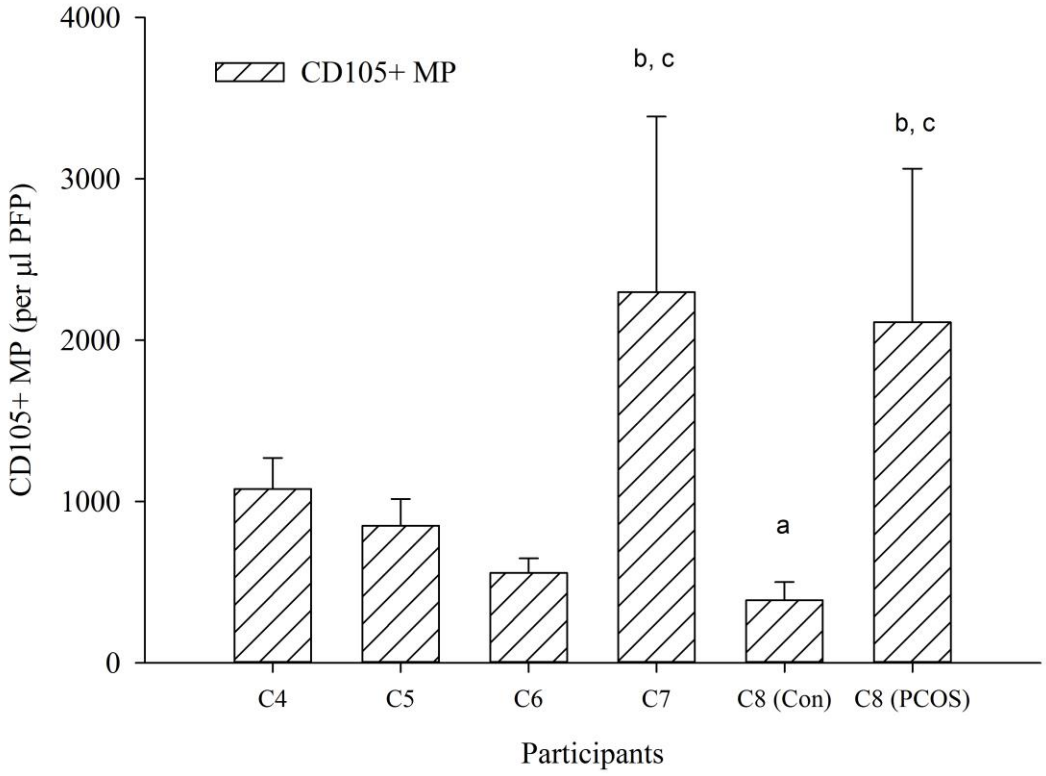


Fig 9.1 CD105+ MP in participants from chapter 4 (C4), chapter 5 (C5), chapter 6 (C6), chapter 7 (C7), chapter 8 (controls, C8 Con) and chapter 8 (PCOS, C8 PCOS) at rest/pre exercise. Data represented as the concentration of CD105+ MP per µl PFP (means ± SEM). [a] significantly different compared to C4 (p = 0.01) . [b] significantly different compared to C6 (p ≤ 0.04) . [c] significantly different to C8 (Con) (p ≤ 0.021). Error bars represent mean ± SEM.

9.3.2 CD106+ MP baseline data

Fig 9.2 shows the resting/pre exercise data for CD105+ MP across each of the experimental chapters using experimental and control groups. Fig 9.1 shows a clear difference in the pattern of CD106+ MP concentration between experimental chapters compared to that of CD105+ MP. The healthy males from chapter 4 were significantly lower in concentration of CD106+ MP compared to chapter 5 ($p = 0.004$), chapter 6 ($p < 0.001$), and chapter 7 ($p = 0.002$). The PCOS females had the highest concentration of CD106+ MP (7626 ± 5428 CD106+ MP per µl PFP), but this was not significantly greater than any groups from other chapters.

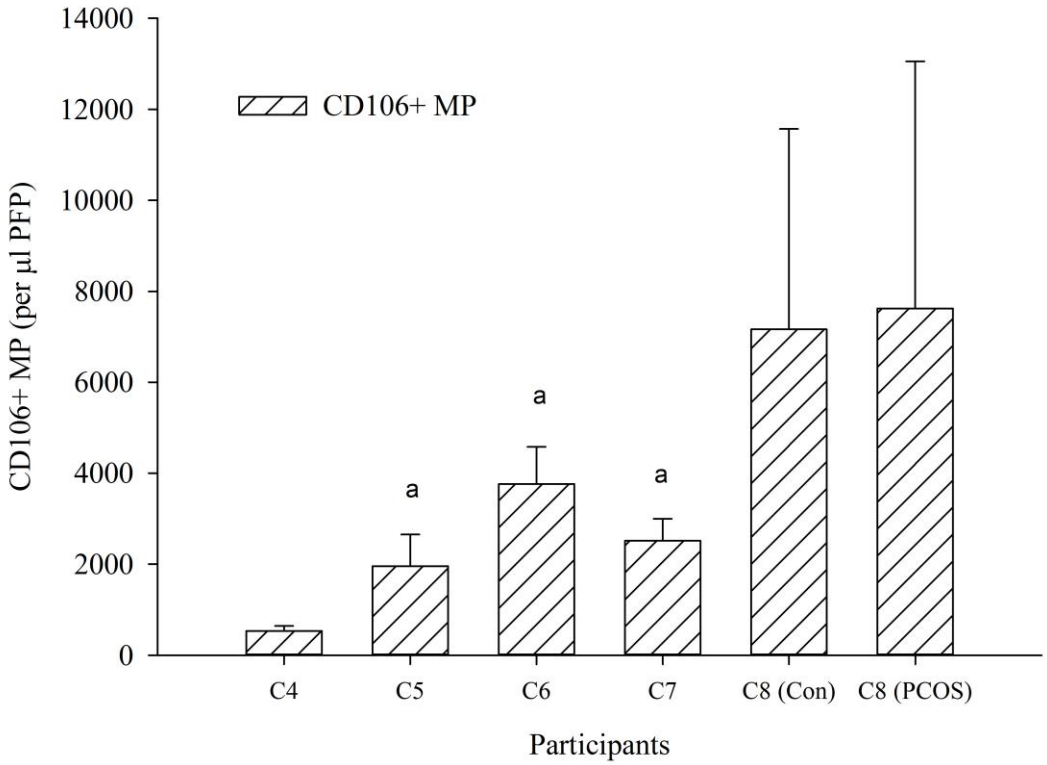


Fig 9.2 CD106+ MP in participants from chapter 4 (C4), chapter 5 (C5), chapter 6 (C6), chapter 7 (C7), chapter 8 (controls, C8 Con) and chapter 8 (PCOS, C8 PCOS) at rest/pre exercise. Data represented as the concentration of CD106+ MP per µl PFP (means ± SEM). [a] significantly different compared to C4 ($p \leq 0.004$). Error bars represent mean ± SEM.

9.3.3 Effects of gender on EMP

Table 9.1 shows the resting/pre exercise data for different groups throughout this thesis in order to determine any possible influence of gender of MP concentration. When combining group data from either healthy, trained participants or overall participants free from any known disease, it can be seen that there was no significant differences between genders. For the first 3 experimental chapters, which involved participants that were accustomed to exercise, the concentrations of + MP were similar between males and females, in particularly CD105+ MP (807 ± 115 CD105+ MP per µl PFP and 849 ± 166 CD105+ MP per µl PFP for males and females respectively, p = 0.85). With the addition of participants that were healthy, but could not be deemed accustomed to exercise, i.e. from chapters 7 and 8, there were greater concentrations of both markers of + MP in females compared to males, but this was not significant.

Table 9.1 Resting/pre exercise + MP concentrations in healthy trained males, healthy trained females, healthy males and healthy females from entire thesis (mean ± SEM).

+ MP (per µl PFP)	Healthy, trained males (n = 28)	Healthy, trained females (n = 10)	P value	Healthy males (n = 34)	Healthy females (n = 24)	P value
CD105+ MP	807 ± 115	849 ± 166	0.85	837 ± 117	1230 ± 475	0.36
CD106+ MP	2149 ± 509	1296 ± 696	0.36	2223 ± 421	4051 ± 1918	0.30

Data represented as means (± SEM). Healthy trained males taken from chapters 4 and 6. Healthy trained females taken from chapter 5. Healthy males taken from chapters 4, 6 and 7. Healthy females taken from chapters 5, 7 and 8.

9.3.4 Effects of acute exercise on EMP

Fig 9.3 displays the general profile of + MP quantification as a result of exercise in the participants from the repeated cycle sprints exercise protocol (C4 and C5) and the long duration cycling exercise (C6). With the exception of CD106+ MP in the long duration exercise protocol, both markers appeared to follow a similar course after exercise. It may be suggested that across studies, exercise has little effect on CD105+ MP and CD106+ MP immediately after exercise, after which these markers appear to rise to an observed peak at 90 min post exercise. A finding consistent from all 3 acute exercise studies is that CD105+ MP and CD106+ MP fall from this observed peak and near basal levels by 180 min post exercise.

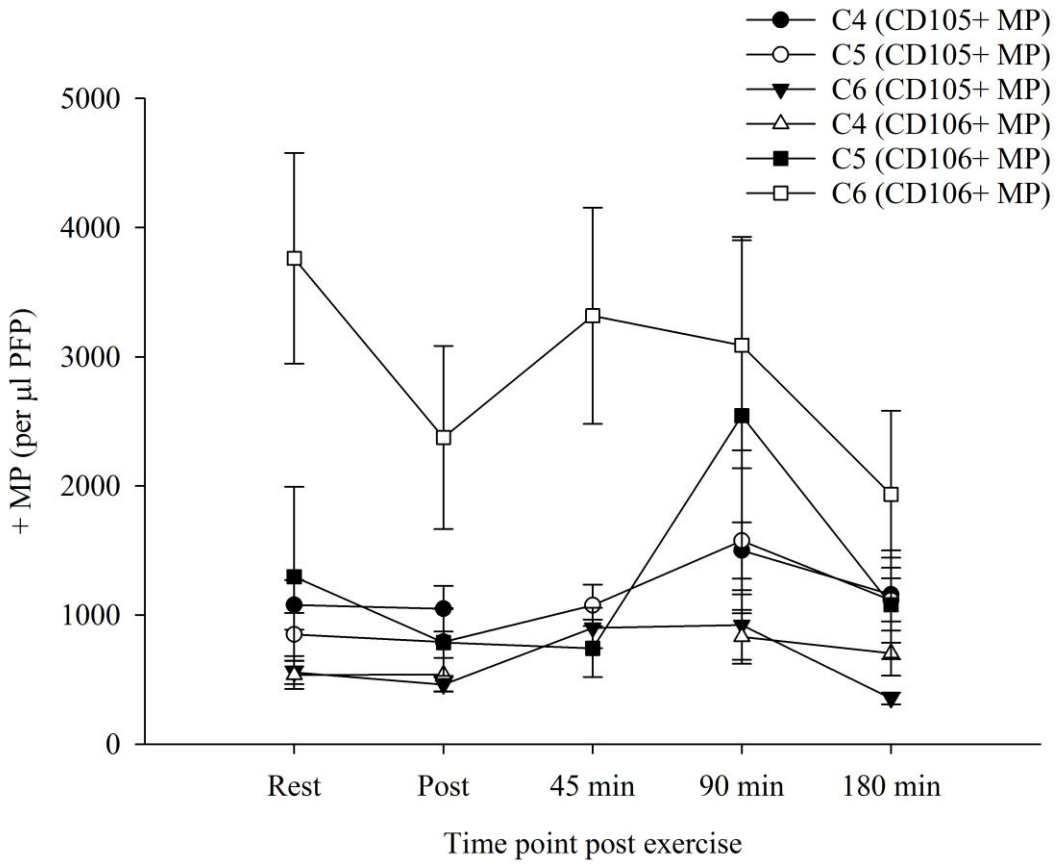


Fig 9.3 CD105+ MP and CD106+ MP in participants from chapter 4 (C4), chapter 5 (C5), and chapter 6 (C6) at rest, and then post, 45, 90 and 180 min post exercise. Data represented as the concentration of CD105+ MP and CD106+ MP per µl PFP (means ± SEM).

9.4 Discussion

This chapter was aimed at comparing data between the experimental chapters and to provide an overview of the response of EMP across the range of studies that have been investigated. Resting data showed that EMP concentrations tended to be higher in individuals that were either sedentary (chapter 7) or known to have a disease, in this instance women with PCOS (chapter 8). In accordance with current literature, this finding would be expected, as it has been shown previously that women with PCOS present with ED compared with healthy controls (El-Kannishy et al., 2010; Sprung et al., 2014), and EMP (CD31+/CD42b-) have been shown to correlate to ED in women with increased obesity and CV risk factors characterised by women with PCOS (Esposito et al., 2006). Surprisingly however, PCOS women in this thesis did not have significantly greater levels of CD105+ MP or CD106+ MP when compared to some of the chapters that involved healthy, trained individuals. This may be in part explained by a relatively small sample size for the PCOS group (n = 11) and further work involving a larger sample size may elicit a significantly greater difference compared to healthy individuals.

It has been previously stated (Toth et al., 2007) that gender may influence the concentration of MP, but this has not been studied extensively and further research is required in order to assess this. Toth et al (2007) discovered that PMP (CD61), P-selectin exposing PMP and CD62E were elevated in healthy females (n = 27) compared to healthy men (n = 18). Additionally, these concentrations seemed to be strongly associated with the menstrual cycle, with greater concentrations present during the luteal phase. Unfortunately, it was not feasible to control for menstrual cycle phase in women throughout this thesis, due in part to the erratic nature experienced in PCOS

women, as all experienced oligo- or anovulation. Table 9.1 confirms that gender had no effect on the resting/pre exercise concentrations of CD105+ MP or CD106+ MP when involving healthy participants from previous chapters. There was a tendency for higher concentrations of each of these markers in females when combining sedentary individuals from chapter 7, but this is speculative considering the low numbers involved. This highlights that further work should concentrate on larger study samples and thus a more controlled study population, with menstrual cycles factored in to the study design where possible in order to test the effects of gender on concentrations of EMP.

The general time course of EMP concentrations over acute exercise bouts can be seen in Fig 9.3. With the exception of CD106+ MP from the healthy males long duration exercise (chapter 6), EMP concentrations can be seen to follow a typical response to exercise, with insignificant change from rest to immediately post exercise. Exercise then induces an increase in levels of EMP from immediately post up to an observed peak at 90 min post exercise. Levels of each marker then appear to fall to basal levels at 180 min post exercise. Future research should attempt to determine a more precise time course of MP release by incorporating more constant blood sample collection, as it is unclear from results in this thesis if EMP levels were still increasing at 90 min, or actually falling.

Finally, the use of EMP as a validated marker of EF may be further strengthened with the results provided in this thesis. Recently, fellow colleagues (Chrismas et al., 2010) conducted a study that comprised of 5 healthy males that were recreationally active, and it could be argued that the participant population were comparable with the participants in chapters 4, 5 and 6, given that no differences in EMP concentration occurred between

genders at rest. Additionally, the mean ages were comparable between Chrismas et al (2010) and those used in this thesis (23 ± 6 years and 23 ± 4 years respectively). Chrismas et al (2010) measured CD105+ MP by FC in accordance with the protocol used throughout this thesis. With respect to the resting concentrations of CD105+ MP, it may suggested that concentrations appear comparable to those in Chrismas et al (2010) when using all participants across chapters 4, 5 and 6 (818 ± 94 CD105+ MP per μl PFP in this thesis). Furthermore, Madden et al (2010) tested concentrations in CD105+ MP in five healthy males using the same protocol as in the previous study discussed and this thesis. It may be suggested again that resting values of healthy participants in this thesis fall close to those in this published research, although detailed results are not available. These results do however support that EMP concentrations can be a reliable source of EF as the concentrations from this thesis appear to fall within close proximity of published research using similar participants at rest.

This chapter was able to show that across multiple studies and using a wide spectrum of human participants, CD105+ MP and CD106+ MP appear to be consistent and a reliable measure of EF at rest/pre exercise and during post exercise recovery. In general, these biomarkers were elevated in participants with PCOS, a condition known to present with ED (Paradisi et al., 2001; Thomson et al., 2012) when compared with healthy volunteers.

Chapter 10: General Discussion

10.1 Introduction

This thesis has investigated a series of acute and chronic exercise sessions in both health and diseased populations. The experiments set out to assess the EMP response to highly strenuous, acute exercise in both males and females. The thesis then looked at acute exercise completed over a longer duration in healthy males. Further to this, subsequent chapters assessed different techniques of EF as a result of exercise in sedentary individuals, and in women with PCOS during an exercise intervention. The purpose of the current section is to further discuss the findings of this thesis and to highlight areas for future research. Prior to this, it is important to re-address the aims and main findings of each chapter.

- ❖ Experiment 1 (chapter 4) – The aim of this chapter was to assess the damage of the endothelium of healthy males by measuring EMP following acute, highly intense cycling, and to see if the ingestion of $NaHCO_3$ affected any possible damage.

- *Highly strenuous repeated sprint cycling was sufficient enough to elicit sufficient endothelial damage in healthy males. Additionally, it was found that the ingestion of $NaHCO_3$ had no effect on the quantity of EMP released after exercise. This then led to the combination of placebo and experimental data, and from this data it was apparent that both CD105+ MP and CD106+ MP rose significantly from rest and immediately post exercise respectively to a peak value at 90 min post exercise. A decline in both markers was evident from 90 min to 180 min post exercise, but there was no statistical significance between these two points.*

❖ Experiment 2 (chapter 5) – The aim of this chapter was to verify the EMP results from experiment 1 and to examine the effects of an identical exercise protocol in healthy females, without the ingestion of $NaHCO_3$. An additional aim was to further assess the damage of the endothelium post exercise by the addition of a blood draw at 45 min post exercise.

- *Highly strenuous sprint cycling was again sufficient to cause ED, as seen by a rise in CD105+ MP at 90 min post exercise in healthy females. For both markers of EF, this population showed signs of endothelial repair as evidenced by a decline in MP at 180 min, but this value was not significant. This chapter also highlighted that shear stress may be an important factor in ED. Although it was not possible to measure this specifically, HR and SBP were significantly affected by exercise that may have contributed to the ED seen. The findings here verified those seen in experiment 1, as an acute exercise bout significantly increased EMP, and showed signs of a return to baseline levels within a relatively short time frame.*

❖ Experiment 3 (chapter 6) – This experiment investigated the effects of a longer duration, high intensity aerobic exercise bout of 90 min cycling on the EMP response in healthy males in order to see the effects of a more prolonged exercise on EF. Following on from the previous chapter, factors of shear stress were measured in order to assess the influence of these factors on the quantity of EMP.

- *The findings from chapters 4 and 5 were verified as EF was significantly affected by exercise, as seen by rises in EMP at several time points post exercise. Additionally, the endothelium was repaired within 180 min of ceasing exercise. The additional blood draw suggested that levels of EMP appear to be*

rising from immediately post exercise, through 45 min to a peak value of 90 min post exercise, consistent with the previous two chapters. Levels of pH, lactate and HR were significantly affected by exercise at each of the time points measured, indicating a possible influence of shear stress on the changes in EF seen as a result of exercise.

❖ Experiment 4 (chapter 7) – This experiment investigated EF in sedentary, but otherwise healthy individuals, through the use of EMP (CD105+ MP and CD106+ MP) and the EndoPAT-2000. The aim of this study was to assess the use of these two assessment methods and if they differed in any way as a result of a moderate intensity acute bout of exercise.

- *Exercise was not strenuous enough to see any impairment in EF as indicated by + EMP levels and the EndoPAT-2000 device. Additionally, there was no correlation between + EMP levels and RHI, although there was a slight relationship reflecting a poorer RHI score with higher + EMP levels, but this was not significant (r = -0.327, p = 0.171).*

❖ Experiment 5 (chapter 8) – This experiment was carried out to investigate if EF could be improved as a result of a supervised, short term, moderate intensity exercise programme in women with PCOS.

- *The main finding of this chapter was that EF was improved in PCOS women as a result of 8 weeks of moderate intensity exercise, and this improvement was not seen in control women. This was evidenced by a significant reduction in CD105 +MP from pre (2113 CD105+ MP per µl PFP) to post (424 CD105+ MP per µl PFP) exercise programme (p = 0.025). Additionally, improvements were seen in SBP, DBP, WC, body mass, BMI and cardiorespiratory fitness.*

- ❖ Experiment 6 (chapter 9) – This chapter was a summary chapter investigating the comparison of data across previous experimental chapters. The aim of this chapter was to determine if resting/pre exercise levels of + EMP differed between populations, and also to represent a typical response in EF as a result of acute exercise.

- *This chapter strengthened the use of CD105+ MP and CD106+ MP as markers of EF in participants of varying health status and gender at rest/pre exercise and during post exercise recovery. This chapter also highlighted the need for additional studies in this area of research with larger participant numbers and a more intense and closely monitored MP pattern post exercise.*

10.2 CD105+ MP and CD106+ MP responses to exercise

It is highly possible that the increases in CD105+ MP and CD106+ MP seen in the acute exercise bouts (chapters 4, 5 and 6) were down to shear stress factors, but these were not studied extensively, as this would have been a very complex measure that was originally not a main consideration of the thesis. An attempt was however made following the first experimental study (chapter 4) to measure haemodynamic variables such as HR and BP in order to suggest reasons for the increase in EMP concentrations as in previous research suggesting similar notions (Chen, Chen & Wang, 2010; Jee & Jin, 2012; Sossdorf et al., 2011), but more detailed research is a necessity. In one of the more recent studies to assess the effects of exercise on endothelial markers, 24 male marathon runners were assessed at different stages of an ultra-marathon race of 308 km in distance (Jee & Jin, 2012). The amount of sCD106 and sCD62E significantly increased from baseline to a peak at 100 km of the race. These increases at the early phase of the race suggest that the endothelium was activated and the release of

endothelial markers were dependent on the exercise intensity. Interestingly, the mean running speeds correlated to the trend of sCD106, suggesting exercise intensity is an important factor in the release of adhesion molecules. Increased blood velocity is a typical characteristic of increased physical activity (Bartzeliotou et al., 2007), and there is also an increased oxygen demand in working muscles and increased cardiac output, which subsequently elevates circulating blood and shear stress on the blood vessel walls (Marsh & Coombes, 2005; Stewart et al., 2004). Such increases in shear force would explain the release in endothelial markers, and especially the increases seen in the early phases of vigorous exercise, and warrants further understanding.

Chapter 9 was conducted in order to compare data between studies, and this thesis primarily observed changes in CD105+ MP and CD106+ MP post exercise, achieving an observed peak 90 min post exercise. This response was seen in the acute bouts of highly strenuous exercise, but not seen in chapter 7, an acute bout of moderate intensity, indicating that the nature of the exercise is a key component in EMP release. This finding as discussed in previous chapters is thought to be as a result of shear stress and increased oxidative stress (Vince et al., 2009b). In relation to EMP concentrations as a result of an exercise programme, chapter 8 was able to show that levels of CD105+ MP, but not CD106+ MP, were able to improve in women with PCOS after a moderate intensity exercise programme. This is a highly novel finding, as to the authors knowledge, there is currently no other study that has assessed EMP in PCOS women across an exercise intervention of any kind. Recently however, and in agreement to this chapter, Sprung et al (2013a) presented an improvement in BA-FMD as a result of moderate intensity exercise in PCOS women compared to control PCOS women receiving no exercise intervention. These findings, alongside those from this thesis, suggest that supervised, moderate intensity exercise training should be implemented as

a treatment for ED and primary prevention of CVD in women with PCOS, and we are able to present justification of EMP as a measure.

10.3 Biomarkers of EF

The investigation of EF throughout this thesis was conducted with CD105+ MP and CD106+ MP, and so it is not known if the use of other markers of the endothelium would have produced similar results. Following a recent position statement from the European Society of Cardiology working group on peripheral circulation (Lekakis et al. 2011), it may be suggested that other EMP markers that were not used throughout this thesis are available in order to further attest for the endothelial origin of MP. This group suggest that CD144, CD146, and CD62E appear strong markers for EMP detection. It was in the author's interest to use more markers, given that the use of limited markers may under represent EF (Haghjooyejavanmard & Nematbakhsh, 2008) but there were constraints on this. At present, a phenotypically uniform marker that encompasses varying disease indications is lacking (Yong, Koh & Shim, 2013). The standardisation in detection of MP is required and would prove useful in the risk stratification and prediction of disease outcomes in patients at risk. However, the markers used in the current thesis do cover the endothelium, as CD106+ MP are specifically expressed on activated endothelium (Madden et al., 2010), and CD105+ MP are expressed on vascular endothelial cells (Dallas et al., 2008). There have been numerous efforts to standardize the enumeration of MP (Robert et al., 2009), but there still exists no standardised methods for the measurements of MP. Additionally, studies looking at the effect of *in vivo* derived MP isolated from plasma may be fraught due by pre-analytic alterations introduced by the collection method (Mullier et al., 2013). In order to account for this, all variables were controlled during this thesis through the adoption of

a standard protocol for MP quantification. An MP region was established using megamix beads (Biocytex, France) according to the current International Society on Thrombosis and Haemostasis Scientific and Standardization Subcommittee protocol (Lacroix et al., 2010). This standard protocol has been well used by fellow research colleagues for MP quantification (Chrismas et al., 2010; Madden et al., 2010; Vince et al., 2009a;b) and should be implemented in future research.

In conclusion, the data presented here has strengthened the notion that ED is a mechanisms induced by highly strenuous exercise, and also presents in women with PCOS, and somewhat in sedentary, otherwise healthy individuals. The data also suggests that exercise training can be hugely beneficial in improving EF in PCOS women, and also the endothelium appears to be at its most damaged within 90 min of ceasing highly strenuous exercise, but the endothelium can be repaired in healthy males and females. However, these findings would need to be evaluated in further studies.

10.4 Recommendations for future research

This thesis has demonstrated that CD105+ MP and CD106+ MP have the potential to be considered as effective markers of EF as a response of acute and chronic exercise in both health and diseased populations. Recommendations for future work in this project include the continuation of the acute exercise bouts with the addition of an appropriate measure of shear stress on MP release, as this remains largely speculative (Chen,Chen & Wang, 2010). The impact of differing modes, duration and intensities of exercise should be explored further, as highly intense exercise has previously been shown to have no impact on EMP (Chaar et al., 2011). Adaptation of the clinical studies (chapters

7 and 8) would be very useful to support and extend the preliminary findings from this thesis, in a field that is ever growing with popularity and research. Predominantly, achieving greater recruitment numbers for the PCOS arm would enable further analysis and sub populations to be formed, enabling correlation of body composition with EMP as seen in previous research (Esposito et al., 2006). The latter study showed that body composition was an important factor in numbers of EMP and PMP, suggesting a key role for WHR, and this should be explored further. In addition, other methods of analysing EF, such as FMD, could be implemented alongside current methods in order to further validate the use of MP biomarkers. Furthermore, more research is needed in order to investigate any differences in resting MP levels between genders, and also test the effect of the menstrual cycle on these concentrations of MP, as this area remains scarce (Toth et al., 2007).

Although the levels of + MP were unexpected in some circumstances, such as CD106+ MP in chapter 6, it does appear that concentrations of these biomarkers can represent EF as one would expect based on the wealth of research that is currently being published. Future research should however attempt to adhere to a standardised technique for MP quantification and implement this in larger population sizes.

Chapter 11: References

Abcam. Flow cytometry guide. http://www.abcam.com/index.html?pageconfig=resource&rid=11446 Accessed 4th August 2014.

Adamopoulos, S., Parissis, J., Kroupis, C., Georgiadis, M., Karatzas, D., Karavolias, G., Koniavitou, K., Coats, A.J., & Kremastinos, D.T. (2001). Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. *Eur Heart J,* **22(9), 791-797.**

Arthur, H.M., Ure, J., Smith, A.J., Renforth, G., Wilson, D.I., Torsney, E., Charlton, R., Parums, D.V., Jowett, T., Marchuk, D.A., Burn, J., & Diamond, A.G. (2000). Endoglin, an ancillary TGFbeta receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. *Dev Biol,* **217(1), 42-53.**

Augustine, D., Ayers, L.V., Lima, E., Newton, L., Lewandowski, A.J., Davis, E.F., Ferry, B. & Leeson, P. (2014). Dynamic release and clearance of circulating microparticles during cardiac stress. *Circ Res*, **114, 109-113.**

Aversa, A., Vitale, C., Volterrani, M., Fabbri, A., Spera, G., Fini, M., & Rosano, G.M. (2008). Chronic administration of Sildenafil improves markers of endothelial function in men with Type 2 diabetes. *Diabet Med,* **25(1), 37-44.**

Axtell., A.L., Gomari, F.A., & Cooke, J.P. (2010). Assessing endothelial vasodilator function with the Endo-PAT 2000. *J Vis Exp*, **15(44), 2167.**

Azevedo, L.C.P. (2012). Microparticles and Exosomes: Are They Part of Important Pathways in Sepsis Pathophysiology?, Severe Sepsis and Septic Shock - Understanding a Serious Killer, Dr Ricardo Fernandez (Ed.), ISBN: **978-953-307-950-9,** InTech, DOI: 10.5772/28136.

Azziz, R., Carmina, E., Dewailly, D., Diamanti-Kandarakis, E., Escobar-Morreale, H.F., Futterweit, W., Janssen, O.E., Legro, R.S., Norman, R.J., Taylor, A.E., & Witchel, S.F. (2009). The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril*, **91, 456-462.**

Azziz, R., Marin, C., Hoq, L., Badamgarav, E., & Song, P. (2005). Health care-related economic burden of the polycystic ovary syndrome during the reproductive life span. *J Clin Endocrinol Metab,* **90(8), 4650-4658.**

Balciunas, M., Bagdonaite, L., Samalavicius, R., & Baublys, A. (2009). Markers of endothelial dysfunction after cardiac surgery: soluble forms of vascular-1 and intercellular-1 adhesion molecules. *Medicina (Kaunas),* **45(6), 434-439.**

Balen, A.H., Laven, J.S.E., Tan, S.L., & Dewailly, D. (2003). Ultrasound assessment of the polycystic ovary: international consensus definitions. *Hum Reprod Upd,* **9(6), 505-514.** doi: 10.1093/humupd/dmg044.

Barac, A., Campia, U., & Panza, J.A. (2007). Methods for evaluating endothelial function in humans. *Hypertension,* **49, 748-760.**

Barringer, T.A., Hatcher, L., & Sasser, H.C. (2011). Potential benefits on impairment of endothelial function after a high-fat meal of 4 weeks of flavonoid supplementation. *J Evid Based Complementary Altern Med, 2011*. doi: 10.1093/ecam/nen048.

Bartzeliotou, A.I., Margeli, A.P., Tsironi, M., Skenderi, K., Bacoula, C., Chrousos, G.P., & Papassotiriou, I. (2007). Circulating levels of adhesion molecules and markers of endothelial activation in acute inflammation induced by prolonged brisk exercise. *Clin Biochem,* **40(11), 765-770.**

Berckmans, R.J., Nieuwland, R., Boing, A.N., Romijn, F.P., Hack, C.E., & Sturk, A. (2001). Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thromb Haemost,* **85(4), 639-646.**

Bernebau, C., Conley, B.A., & Vary, C.P.H. (2007). Novel biochemical pathways of endoglin in vascular cell physiology. *J Cell Biochem,* **102(6), 1375-1388.**

Bevers, E.M., Comfurius, P., Dekkers, D.W., & Zwaal, R. F. (1999). Lipid translocation across the plasma membrane of mammalian cells. *Biochim Biophys Acta,* **1439(3), 317-330.**

Blazquez-Medela, A.M., Garcia-Ortiz, L., Gomez-Marcos, M.A., Recio-Rodriguez, J.I., Sanchez-Rodriguez, A., Lopez-Novoa, J.M., & Martinez-Salgado, C. (2010). Increased plasma soluble endoglin levels as an indicator of cardiovascular alterations in hypertensive and diabetic patients. *BMC Med,* **8, 86.**

Boulanger, C.M., & Dignat-George, F. (2011). Microparticles: An Introduction. *Arterioscler Thromb Vasc Biol,* **31(1), 2-3.** doi: 10.1161/atvbaha.110.220095.

Boulanger, C.M. (2010). Microparticles, vascular function and hypertension. *Curr Opin Nephrol Hypertens,* **19(2), 177-180.**

Boulanger, C.M., Amabile, N., & Tedgui, A. (2006). Circulating microparticles: a potential prognostic marker for atherosclerotic vascular disease. *Hypertension,* **48(2), 180-186.**

Bourdeau, A., Dumont, D.J., & Letarte, M. (1999). A murine model of hereditary hemorrhagic telangiectasia. *J Clin Invest,* **104(10), 1343-1351.**

Brinkworth, G.D., Noakes, M., Moran, L.J., Norman, R., & Clifton, P.M. (2006). Flow-mediated dilatation in overweight and obese women with polycystic ovary syndrome. BJOG : *Int J Gynaecol Obstet,* **113(11), 1308-14.**

Brodsky, S.V., Zhang, F., Nasjletti, A., & Goligorsky, M.S. (2004). Endothelium-derived microparticles impair endothelial function in vitro. *Am J Physiol Heart Circ Physiol,* **286(5), H1910-1915.**

Brown, A.J., Setji, T.L., Sanders, L.L., Lowry, K.P., Otvos, J.D., Kraus, W.E., & Svetkey, P.L. (2009). Effects of exercise on lipoprotein particles in women with polycystic ovary syndrome. *Med Sci Sports Exerc,* **41, 497–504.**

Bruyndonckx, L., Hoymans, V.Y., Van Craenenbroeck, A.H., Vissers, D.K., Vrints, C.J., Ramet, J., & Conraads, V.M. (2013). Assessment of endothelial dysfunction in childhood obesity and clinical use. *Oxid Med Cell Longev,* **2013, 19 pages.**

Burbridge, M.F., West, D.C., Atassi, G., & Tucker, G.C. (1999). The effect of extracellular pH on angiogenesis in vitro. *Angiogenesis,* **3(3), 281-288.**

Burger, D., Schock, S., Thompson, C.S., Montezano, A.C., Hakim, A.M., & Touyz, R. M. (2013). Microparticles: biomarkers and beyond. *Clin Sci (Lond),* **124(7), 423-441.**

Burger, D., & Touyz, R.M. (2012). Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells. *J Am Soc Hypertens,* **6(2), 85-99.**

Burghen, G.A., Givens, J.R., & Kitabchi, A.E. (1980). Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. *J Clin Endocrinol Metab,* **50(1), 113-116.**

Burrows, F.J., Derbyshire, E.J., Tazzari, P.L., Amlot, P., Gazdar, A.F., King, S.W., Letarte, M., Vitetta, E.S., & Thorpe, P.E. (1995). Up-regulation of endoglin on vascular endothelial cells in human solid tumors: implications for diagnosis and therapy. *Clin Cancer Res,* **1(12), 1623-1634.**

Camarda, S.R., Tebexreni, A.S., Pafaro, C.N., Sasai, F.B., Tambeiro, V.L., Juliano, Y., & Barros Neto, T.L. (2008). Comparison of maximal heart rate using the prediction equations proposed by Karvonen and Tanaka. *Arq Bras Cardiol,* **91(5), 311-314.**

Carlos, T.M., Schwartz, B.R., Kovach, N.L., Yee, E., Rosa, M., Osborn, L., Chi-Rosso, G., Newman, B., Lobb, R., & Harlan, J.M. (1990). Vascular cell adhesion

molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. *Blood,* **76(5), 965-970.**

Carmina, E., Bucchieri, S., Esposito, A., Del Puente, A., Mansueto, P., Orio, F., Di Fede, G., & Rini, G. (2007). Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. *J Clin Endocrinol Metab,* **92(7), 2500-2505.**

Carmina, E., Orio, F., Palomba, S., Longo, R.A., Cascella, T., Colao, A., Lombardi, G., Rini, G.B., & Lobo, R.A. (2006). Endothelial dysfunction in PCOS: role of obesity and adipose hormones. *Am J Med,* **119(4), 356 e351-356.**

Carmina, E., Chu, M.C., Longo, R.A., Rini, G.B., & Lobo, R.A. (2005). Phenotypic variation in hyperandrogenic women influences the findings of abdominal metabolic and cardiovascular risk parameters. *J Clin Endocrinol Metab,* **90, 2545-2549.**

Carter, R.A., & Wicks, I.P. (2001). Vascular cell adhesion molecule 1 (CD106): a multifaceted regulator of joint inflammation. *Arthritis Rheum,* **44(5), 985-994.**

Celermajer, D.S. (2008). Reliable endothelial function testing: at our fingertips? *Circulation,* **117(19), 2428-2430.** doi: 10.1161/circulationaha.108.775155.

Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Spiegelhalter, D.J., Miller, O.I., Sullivan, I.D., Lloyd, J.K., & Deanfield, J.E. (1992). Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet,* **340(8828), 1111-1115.**

Chaar, V., Romana, M., Tripette, J., Broquere, C., Huisse, M.G., Hue, O., Hardy-Dessources, M.D., & Connes, P. (2011). Effect of strenuous physical exercise on circulating cell-derived microparticles. *Clin Hemorheol Microcirc,* **47(1), 15-25.**

Chandler, W.L., Yeung, W., & Tait, J.F. (2011). A new microparticle size calibration standard for use in measuring smaller microparticles using a new flow cytometer. *J Thromb Haemost,* **9, 1216–24.**

Chereau, A. (1844). Memories pour servir a l'etude des maladies des ovaries. In Masson & Cie. (1844). **Paris: Fortin.**

Christakou, C., Economou, F., Livadas, S., Piperi, C., Adamapoulos, C., Marinakis, E., & Diamanti-Kandarakis, E. (2011). Strong and positive association of Endothelin-1 with AGEs in PCOS: A causal relationship or a bystander. *Hormones,* **10(4), 292-297.**

Chen, Y.W., Chen, J.K., & Wang, J.S. (2010). Strenuous exercise promotes shear-induced thrombin generation by increasing the shedding of procoagulant microparticles from platelets. *Thromb Haemost,* **104(2), 293-301.**

Chen, M.J., Yang, W.S., Yang, J.H., Chen, C.L., Ho, H.N., & Yang, Y.S. (2007). Relationship between androgen levels and blood pressure in young women with polycystic ovary syndrome. *Hypertension,* **49(6), 1442-1447.**

Chironi, G.N., Boulanger, C.M., Simon, A., Dignat-George, F., Freyssinet, J.M., & Tedgui, A. (2009). Endothelial microparticles in diseases. *Cell Tissue Res,* **335(1), 143-151.**

Chrismas, B.C., Midgley, A.W., Taylor, L., Vince, R.V., Laden, G., & Madden, L.A. (2010). Acute antioxidant pre-treatment attenuates endothelial microparticle release after decompression. *Diving Hyperb Med,* **40(4), 184-188.**

Chung, J., Nguyen, A.K., Henstridge, D.C., Holmes, A.G., Chan, M.H.S., Mesa, J., Lancaster, G.I., Southgate, R.J., Bruce, C.R., Duffy, S.J., Horvath, I., Mestril, R., Watt, M.J., Hooper, P.L., Kingwell, B.A., Vigh, L., Hevener, A., & Febbraio, M.A. (2008). HSP72 protects against obesity-induced insulin resistance. *Proc Natl Acad Sci USA,* **105(5), 1739-1744.** doi: 10.1073/pnas.0705799105.

Clausen, P., Jacobsen, P., Rossing, K., Jensen, J.S., Parving, H.H., & Feldt-Rasmussen, B. (2000). Plasma concentrations of VCAM-1 and ICAM-1 are elevated in patients with Type 1 diabetes mellitus with microalbuminuria and overt nephropathy. *Diabet Med,* **17(9), 644-649.**

Combes, V., Simon, A.C., Grau, G.E., Arnoux, D., Camoin, L., Sabatier, F., Mutin, M., & Dignat-George, F. (1999). In vitro generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. *J Clin Invest,* **104(1), 93-102.**

Cui, W., & Song, Y. (2009). Potential clinical usefulness of endothelial adhesion molecules in predicting risk of type 2 diabetes. *N A J Med Sci*, **2(2), 60-63.**

Curtis, A.M., Edelberg, J., Jonas, R., Rogers, W.T., Moore, J.S., Syed, W., & Mohler, E.R. (2013). Endothelial microparticles: sophisticated vesicles modulating vascular function. *Vasc Med,* **18(4), 204-214**. doi: 10.1177/1358863x13499773.

D'Arcangelo, D., Facchiano, F., Barlucchi, L.M., Melillo, G., Illi, B., Testolin, L., Gaetano, C., & Capogrossi, M.C. (2000). Acidosis inhibits endothelial cell apoptosis and function and induces basic fibroblast growth factor and vascular endothelial growth factor expression. *Circ Res,* **86(3), 312-318.**

Dallas, N.A., Samuel, S., Xia, L., Fan, F., Gray, M.J., Lim, S.J., & Ellis, L.M. (2008). Endoglin (CD105): A marker of tumor vasculature and potential target for therapy. *Clin Cancer Res,* **14, 1931-1937.**

Davies, P.F. (2007). Endothelial mechanism of flow-mediated athero-protection and susceptibility . *Circ Res,* **101(1), 10-12.**

Dawson, A.J., Sathyapalan, T., Smithson, J.A., Vince, R.V., Coady, A.M., Ajjan, R., Kilpatrick, E.S., & Atkin, S.L. (2014). A comparison of cardiovascular risk indices in patients with polycystic ovary syndrome with and without coexisting nonalcoholic fatty liver disease. *Clin Endocrinol*, **80(6), 843-849.**

Dawson, E.A., Whyte, G.P., Black, M.A., Jones, H., Hopkins, N., Oxborough, D., Gaze, D., Shave, R.E., Wilson, M., George, K.P., & Green, D.J. (2008). Changes in vascular and cardiac function after prolonged strenuous exercise in humans. *J Appl Physiol*, **105, 1562-1568.**

De Vivo, A., Baviera, G., Giordano, D., Todarello, G., Corrado, F., & D'Anna, R. (2008). Endoglin, PlGF and sFlt-1 as markers for predicting pre-eclampsia. *Acta Obstet Gynecol Scand,* **87(8), 837-842.**

Deanfield, J.E., Halcox, J.P., & Rabelink, T.J. (2007). Endothelial function and dysfunction: testing and clinical relevance. *Circulation,* **115(10), 1285-1295.** doi: 10.1161/circulationaha.106.652859.

Diamanti-Kandarakis, E., Christakou, C., & Marinakis, E. (2012). Phenotypes and environmental factors: their influence in PCOS. *Curr Pharmac Des*, **18, 270-282.**

Diamanti-Kandarakis, E. (2008). Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. *Expert Rev Mol Med*, **10, e3.**

Diamanti-Kandarakis, E., Alexandraki, K., Piperi, C., Protogerou, A., Katsikis, I., Paterakis, T., Lekakis, J., Panidis, D. (2006). Inflammatory and endothelial markers in women with polycystic ovary syndrome. *Eur J Clin Invest,* **36:691–697.**

Diamanti-Kandarakis, E., Alexandraki, K., Protogerou, A., Piperi, C., Papamichael, C., Aessopos, A., Lekakis, J., & Mavrikakis, M. (2005). Metformin administration improves endothelial function in women with polycystic ovary syndrome. *Eur J Endocrinol*, **152(5), 749-756.**

Dick, H.J.T., Daeson, E.A., Tinken, T.M., Cable, T. & Green, D.J. (2009). Retrograde flow and shear rate acutely impair endothelial function in humans. *Hypertension*, **53, 986-992.**

Dignat-George, F., & Boulanger, C.M. (2011). The many faces of endothelial microparticles. *Arterioscler Thromb Vasc Biol,* **31(1), 27-33.**

Dohi, Y., Ohashi, M., Sugiyama, M., Takase, H., Sato, K., & Ueda, R. (2003). Circulating thrombomodulin levels are related to latent progression of atherosclerosis in hypertensive patients. *Hypertens Res,* **26, 479-483.**

Dokras, A., Clifton, S., Futterwelt, W., & Wild, R. (2012). Increased prevalence of anxiety symptoms in women with polycystic ovary syndrome: systematic review and meta-analysis. *Fertil Steril*, **97(1), 225-230.**

Dokras, A., Bochner, M., Hollinrake, E., Markham, S., VanVoorhis, B., & Jagasia, D.H. (2005). Screening women with polycystic ovary syndrome for metabolic syndrome. *Obstet Gynecol,* **106(1), 131-137** 110.1097/1001.AOG.0000167408.0000130893.0000167406b.

Donato, A.J., Pierce, G.L., Lesniewski, L.A., & Seals, D.R. (2009). Role of NFkappaB in age-related vascular endothelial dysfunction in humans. *Aging (Albany NY),* **1(8), 678-680.**

Doshi, S.N., Naka, K.K., Payne, N., Jones, C.J., Ashton, M., Lewis, M.J., & Goodfellow, J. (2001). Flow-mediated dilation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin Sci,* **101(6), 629-635.**

Drust, B., Waterhouse, J., Atkinson, G., Edwards, B & Reilly, T. (2005). Circadian rhythms in sports performance-an update. *Chronobiol Int*, **22, 21-44.**

Dunaif, A. (1997). Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev,* **18(6), 774-800.**

Dunaif, A., Segal, K.R., Futterweit, W., & Dobrjansky, A. (1989). Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes,* **38(9), 1165-1174.**

Ehrmann, D.A. (2005). Medical Progress: Polycystic ovary syndrome. *N Engl J Med*, **352(12), 1223-1236.**

El-Kannishy, G., Kamal, S., Mousa, A., Saleh, O., El Badrawy, A., El farahaty, R., & Shokeir, T. (2010). Endothelial function in young women with polycystic ovary syndrome (PCOS): implications of body mass index (BMI) and insulin resistance. *Obes Res Clin Pract,* **4(1), e49-e56.**

Enjeti, A.K., Lincz, L.F., & Seldon, M. (2007). Detection and measurement of microparticles: an evolving research tool for vascular biology. *Semin Thromb Hemost,* **33(8), 771-779.**

Esposito, K., Ciotola, M., Schisano, B., Gualdiero, R., Sardelli, L., Misso, L., Giannetti, G., & Giugliano, D. (2006). Endothelial microparticles correlate with endothelial dysfunction in obese women. *J Clin Endocrinol Metab,* **91(9), 3676-3679.** doi: 10.1210/jc.2006-0851.

Feletou, M., & Vanhoutte, P.M. (2006). Endothelial dysfunction: a multifaceted disoder. *Am J Physiol Heart Circ Physiol,* **291, H985-H1002.**

Flammer, A.J., Anderson, T., Celermajer, D.S., Creager, M.A., Deanfield, J., Ganz, P., Hamburg, N.H., Luscher, T.F., Shechter, M., Taddei, S., Vita, J.A., & Lerman, A. (2012). The assessment of endothelial function: from research into clinical practice. *Circulation,* **126, 753-767.**

Flammer, A.J., & Luscher, T.F. (2010). Three decades of endothelium researcg: from the detection of nitric oxide to the everyday implementation of endothelial function measurements in cardiovascular diseases. *Swiss Med Wkly,* **140, w13122.**

Flaumenhaft, R. (2006). Formation and fate of platelet microparticles. *Blood Cells Mol Dis,* 36, 182–187.

Foster, C., Florhaug, J.A., Franklin, J., Gottschall, L., Hrovatin, L.A., Parker, S., Doleshal., P., & Dodge, C. (2001). A new approach to monitoring exercise training. *J Strength Cond Res,* **15(1), 109-115.**

Fotis, L., Giannakopoulos, D., Stamogiannou, L., & Xatzipsalti, M. (2012). Intercellular cell adhesion molecule-1 and vascular cell adhesion molecule-1 in children. Do they play a role in the progression of atherosclerosis?. *Hormones,* **11(2), 140-146.**

Freyssinet, J.M., & Toti, F. (2010). Formation of procoagulant microparticles and properties. *Thromb Res,* **125 Suppl 1, S46-48.**

Freyssinet, J.M. (2003). Cellular microparticles: what are they bad or good for? *J Thromb Haem,* **1(7), 1655-1662.**

Freyssinet, J.M., Toti, F., Hugel, B., Gidon-Jeangirard, C., Kunzelmann, C., Martínez, M.C., & Meyer, D. (1999). Apoptosis in vascular disease. *Thromb Haemost*. **82, 727–735.**

Fukumura, D., Xu, L., Chen, Y., Gohongi, T., Seed, B., & Jain, R.K. (2001). Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. *Cancer Res,* **61(16), 6020-6024.**

Furchgott, R.F., & Zawadzki, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature,* **288(5789), 373-376.**

Gad, A., Alwakeel, H., Bekheet, I., Mansour, I., El Ilbrashy, I., & Ibrahim, A. (2014). Study of platelet and endothelial microparticles in patients with type 2 diabetes mellitus. *Life Si J,* **11(3), 327-334.**

Galley, H.F., & Webster, N.R. (2004). Physiology of the endothelium. *Brit J Anaesth,* **93(1), 105-113.**

Gearing, A.J., & Newman, W. (1993). Circulating adhesion molecules in disease. *Immunol Today,* **14, 506-512.**

Gelderman, M.P., & Simak, J. (2008). Flow cytometric analysis of cell membrane microparticles. *Methods Mol Biol,* **484, 79-93.**

George, J.N., Thoi, L.L., McManus, L.M., & Reimann, T.A. (1982). Isolation of human platelet membrane microparticles from plasma and serum. *Blood,* **60(4), 834-840.**

Ghisi, G.L., Durieux, A., Pinho, R., & Benetti, M. (2010). Physical exercise and endothelial dysfunction. *Arq Bras Cardiol,* **95(5), e130-137.**

Giallauria, F., Orio, F., Palomba, S., Lombardi, G., Colao, A., & Vigorito, C. (2008a). Cardiovascular risk in women with polycystic ovary syndrome. *J Cardiovasc Med,* **9(10), 987-992.** doi: 10.2459/JCM.0b013e32830b58d4.

Giallauria, F., Palomba, S., Maresca, L., Vuolo, L., Tafuri, D., Lombardi, G., Colao, A., Vigorito, C., & Orio, F. (2008b). Exercise training improves autonomic function and inflammatory pattern in women with polycystic ovary syndrome (PCOS). *Clin Endocrinol,* **69(5), 792-798.** doi: 10.1111/j.1365-2265.2008.03305.x

GillingSmith, C., Story, H., Rogers, V., & Franks, S. (1997). Evidence for a primary abnormality of thecal cell steroidogenesis in the polycystic ovary syndrome. *Clin Endocrinol,* **47(1), 93-99.**

Golias, C., Tsoutsi, E., Matziridis, A., Makridis, P., Batistatou, A., & Charalabopoulos, K. (2007). Review. Leukocyte and endothelial cell adhesion molecules in inflammation focusing on inflammatory heart disease. *In Vivo,* **21(5), 757-769.**

Goodarzi, M.O., Dumesic, D.A., Chazenbalk, G., & Azziz, R. (2011). Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol,* **7, 219-231.**

Goodarzi, M.O., Erickson, S., Port, S.C., Jennrich, R.I., & Korenman, S.G. (2003). Relative impact of insulin resistance and obesity on cardiovascular risk factors in polycystic ovary syndrome. *Metabolism,* **52(6), 713-719.**

Green, D.J., O'Driscoll, G., Joyner, M.J., & Cable, N.T. (2008). Exercise and cardiovascular risk reduction: time to update the rationale for exercise? *J Appl Physiol,* **105(2), 766-768.**

Grundy, S.M. (2002). Obesity, metabolic syndrome, and coronary atherosclerosis. *Circulation,* **105(23), 2696-2698.**

Guiraud, T., Gayda, M., Juneau, M., Bosquet, L., Meyer, P., Theberge-Julien, G., Galinier, M., Nozza, A., Lambert, J., Rheaume, E., Tardiff, J.C., & Nigam, A. (2013). A single bout of high-intensity interval exercise does not increase endothelial or platelet microparticles in stable, physically fit men with coronary heart disease. *Can J Cardiol,* **29(10), 1285-1291.**

Halcox, J.P.J., Donald, A.E., Ellins, E., Witte, D.R., Shipley, M.J., Brunner, E.J., Marmot, M.G., & Deanfield, J.E. (2009). Endothelial function predicts progression of carotid intima-media thickness. *Circulation,* **119(7), 1005-1012.** doi: 10.1161/circulationaha.108.765701.

Haghjooyejavanmard, S. & Nematbakhsh, M. (2008) Endothelial function and dysfunction: clinical significance and assessment. *J Res Med Sci,* **13, 207-221.**

Hallmark, R., Patrie, J.T., Liu, Z., Gaesser, G.A., Barrett, E.J. & Weltman, A. (2014). The effect of exercise intensity on endothelial function in physically inactive lean and obese adults. *PLoS ONE,* **9(1), e85450.**

Hamburg, N.M., & Benjamin, E.J. (2009). Assessment of endothelial function using digital pulse amplitude tonometry. *Trends Cardiovascular Med,* **19(1), 6-11.**

Hamburg, N.M., Larson, M.G., Vita, J.A., Vasan, R.S., Keyes, M.J., Widlansky, M.E., Fox, C.S., Mitchell, G.F., Levy, D., Meigs, J.B., & Benjamin, E. J. (2008a). Metabolic syndrome, insulin resistance, and brachial artery vasodilator function in Framingham offspring participants without clinical evidence of cardiovascular disease. *Am J Cardiol,* **101(1), 82-88.**

Hamburg, N.M., Keyes, M.J., Larson, M.G., Vasan, R.S., Schnabel, R., Pryde, M.M., Mitchell, G.F., Sheffy, J., Vita, J.A., & Benjamin, E.J. (2008b). Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham heart study. *Circulation,* **117(19), 2467-2474.** doi: 10.1161/circulationaha.107.748574.

Hansen, D., Steven, A., Eijnde, B.O., & Dendale, P. (2012). Endurance exercise intensity determination in the rehabilitation of coronary artery disease patients. A critique re-appraisal of current evidence. *Sports Med*, **42(1), 11-30.**

Hargett, L.A., & Bauer, N.N. (2013). On the origin of microparticles: from "platelet dust" to mediators of intercellular communication. *Pulm Circ*, **3(2), 329-340.**

Harrison, C.L., Lombard, C.B., Moran, L.J., & Teede, H.J. (2011). Exercise therapy in polycystic ovary syndrome: a systematic review. *Human Reprod Update*, **17(2), 171-183.** doi: 10.1093/humupd/dmq045.

Harrison, M., Murphy, R.P., O'Connor, P.L., O'Gorman, D.J., McCaffrey, N., Cummins, P.M., & Moyna, N.M. (2009). The endothelial microparticle response to a high fat meal is not attenuated by prior exercise. *Eur J Appl Physiol*, **106(4), 555-562.** doi: 10.1007/s00421-009-1050-5.

Harvey, P.J., Morris, B.L., Kubo, T., Picton, P.E., Su, W.S., Notarius, C.F., & Floras, J.S. (2005). Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women. *J Hypertens*, **23(2), 285-292.**

Helal, O., Defoort, C., Robert, S., Marin, C., Lesavre, N., Lopez-Miranda, J., Riserus, U., Basu, S., Lovegrove, J., McMonagle, J., Roche, H.M., Dignat-George, F., & Lairon, D. (2011). Increased levels of microparticles originating from endothelial cells, platelets and erythrocytes in subjects with metabolic syndrome: relationship with oxidative stress. *Nutr Metab Cardiovasc Dis*, **21(9), 665-671.**

Higashi, Y., Sasaki, S., Kurisu, S., Yoshimizu, A., Sasaki, N., Matsuura, H., Kajiyama, G., & Oshima, T. (1999). Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects. *Circulation*, **100, 1194-1202.**

Himelein, M.J. & Thatcher, S.S. (2006). Polycystic ovary syndrome and mental health: A review. *Obstet Gynecol Surv*, **61(11), 723-732.**

Hoeger, K.M., & Oberfield, S.E. (2012). Do women with PCOS have a unique predisposition to obesity? *Fertil Steril*, **97(1), 13-17.**

Hoffman, L.K., & Ehrmann, D.A. (2008). Cardiometabolic features of polycystic ovary syndrome. *Nat Clin Pract Endocrinol Metab*, **4(4), 215-222.**

Horstman, L.L., & Ahn, Y.S. (1999). Platelet microparticles: a wide-angle perspective. *Crit Rev Oncol Hematol*, **30(2), 111-142.**

Horstman, L.L., Jy, W., Jimanez, J.J. & Ahn, Y.S. (2004). Endothelial microparticles as markers of endothelial dysfunction. *Front Biosci*, **9, 1118-1135.**

Hugel, B., Martínez, M.C., Kunzelmann, C., & Freyssinet, J.M. (2005). Membrane microparticles: two sides of the coin. *Physiology,* **20(1), 22-27.** doi: 10.1152/physiol.00029.2004.

Hugel, B., Socie, G., Vu, T., Toti, F., Gluckman, E., Freyssinet, J.M., & Scrobohaci, M.I. (1999). Eleveated levels of circulating procoagulant microparticles in patients with paroxysmal nocturnal hemoglobinuria and aplastic anemia. *Blood,* **93, 3451-3456.**

Hwang, I.C., Kim, K.H., Choi, W.S., Kim, H.J., Im, M.S., Kim, Y.J., Kim, S.H., Kim, M.A., Sohn, D.W., & Zo, J.H. (2012). Impact of acute exercise on brachial artery flow-mediated dilatation in young healthy people. *Cardiovasc Ultrasound,* **10(39), 1476-7120.**

Iiyama, K., Hajra, L., Iiyama, M., Li, H., DiChiara, M., Medoff, B.D., & Cybulsky, M.I. (1999). Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. *Circ Res,* **85(2), 199-207.**

Ilie, I.R., Pepene, C.E., Duncea, I., & Ilie, R. (2008). Vascular abnormalities and low-grade chronic inflammation in women with polycystic ovary syndrome: relationships with insulin resistance, obesity and hyperandrogenemia. *Cent Eur J Med,* **3, 257–270.**

Italiano, J.E., Jr., Mairuhu, A.T., & Flaumenhaft, R. (2010). Clinical relevance of microparticles from platelets and megakaryocytes. *Curr Opin Hematol,* **17(6), 578-584.**

Jae, S.Y., Ahn, E.S., Heffernan, K.S., Woods, J.A., Lee, M.K., Park, W.H., & Fernhall, B. (2007). Relation of heart rate recovery after exercise to C-reactive protein and white blood cell count. *Am J Cardiol,* **99(5), 707-710.**

Jayachandran, M., Litwiller, R.D., Owen, W.G. & Miller, V.M. (2009). Circulating microparticles and endogenous estrogen in newly menopausal women. *Climacteric,* **12, 177-184.**

Jee, H., Park, J., Oh, J.G., Lee, Y.H., Shin, K.A., & Kim, Y.J. (2013). Effect of a prolonged endurance marathon on vascular endothelial and inflammation markers in runners with exercise-induced hypertension. *Am J Phys Med Rehabil,* **92(6), 513-522.**

Jee, H. & Jin, Y. (2012). Effects of prolonged endurance exercise on vascular endothelial and inflammation markers. *J Sports Sci Med,* **11, 719-726.**

Jenkins, N.T., Padilla, J., Boyle, L.J., Credeur, D.P., Laughlin, M.H., & Fadel, P.J. (2013). Disturbed blood flow acutely induces activation and apoptosis of the human vascular endothelium. *Hypertension,* **61, 615-621.**

Jensen, L., Bangsbo, J., & Hellsten, Y. (2004). Effect of high intensity training on capillarization and presence of angiogenic factors in human skeletal muscle. *J Physiol,* **557(Pt 2), 571-582.**

Jimenez, J.J., Jy, W., Mauro, L.M., Soderland, C., Horstman, L.L., & Ahn, Y.S. (2003). Endothelial cells release phenotypically and quantitatively distinct microparticles in activation and apoptosis. *Thromb Res,* **109(4), 175-180.**

Johnson-Leger,C., Aurrand-Lions, M & Imhof, B.A. (2000). The parting of the endothelium: miracle, or simply a junctional affair?. *J Cell Sci,* **113, 921-933.**

Jungersten, L., Ambring, A., Wall, B. & Wennmalm, A. (1997). Both physical fitness and acute exercise regulate nitric oxide formation in healthy humans. *J Appl Physiol,* **82, 760-764.**

Jy, W., Horstman, L.L., Jimenez, J.J., & Ahn, Y.S. (2004). Measuring circulating cell-derived microparticles. *Thromb Haemost,* **2(10), 1842-1843.**

Jy , W., Horstman, L.L., Arce, M., & Ahn, Y.S. (1992). Clinical significance of platelet microparticles in autoimmune thrombocytopenias. *J Lab Clin Med,* **119, 334-345.**

Kapur, N.K., Morine, K.J., & Letarte, M. (2013). Endoglin: a critical mediator of cardiovascular health. *Vasc Health Risk Manag,* **9, 195-206.**

Karu, T., Nurmekivi, A., Lemberg, H., Pihl, E., & Jurimae, T. (2000). Relationship between perceived readiness to run and physiological variables during repeated 2000 m bouts in middle-distance runners. *Scand J Med Sci Sports,* **10(1), 33-36.**

Kato, G.J., Martyr, S., Blackwelder, W.C., Nichols, J.S., Coles, W.A., Hunter, L.A., Brennan, M.L., Hazen, S.L., & Gladwin, M.T. (2005). Levels of soluble endothelium-derived adhesion molecules in patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction, and mortality. *Br J Haematol,* **130(6), 943-953.**

Kavanagh, T., Mertens, D.J., Hamm, L.F., Beyene, J., Kennedy, J., Corey, P., & Shephard, R.J. (2003). Peak oxygen intakeand cardiac mortality in women referred for cardiac rehabilitation. *J Am Coll Cardiol,* **42, 2139-2143.**

Kellum, J.A., Song, M., & Li, J. (2004). Science review: extracellular acidosis and the immune response: clinical and physiologic implications. *Crit Care,* **8(5), 331-336.**

Kelly, C.C.J., Lyall, H., Petrie, J.R., Gould, G.W., Connell, J.M.C., & Sattar, N. (2001). Low grade chronic inflammation in women with polycystic ovarian syndrome. *J Clin Endocrinol Metab,* **86(6), 2453-2455.** doi: 10.1210/jc.86.6.2453.

Kerschner, A., Lester, W., Stuart, S.P., & Dokras, A. (2009). Risk of depression and other mental health disorders in women with polycystic ovary syndrome: a longitudinal study. *Fertil Steril*, **91(1), 207-212.**

Ketel, I.J., Stehouwer, C.D., Serne, E.H., Korsen, T.J., Hompes, P.G., Smulders, Y.M., de Jongh, R.T., Homburg, R., & Lambalk, C.B. (2008). Obese but not normal-weight women with polycystic ovary syndrome are characterized by metabolic and microvascular insulin resistance. *J Clin Endocrinol Metab,* **93(9), 3365-3372.**

Kiddy, D.S., Sharp, P.S., White, D.M., Scanlon, M.F., Mason, H.D., Bray, C.S., Polson, D.W., Reed, M.J., & Franks, S. (1990). Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome—an analysis of 263 consecutive cases. *Clin Endocrinol*, **32:213–220.**

Kim, J.S., & Ligler, F.S. (2010). Utilization of microparticles in next generation assays for microflow cytometers. *Anal Bioanal Chem*, **398, 2373-2382.**

Kim, H.K., Song, K.S., Lee, E.S., Lee, Y.J., Park, Y.S., Lee, K.R., & Lee, S.N. (2002). Optimized flow cytometric assay for the measurement of platelet microparticles in plasma: pre-analytic and analytic considerations. *Blood Coagul Fibrinolysis,* **13(5), 393-397.**

Kravariti, M., Naka, K.K., Kalantaridou, S.N., Kazakos, N., Katsouras, C.S., Makrigiannakis, A., Paraskevaidis, E.A., Chrousos, G.P., Tsatsoulis, A., & Michalis, L.K. (2005). Predictors of endothelial dysfunction in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab,* **90(9), 5088-5095.**

Kurose, S., Tsutsumi, H., Yamanaka, Y., Shinno, H., Miyauchi, T., & Tamanoi, A. (2012). Improvement in endothelial function by lifestyle modification focused on exercise training is associated with insulin resistance in obese patients. *Obes Res Clin Prac*, **8(1), 106-114.**

Kuvin, J.T., Patel, A.R., Sliney, K.A., Pandian, N.G., Sheffy, J., Schnall, R.P., Karas, R.H., & Udelson, J.E. (2003). Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J,* **146(1), 168-174.**

Lacroix, R., Poncelet, P., Harhouri, K., Bouriche, T., Judicone, C., Wischhusen, J., Arnauld, L., & Dignat-George, F. (2012). High-sensitivity flow cytometry

provides access to standardized measurement of small-size microparticles – brief report. *Arterioscler Thromb Vasc Biol,* **32, 1054–8.**

Lacroix, R., Robert, S., Poncelet, P., & Dignat-George, F. (2010). Overcoming limitations of microparticle measurement by flow cytometry. *Semin Thromb Hemost,* **36(8), 807-818.**

Legro, R. S., Kunselman, A. R., Dodson, W. C., & Dunaif, A. (1999). Prevalence and Predictors of Risk for Type 2 Diabetes Mellitus and Impaired Glucose Tolerance in Polycystic Ovary Syndrome: A Prospective, Controlled Study in 254 Affected Women. *Journal of Clinical Endocrinology & Metabolism, 84*(1), 165-169.

Lehoux, S., Castier, Y., & Tedgui, A. (2006). Molecular mechanisms of the vascular responses to haemodynamic forces. *J Intern Med,* **259(4), 381-392.**

Lekakis, J., Abraham, P., Balbarini, A., Blann, A., Boulanger, C.M., Cockcroft, J., Cosentino, F., Deanfield, J., Gallino, A., Ikonomidis, I., Kremastinos, D., Landmesser, U., Protogerou, A., Stefanadis, C., Tousoulis, D., Vassalli, G., Vink, H., Werner, N., Wilkinson, I., & Vlachopoulos, C. (2011). Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. *Eur J Cardiovasc Prev Rehabil,* **18(6), 775-789.**

Lerman, A., & Burnett, J.C.Jr. (1992). Intact and altered endothelium in regulation of vasomotion. *Circulation,* **86(6), 12-19.**

Leroyer, A.S., Tedgui, A., & Boulanger, C.M. (2008). Role of microparticles in atherothrombosis. *J Intern Med,* **263(5), 528-537.**

Li, D.Y., Sorensen, L.K., Brooke, B.S., Urness, L.D., Davis, E.C., Taylor, D.G., Boak, B.B., & Wendel, D.P. (1999). Defective angiogenesis in mice lacking endoglin. *Science,* **284(5419), 1534-1537.**

Liao, Y., Feng, Y., Chen, L.L., Zeng, T.S., Yu, F., & Hu, L.J. (2014). Coronary heart disease risk equivalence in diabetes and arterial diseases characterized by endothelial function and endothelial progenitor cell. *J Diabetes Complicat*, **28(2), 214-218.**

Libby, P., Ridker, P.M., & Hansson, G.K. (2009). Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol*, **54(23), 2129-2138.**

Liu, K.H., Chan, Y.L., Chan, J.C., & Chan, W.B. (2005). Association of carotid intima-media thickness with mesenteric, preperitoneal and subcutaneous fat thickness. *Atherosclerosis,* **179(2), 299-304.**

Lobo, R. A., & Carmina, E. (2000). The importance of diagnosing the polycystic ovary syndrome. *Annals of Internal Medicine,* **132(12), 989-993.**

Lopez-Novoa, J.M., & Bernabeu, C. (2010). The physiological role of endoglin in the cardiovascular system. *Am J Physiol Heart Circ Physiol,* **299(4), H959-974.**

Lord, J., Thomas, R., Fox, B., Acharya, U., & Wilkin, T. (2006). The central issue? Visceral fat mass is a good marker of insulin resistance and metabolic disturbance in women with polycystic ovary syndrome. *BJOG,* **113(10), 1203-1209.** doi: 10.1111/j.1471-0528.2006.00973.

Lovren, F., & Verma, S. (2013). Evolving role of microparticles in the pathophysiology of endothelial dysfunction. *Clin Chem,* **59(8), 1166-1174.**

Lowenstein, L., Damti, A., Pillar, G., Shott, S., & Blumenfeld, Z. (2007). Evaluation of endothelial function in women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol,* **134(2), 208-212.**

Madden, L.A., Chrismas, B.C., Mellor, D., Vince, R.V., Midgley, A.W., McNaughton, L.R., Atkin, S.L., & Laden, G. (2010). Endothelial function and stress response after simulated dives to 18 msw breathing air or oxygen. *Aviat Space Environ Med,* **81(1), 41-45.** doi: 10.3357/asem.2610.2010.

Madden, L.A., Sandstrom, M.E., Lovell, R.J., & McNaughton, L. (2008). Inducible heat shock protein 70 and its role in preconditioning and exercise. *Amino Acids, **34*(4), 511-516.** doi: 10.1007/s00726-007-0004-7.

Maiorana, A., O'Driscoll, G., Cheetham, C., Dembo, L., Stanton, K., Goodman, C., Taylor, R., & Green, D. (2001). The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. *J Am Coll Cardiol,* **38(3), 860-866.**

Malyszko, J. (2010). Mechanism of endothelial dysfunction in cnronic kidney disease. *Clinica Chimica Acta*, **411, 1412-1420.**

March, W.A., Moore, V.M., Willson, K.J., Phillips, D.I.W., Norman, R.J., & Davies, M.J. (2010). The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod,* **25(2), 544-551.** doi: 10.1093/humrep/dep399.

Marfell-Jones, M., Olds, T., Stewart, A., & Carter, L. (2006), *International Standards for Anthropometric Assessment*, Trans.

Marsh, S.A., & Coombes, J.S. (2005). Exercise and the endothelial cell. *Int J Cardiol,* **99(2), 165-169.**

Maruyama, K., Kadono, T., & Morishita, E. (2012). Plasma levels of platelet-derived microparticles are increased after anaerobic exercise in healthy subjects. *J Atheroscler Thromb*, **19(6):585-7.** Epub 2012 Feb 23.

Mather, K.J., Verma, S., Corenblum, B., & Anderson, T.J. (2000). Normal endothelial function despite insulin resistance in healthy women with the polycystic ovary syndrome. *J Clin Endocrinol Metab,* **85(5), 1851-1856.**

Mause, S.F., & Weber, C. (2010). Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ Res,* **107(9), 1047-1057.**

McCrohon, J.A., Woo, K.S. & Celermajer, D.S. (2000). A comparison of endothelial function in Caucasian and Chinese women before and after the menopause. *Maturitas,* **35(1), 31-37.**

McNaughton, L.R., Siegler, J., & Midgley, A. (2008). Ergogenic effects of sodium bicarbonate. *Curr Sports Med Rep,* **7(4), 230-236.**

Meyer, C., McGrath, B.P., & Teede, H.J. (2005). Overweight women with polycystic ovary syndrome have evidence of subclinical cardiovascular disease. *J Clin Endocrinol Metab,* **90(10), 5711-5716.**

Midgley, A.W., Carroll, S., Marchant, D., McNaughton, L.R., & Siegler, J. (2009). Evaluation of true maximal oxygen uptake based on a novel set of standardized criteria. *Appl Physiol Nutr Metab,* **34(2), 115-123.**

Miller, D.W., Graulich, W., Karges, B., Stahl, S., Ernst, M., Ramaswamy, A., Sedlacek, H.H., Muller, R., & Adamkiewicz, J. (1999). Elevated expression of endoglin, a component of the TGF-beta-receptor complex, correlates with proliferation of tumor endothelial cells. *Int J Cancer,* **81(4), 568-572.**

Moens, A.L., Goovaerts, I., Claeys, M.J., & Vrints, C.J. (2005). Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? *Chest,* **127(6), 2254-2263.**

Moerland, M., Kales, A.J., Schrier, L., van Dongen, M.G., Bradnock, D., & Burggraaf, J. (2012). Evaluation of the EndoPAT as a tool to assess endothelial function. *Int J Vasc Med,* **2012, 904141.** doi: 10.1155/2012/904141.

Moncada, S., & Higgs, A. (1993). The L-arginine-nitric oxide pathway. *N Engl J Med,* **329(27), 2002-2012.**

Montoro-Garcia, S., Shantsila, E., Marin, F., Blann, A., & Lip, G.Y.H. (2011). Circulating microparticles: new insights into the biochemical basis of

microparticle release and activity. *Basic Res Cardiol,* **106(6), 911-923.** doi: 10.1007/s00395-011-0198-4.

Moran, L.J., Cameron, J.D., Strauss, B.J., & Teede, H.J. (2011). Vascular function in the diagnostic categories of polycystic ovary syndrome. *Hum Reprod,* **26(8), 2192-2199.**

Moran, L.J., Pasquali, R., Teede, H.J., Hoeger, K.M., & Norman, R.J. (2009). Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. *Fertil Steril,* **92:1966–1982.**

Moran, L.J., Brinkworth, G., Noakes, M., & Norman, R.J. (2006). Effects of lifestyle modification in polycystic ovarian syndrome. *Reprod Biomed Online,* **12:569–578.**

Moran, L.J., Noakes, M., Clifton, P.M., Tomlinson, L., & Norman, R.J. (2003). Dietary composition in restoring reproductive and metabolic physiology in overweight women with polycystic ovary syndrome. *J Clin Endocrinol Metab,* **88(2), 812-819.** doi: 10.1210/jc.2002-020815.

Morel, O., Jesel, L., Freyssinet, J.M., & Toti, F. (2011). Cellular mechanisms underlying the formation of circulating microparticles. *Arterioscler Thromb Vasc Biol,* **31(1), 15-26.**

Morikawa, S., Inubushi, T., Kito, K., & Tabata, R. (1994). Imaging of phosphoenergetic state and intracellular pH in human calf muscles after exercise by 31P NMR spectroscopy. *Magn Reson Imaging,* **12(7), 1121-1126.**

Moro, C., Pasarica, M., Elkind-Hirsch, K., & Redman, L.M. (2009). Aerobic exercise training improves atrial natriuretic peptide and catecholamine-mediated lipolysis in obese women with polycystic ovary syndrome. *J Clin Endocrinol Metab,* **94(7), 2579-2586.** doi: 10.1210/jc.2009-0051

Mozaffarian, D., Wilson, P.W., & Kannel. (2008). Beyond established and novel risk factors: lifestyle risk factors for cardiovascular disease, *Circulation,* **117(23), 3031-3038.**

Mullier, F., Bailly, N., Chatelain, C., Chatelain, B., & Dogne, J.M. (2013). Pre-analytical issues in the measurement of circulating microparticles: current recommendations and pending questions. *J Thromb Haemost,* **11, 693–696.**

Nachtigal, P., Zemankova Vecerova, L., Rathouska, J., & Strasky, Z. (2012). The role of endoglin in atherosclerosis. *Atherosclerosis,* **224(1), 4-11.**

Nardo, L.G., & Gelbaya, T.A. (2008). Evidence-based approach for the use of ultrasound in the management of polycystic ovary syndrome. *Minerva Ginecol,* **60(1), 83-89.**

Navasiolava, N.M., Dignat-George, F., Sabatier, F., Larina, I.M., Demiot, C., Fortrat, J.O., Gauquelin-Koch, G., Kozlovskaya, I.B., & Custaud, M.A. (2010). Enforced physical inactivity increases endothelial microparticle levels in healthy volunteers. *Am J Physiol Heart Circ Physiol,* **299(2), H248-256.**

Niebauer, J. & Cooke, J.P. (1996). Cardiovascular effects of exercise: role of endothelial shear stress. *J Am Coll Cardiol,* **28, 1652-1660.**

Nielsen, H.G., & Lyberg, T. (2004). Long-distance running modulates the expression of leucocyte and endothelial adhesion molecules. *Scand J Immunol,* **60(4), 356-362.**

Norman, R.J., Dewailly, D., Legro, R.S., & Hickey, T.E. (2007). Polycystic ovary syndrome. *Lancet,* **370(9588), 685-697.**

Norton, K., Norton, L., & Sadgrove, D. (2010). Position statement on physical activity and exercise intensity terminology. *J Sci Med Sport,* **13(5), 496-502.**

Onkelinx, S., Cornelissen, V., Goetschalckx, K., Thomaes, T., Verhamme, P., & Vanhees, L. (2012). Reproducibility of different methods to measure the endothelial function. *Vasc Med,* **17(2), 79-84.**

Oral, B., Mermi, B., Dilek, M., Alanoglu, G., & Sutcu, R. (2009). Thrombin activatable fibrinolysis inhibitor and other hemostatic parameters in patients with polycystic ovary syndrome. *Gynecol Endocrinol,* **25(2), 110-116.**

Orio, F., Palomba, S., & Colao, A. (2006). Cardiovascular risk in women with polycystic ovary syndrome. *Fertil Steril,* **86, S20-S21.** doi: 10.1016/j.fertnstert.2006.03.003.

Orio, F. Jr., Palomba, S., Cascella, T., De Simone, B., Manguso, F., Savastano, S., Russo, T., Tolino, A., Zullo, F., Lombardi, G., Azziz, R., & Colao, A. (2005). Improvement in endothelial structure and function after metformin treatment in young normal-weight women with polycystic ovary syndrome: Results of a 6-month study. *J Clin Endocrinol Metab,* **90:6072–6076.**

Orio, F., Palomba, S., Spinelli, L., Cascella, T., Tauchmanova, L., Zullo, F., Lombardi, G., & Colao, A. (2004). The cardiovascular risk of young women with polycystic ovary syndrome: an observational, analytical, prospective case-control study. *J Clin Endocrinol Metab,* **89(8), 3696-3701.** doi: 10.1210/jc.2003-032049.

Padilla, J., Harris, R.A., Fly, D.A., Rink, L.D. & Wallace, J.P. (2006). A comparison between active and reactive hyperaemia induced brachial artery vasodilation. *Clin Sci*, **110, 387-392.**

Palomba, S., Falbo, A., Giallauria, F., Russo, T., Rocca, M., Tolino, A., Zullo, F., & Orio, F. (2010). Six weeks of structured exercise training and hypocaloric diet increases the probability of ovulation after clomiphene citrate in overweight and obese patients with polycystic ovary syndrome: a randomized controlled trial. *Hum Reprod,* **25(11), 2783-2791.**

Palomba, S., Giallauria, F., Falbo, A., Russo, T., Oppedisano, R., Tolino, A., Colao, A., Vigorito, C., Zullo, F., & Orio, F. (2008). Structured exercise training programme versus hypocaloric hyperproteic diet in obese polycystic ovary syndrome patients with anovulatory infertility: a 24-week pilot study. *Hum Reprod*, **23, 642–650.**

Paradisi, G., Steinberg, H.O., Hempfling, A., Cronin, J., Hook, G., Shepard, M.K., & Baron, A.D. (2001). Polycystic ovary syndrome is associated with endothelial dysfunction. *Circulation,* **103(10), 1410-1415.** doi: 10.1161/01.cir.103.10.1410.

Pasternak, R.C., Criqui, H.M., Benjamin, E.J., Fowkes, F.G., Isselbacher, E.M., McCullough, P.A., Wolf, P.A., & Zheng, Z.J. (2004). American heart association. Atherosclerotic vascular disease conference. Writing group I: epidemiology, *Circulation,* **109(21), 2605-2612.**

Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon, R.O., Criqui, M., Fadl, Y.Y., Fortmann, S.P., Hong, Y., Myers, G.L., Rifai, N., Smith Jr, S.C., Taubert, K., Tracy, R.P., & Vinicor, F. (2003). Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a tatement for healthcare professionals from the centers for disease control and prevention and the american heart association. *Circulation,* **107(3), 499-511.** doi: 10.1161/01.cir.0000052939.59093.45

Peart, D.J., McNaughton, L.R., Midgley, A.W., Taylor, L., Towlson, C., Madden, L.A., & Vince, R.V. (2011). Pre-exercise alkalosis attenuates the heat shock protein 72 response to a single-bout of anaerobic exercise. *J Sci Med Sport,* **14(5), 435-440.**

Perez-Gomez, E., Del Castillo, G., Juan Francisco, S., Lopez-Novoa, J.M., Bernabeu, C., & Quintanilla, M. (2010). The role of the TGF-beta coreceptor endoglin in cancer. *ScientificWorldJournal,* **10, 2367-2384.**

Peter, K., Weirich, U., Nordt, T.K., Ruef, J., & Bode, C. (1999). Soluble vascular cell adhesion molecule-1 (VCAM-1) as potential marker of atherosclerosis. *Thromb Haemost,* **82 Suppl 1, 38-43.**

Phipps, W. R. (2001). Polycystic ovary syndrome and ovulation induction. *Obstet Gynecol Clin North Am,* **28(1), 165-182.**

Piccin, A, Murphy, W.G., & Smith, O.P. (2007). Circulating microparticles: pathophysiology and clinical implications. *Blood Rev,* **21(3), 157-171.**

Preston, R.A., Jy, W., Jimenez, J.J., Mauro, L.M., Horstman, L.L., Valle, M., Aime, G., & Ahn, Y.S. (2003). Effects of severe hypertension on endothelial and platelet microparticles. *Hypertension,* **41(2), 211-217.**

Proulle, V., Hugel, B., Guillet, B., Trichet, C., Rafowicz, A., Lambert, T., Freyssinet, J.M., & Dreyfus, M. (2004). Injection of recombinant activated factor VII can induce transient increase in circulating procoagulant microparticles. *Thromb Haemost,* **91(5), 873-878.**

Pyke, K.E., & Tschakovsky, M.E. (2005). The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol,* **568(Pt 2), 357-369.**

Ramirez-Velez, R., Ramirez, J.H., & Aguilar, C. (2010). Effect of prolonged strenuous exercise on endothelial function in non-elite runners. *Rev Bras Cardiol,* **23(3), 157-161.**

Rand, M.L., Wang, H., Bang, K.W., Packham, M.A., & Freedman, J. (2006). Rapid clearance of procoagulant platelet-derived microparticles from the circulation of rabbits. *J Thromb Haemost,* **4:1621–1623.**

Randeva, H.S., Tan, B.K., Weickert, M.O., Lois, K., Nestler, J.E., Sattar, N., & Lehnert, H. (2012). Cardiometabolic aspects of the polycystic ovary syndrome. *Endocr Rev,* **33(5), 812-841.**

Randeva, H.S., Lewandowski, K.C., Drzewoski, J., Brooke-Wavell, K., O'Callaghan, C., Czupryniak, L., Hillhouse, E.W., & Prelevic, G.M. (2002). Exercise decreases plasma total homocysteine in overweight young women with polycystic ovary syndrome. *J Clin Endocrinol Metab,* **87, 4496–4501.**

Rank, A., Nieuwland, R., Crispin, A., Grutzner, S., Iberer, M., Toth, B. & Pihusch, R. (2011). Clearance of platelet microparticles in vivo. *Platelets,* **22(2), 111-116.**

Rehman, J., Mills, P.J., Carter, S.M., Chou, J., Thomas, J., & Maisel, A.S. (1997). Dynamic exercise leads to an increase in circulating ICAM-1: further evidence for adrenergic modulation of cell adhesion. *Brain Behav Immun,* **11(4), 343-351.**

Robert, S., Poncelet, P., Lacroix, R., Arnauld, L., Giraudo, L., Hauchard, A., Sampol, J., & Dignat-George, F. (2009). Standardization of platelet-derived microparticle counting using calibrated beads and a Cytomics FC500 routine flow cytometer: a first step towards multicenter studies? *J Thromb Haemost,* **7, 190–197.**

Roessler, K.K., Birkebaek, C., Ravn, P., Andersen, M.S., & Glintborg, D. (2013). Effects of exercise and group counselling on body composition and VO2max in overweight women with polycystic ovary syndrome. *Acta Obstet Gynecol Scand,* **92, 272-277.**

Roos, M.A., Gennero, L., Denysenko, T., Reguzzi, S., Cavallo, G., Pescarmona, G.P., & Ponzetto, A. (2010). Microparticles in physiological and in pathological conditions. *Cell Biochem Funct,* **28(7), 539-548.**

Ross, M.D. (2011). The influence of an acute bout of resistance exercise on circulating endothelial progenitor cells and endothelial microparticles in trained men. Masters thesis, Waterford institute of technology.

Ross, R. (1999). Atherosclerosis - an inflammatory disease. *N Engl J Med,* **340, 115-126.**

Rotterdam. (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. (2004). *Fertil Steril,* **81(1), 19-25.**

Rozanski, A., Quereshi, E., Bauman, M., Reed, G., Pillar, G., & Diamond, G.A. (2001). Peripheral arterial responses to treadmill exercise among healthy subjects and atherosclerotic patients. *Circulation,* **103, 2084-2089.**

Salomaa, V., & Wu, K.K. (1999). Soluble thrombomodulin as predictor of incident coronary heart disease. *Lancet,* **354(9190), 1646.**

Salomaa, V., Matei, C., Aleksic, N., Sansores-Garcia, L., Folsom, A.R., Juneja, H., Chambless, L.E., & Wu, K.K. (1999). Soluble thrombomodulin as a predictor of incident coronary heart disease and symptomless carotid artery atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) Study: a case cohort study. *Lancet,* **353(9166), 1729-1734.**

Sam, S. (2007). Obesity and polycystic ovary syndrome. *Obes Manag,* **3(2), 69-73.**

Sathyapalan, T., & Atkin, S.L. (2012). Recent advances in cardiovascular aspects of polycystic ovary syndrome. *Eur J Endocrinol,* **166(4), 575-583.**

Shah, M.D., Bergeron, A.L., Dong, J.F., & Lopez, J.A. (2008). Flow cytometric measurement of microparticles: pitfalls and protocol modifications. *Platelets,* **19(5), 365-372.**

Shantsila, E., Kamphuisen, P.W., & Lip, G.Y. (2010). Circulating microparticles in cardiovascular disease: implications for atherogenesis and atherothrombosis. *J Thromb Haemost,* **8(11), 2358-2368.**

Shephard, R.J., Gannon, G., Hay, J.B., & Shek, P.N. (2000). Adhesion molecule expression in acute and chronic exercise. *Crit Rev Immunol*, **20, 245-266.**

Shet, A.S. (2008). Characterizing blood microparticles, **4(4), 769-774.**

Shimokawa, H. (1999). Primary endothelial dysfunction: atherosclerosis. *J Mol Cell Cardiol*, **31, 23-37.**

Siegler, J.C., Midgley, A.W., Polman, R.C., & Lever, R. (2010). Effects of various sodium bicarbonate loading protocols on the time-dependent extracellular buffering profile. *J Strength Cond Res,* **24(9), 2551-2557.**

Siegler, J.C., Keatley, S., Midgley, A.W., Nevill, A.M., & McNaughton, L.R. (2008). Pre-exercise alkalosis and acid-base recovery. *Int J Sports Med,* **29(7), 545-551.**

Silvestro, A., Scopacasa, F., Oliva, G., de Cristofaro, T., Iuliano, L., & Brevetti, G. (2002). Vitamin C prevents endothelial dysfunction induced by acute exercise in patients with intermittent claudication. *Atherosclerosis,* **165(2), 277-283.**

Sjogren, P., Cederholm, T., Heimburger, M., Stenvinkel, P., Vedin, I., Palmblad, J., & Hellenius, M.L. (2010). Simple advice on lifestyle habits and long-term changes in biomarkers of inflammation and vascular adhesion in healthy middle-aged men. *Eur J Clin Nutr*, **64(12), 1450-1456.**

Smalheiser, N.R. (2009). Do neural cells communicate with endothelial cells via secretory exosomes and microvesicles? *Cardiovasc Psychiatry Neurol*, **2009, 383086.**

Smith, M., Hanson, N., Lucas, A. & Devor, S. (2013). Endothelial response of running a marathon: a tale of three runners. *Int J Ex Sci*, **6(13), 236-241.**

Sorensen, M.B., Franks, S., Robertson, C., Pennell, D.J., & Collins, P. (2006). Severe endothelial dysfunction in young women with polycystic ovary syndrome is only partially explained by known cardiovascular risk factors. *Clin Encocrinol (Oxf),* **65(5), 655-659.**

Sorensen, K.E., Celermajer, D.S., Spiegelhalter, D.J., Georgakopoulos, D., Robinson, J.T.O., & Deanfield, J.E. (1995). Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. *Br Heart J,* **74(3), 247-253.**

Sossdorf, M., Otto, G.P., Claus, R.A., Gabriel, H.H., & Losche, W. (2011). Cell-derived microparticles promote coagulation after moderate exercise. *Med Sci Sports Exerc,* **43(7), 1169-1176.**

Sossdorf, M., Otto, G.P., Claus, R.A., Gabriel, H.H. & Losche, W. (2010). Release of pro-coagulant microparticles after moderate endurance exercise. *Platelets*, **21(5), 389-391.**

Sowers, J.R. (1998). Obesity and cardiovascular disease. *Clin Chem*, **44(8), 1821-1825.**

Sprung, V.S., Jones, H., Pugh, C.J.A., Aziz, N.F., Daousi, C., Kemp, G.J., Green, D.J., Cable, N.T., & Cuthbertson, D.J. (2014). Endothelial dysfunction in hyperandrogenic polycystic ovary syndrome is not explained by either obesity or ectopic fat deposition. *Clin Sci*, **126, 67-74.**

Sprung, V.S., Cuthbertson, D.J., Pugh, C.J.A., Aziz, N., Kemp, G.J., Daousi, C., Green, D.J., Cable, N.T., & Jones, H. (2013a). Exercise training in polycystic ovarian syndrome enhances flow-mediated dilation in the absence of changes in fatness. *Med Sci Sports Exerc,* **45(12), 2234-2242.**

Sprung, V.S., Atkinson, G., Cuthbertson, D.J., Pugh, C.J.A., Aziz, N., Green, D.J., Cable, N.T., & Jones, H. (2013b). Endothelial function measured using flow-mediated dilation in polycystic ovary syndrome: a meta-analysis of the observational studies. *Clin Endocrinol*, **78, 438-446.**

Sprung, V.S., Cuthbertson, D.J., Pugh, C.J.A., Daousi, C., Atkinson, G., Aziz, N.E., Kemp, G.J., Green, D.J., Cable, N.T., & Jones, H. (2013c). Nitric oxide-mediated cutaneous microvascular function is impaired in polycystic ovary syndrome but can be improved by exercise training. *J Physiol*, **591, 1475-1487.**

Stachenfeld, N.S., Yeckel, C.W., & Taylor, H.S. (2010). Greater exercise sweating in obese women with polycystic ovary syndrome compared with obese controls. *Med Sci Sports Exerc,* **42(9), 1660-1668.** doi: 10.1249/MSS.0b013e3181d8cf68.

Stein, I.F., & Leventhal, M.L. (1935). Amenorrhea asociated with bilateral polycystic ovaries. *Am J Obstet Gynecol,* **29, 181-191.**

Stener-Victorin, E., Jedel, E., Janson, P.O., & Sverrisdottir, Y.B. (2009). Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. *Am J Physiol Regul Integr Comp Physiol,* **297, R387–R395.**

Stenina, O.I. (2003). Regulation of gene expression in vascular cells by coagulation proteins. *Curr Drug Targets,* **4(2), 143-158.**

Stewart, K.J., Sung, J., Silber, H.A., Fleg, J.L., Kelemen, M.D., Turner, K.L., Bacher, A.C., Dobrosielski, D.A., DeRegis, J.R., Shapiro, E.P., & Ouyang, P. (2004). Exaggerated exercise blood pressure is related to impaired endothelial vasodilator function. *Am J Hypertens,* **17(4), 314-320.**

Stout, M. (2009). Flow-mediated dilatation: a review of techniques and applications. *Echocardiography,* **26(7), 832-841.**

Strukova, S. (2006). Blood coagulation-dependent inflammation. Coagulation-dependent inflammation and inflammation-dependent thrombosis. *Front Biosci,* **11, 59-80.**

Suzuki, K., Totsuka, M., Nakaji, S., Yamada, M., Kudoh, S., Liu, Q., Sugawara, K., Yamaya, K., & Sato, K. (1999). Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. *J Appl Physiol,* **887(4), 1360-1367.**

Swampillai, J., Doshi, S., Fraser, A.G., Goodfellow, J., & Jones, C.Jh. (2005). Review: Clinical assessment of endothelial function — an update. *Br J Diabetes Vasc Dis,* **5(2), 72-76.** doi: 10.1177/14746514050050020401.

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, 1535–1538.**

Tarkun, I., Arslan, B.C., Canturk, Z., Turemen, E., Sahin, T., & Duman, C. (2004). Endothelial dysfunction in young women with polycystic ovary syndrome: relationship with insulin resistance and low-grade chronic inflammation. *J Clin Endocrinol Metab,* **89(11), 5592-5596.**

Teede, H.J., Hutchison, S.K., Zoungas, S. (2007). The management of insulin resistance in polycystic ovary syndrome. *Trends Endocrinol Metab*, **18:273–279.**

Thatte, H.S., Rhee, J.H., Zagarins, S.E., Treanor, P.R., Birjiniuk, V., Crittenden, M.D., & Khuri, S.F. (2004). Acidosis-induced apoptosis in human and porcine heart. *Ann Thorac Surg,* **77(4), 1376-1383.**

Thery, C., Ostrowski, M., & Segura, E. (2009). Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol,* **9(8), 581-593.**

Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. (2002). *Circulation,* **106(25), 3143-3421.**

Thomson, R.L., Brinkworth, G.D., Noakes, M., Clifton, P.M., Norman, R.J., & Buckley, J.D. (2012). The effect of diet and exercise on markers of endothelial function in overweight and obese women with polycystic ovary syndrome. *Hum Reprod,* **27(7), 2169-2176.**

Thomson, R.L., Buckley, J.D., Lim, S.S., Noakes, M., Clifton, P.M., Norman, R.J., & Brinkworth, G.D. (2010). Lifestyle management improves quality of life and depression in overweight and obese women with polycystic ovary syndrome. *Fertil Steril,* **94(5), 1812-1816.** doi: 10.1016/j.fertnstert.2009.11.001.

Thomson, R.L., Buckley, J.D., Noakes, M., Clifton, P.M., Norman, R.J., & Brinkworth, G.D. (2008). The effect of a hypocaloric diet with and without exercise training on body composition, cardiometabolic risk profile, and reproductive function in overweight and obese women with polycystic ovary syndrome. *J Clin Endocrinol Metab,* **93(9), 3373-3380.** doi: 10.1210/jc.2008-0751.

Toth, B., Nikolajek, K., Rank, A., Nieuwland, R., Lohse, P., Pihusch, V., Friese, K., & Thaler, C.J. (2007). Gender-specific and menstrual cycle dependent differences in circulating microparticles. *Platelets,* **18(7), 515-521.**

Tousoulis, D., Davies, G., Stefanadis, C., Toutouzas, P., & Ambrose, J.A. (2003). Inflammatory and thrombotic mechanisms in coronary atherosclerosis. *Heart,* **89(9), 993-997.**

Ungvari, Z., Wolin, M.S., & Csiszar, A. (2006). Mechanosensitive production of reactive oxygen species in endothelial and smooth muscle cells: role in microvascular remodeling? *Antioxid Redox Signal,* **8(7-8), 1121-1129.**

Vallisneri, A. Storia della generazione dell'uomo e dell'animale (1971). Cited in Cooke, I.D., & Lunenfield, B. (1989). *Res Clin Forums,* **11, 109-113.**

van den Oever, I.A., Raterman, H.G., Nurmohamed, M.T., & Simsek, S. (2010). Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. *Mediators Inflamm,* **2010, 792393.**

van lerssel, S.H.,Hoymans, V.Y., Van Craenenbroeck, E.M., Van Tendeloo, V.F., Vrints, C.J., Jorens, P.G., & Conrads, V.M. (2012). Endothelial microparticles (EMP) for the assessment of endothelial function: an in vitro and in vivo study on possible interference of plasma lipids. *PLoS ONE,* **7(2), e31496.**

van lerssel, S.H., Van Craenenbroeck, E.M., Conraads, V.M., Van Tendeloo, V.F., Vrints, C.J., Jorens, P.G., & Hoymans, V.Y. (2010). Flow cytometric detection of endothelial microparticles (EMP): effects of centrifugation and storage alter with the phenotype studied. *Thromb Res,* **125(4), 332-339.**

van Meer, G., Voelker, D.R., & Feigenson, G.W. (2008). Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol,* **9(2), 112-124.**

Verma, S., Buchanan, M.R., & Anderson, T.J. (2003). Endothelial function testing as a biomarker of vascular disese. *Circulation,* **108, 2054-2059.**

Verma, S., & Anderson, T.J. (2002). Fundamentals of endothelial function for the clinical cardiologist. *Circulation,* **105(5), 546-549.**

Vigorito, C., Giallauria, F., Palomba, S., Cascella, T., Manguso, F., Lucci, R., De Lorenzo, A., Tafuri, D., Lombardi, G., Colao, A., & Orio, F. (2007). Beneficial effects of a three-month structured exercise training program on cardiopulmonary functional capacity in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab,* **92(4), 1379-1384.** doi: 10.1210/jc.2006-2794.

Vince, R.V., Chrismas, B., Midgley, A.W., McNaughton, L.R., & Madden, L.A. (2009a). Hypoxia mediated release of endothelial microparticles and increased association of S100A12 with circulating neutrophils. *Oxid Med Cell Longev,* **2(1), 2-6.**

Vince, R.V., McNaughton, L.R., Taylor, L., Midgley, A.W., Laden, G., & Madden, L.A. (2009b). Release of VCAM-1 associated endothelial microparticles following simulated SCUBA dives. *Eur J Appl Physiol,* **105(4), 507-513.** doi: 10.1007/s00421-008-0927-z.

Vita, J.A. (2011). Endothelial function. *Circulation,* **124(25), e906-e912.** doi: 10.1161/circulationaha.111.078824.

Vogel, R.A., Corretti, M.C., & Plotnick, G.D. (2000). A comparison of brachial artery flow-mediated vasodilation using upper and lower arm arterial occlusion in subjects with and without coronary risk factors. *Clin Cardiol,* **23(8), 571-575.**

Wagner, D.D., & Frenette, P.S. (2008). The vessel wall and its interactions. *Blood,* **111, 5271.**

Wahl, P., Zinner, C., Achtzehn, S., Behringer, M., Bloch, W., & Mester, J. (2011). Effects of acid-base balance and high or low intensity exercise on VEGF and bFGF. *Eur J Appl Physiol,* **111(7), 1405-1413.**

Wang, J.M., Su, C., Wang, Y., Huang, Y.J., Yang, Z., Chen, L., Wu, F., Xu, S.Y., & Tao, J. (2009). Elevated circulating endothelial microparticles and brachial-ankle pulse wave velocity in well-controlled hypertensive patients. *J Hum Hypertens,* **23(5), 307-315.**

Widlansky, M.E., Gokce, N., Keaney, J.F., Jr., & Vita, J.A. (2003). The clinical implications of endothelial dysfunction. *J Am Coll Cardiol,* **42(7), 1149-1160.**

Wild, R.A., Carmina, E., Diamanti-Kandarakis, E., Dokras, A., Escobar-Morreale, H.F., Futterweit, W., Lobo, R., Norman, R.J., Talbott, E., & Dumesic, D.A. (2010). Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *J Clin Endocrinol Metab,* **95(5), 2038-2049.**

Wolf, P. (1967). The nature and significance of platelet products in human plasma. *Br J Haematol,* **13(3), 269-288.**

Wolinsky, H. (1980). A proposal linking clearance of circulating lipoproteins to tissuemetabolic activity as a basis for understanding atherogenesis. *Circ Res,* 47, 301.

Wong, S.H., Hamel, L., Chevalier, S., & Philip, A. (2000). Endoglin expression on human microvascular endothelial cells association with betaglycan and formation of higher order complexes with TGF-beta signalling receptors. *Eur J Biochem,* **267(17), 5550-5560.**

Woo, K.S., McCrohon, J.A., Chook, P., Adams, M.R., Robinson, J.T., McCreadie, R.J., Lam, C.W., Feng, J.Z. & Celermajer, D.S. (1997). Chinese adults are less susceptible than whites to age-related endothelial dysfunction. *J Am Coll Cardiol,* **30(1), 113-118.**

Woywodt, A., Bahlmann, F.H., De Groot, K., Haller, H., & Haubitz, M. (2002). Circulating endothelial cells: life, death, detachment and repair of the endothelial cell layer. *Nephrol Dial Transplant,* **17(10), 1728-1730.**

Xu, J., & Zou, M.H. (2009). Molecular insights and therapeutic targets for diabetic endothelial dysfunction. *Circulation,* **120(13), 1266-1286.** doi: 10.1161/circulationaha.108.835223

Yarbrough, B., & Bradham, A. (1998). Heat illness. In Rosen, P., Barkin, R.M., eds. Emergency medicine concepts and clinical practice, 4th ed. St. Louis, Mosby, **986-1000.**

Yildirim, B., Sabir, N., & Kaleli, B. (2003). Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome. *Fertil Steril,* **79(6), 1358-1364.**

Yildiz, B. O., Knochenhauer, E. S., & Azziz, R. (2008). Impact of obesity on the risk for polycystic ovary syndrome. *J Clin Endocrinol Metab,* **93(1), 162-168.**

Yong, P. J., Koh, C. H., & Shim, W. S. (2013). Endothelial microparticles: missing link in endothelial dysfunction? *Eur J Prev Cardiol,* **20(3), 496-512.**

Zwaal, R. F., & Schroit, A. J. (1997). Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood,* **89(4), 1121-1132.**