Women's health in sport: The prevalence and impact of heavy menstrual bleeding and iron deficiency.

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I, Georgie Bruinvels confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Menstruation is the leading cause of iron deficiency anaemia in pre-menopausal women. When combined with regular exercise, iron deficiency (ID) risk in menstruating women is increased. This may be exacerbated in those with heavy menstrual bleeding (HMB), which despite no validated diagnosis, is thought common in the general population but is under-investigated in exercisers. Accordingly, the potential relationship between menstruation, ID and performance remains unknown. The aims of this research were to: a) identify HMB prevalence (utilising a diagnostic series) and association with fatigue and perceived disruption to exercise training/performance in exercising women; b) evaluate the impact and existing diagnosis of ID.

A 'Female Health Questionnaire' was developed to identify HMB amongst other factors in an exercising population (n=789), elite athletes (n=90) and London Marathon runners (n=1073). The relationships between iron status, HMB and fatigue or the perception that the menstrual cycle disrupts exercise training/performance was then investigated in exercising women (n=271). Finally, a clinical trial assessed the impact of intravenous iron repletion (single dose of 20 $mg \cdot kg^{-1}$) on exercise and aerobic capacity, haematological markers, fatigue and mood disturbance in non-elite, iron deficient (serum ferritin $\leq 30 \mu g \cdot L^{-1}$), exercising women (n=32).

HMB was identified to be common (37% elite athletes, 36% marathon runners), and associated with perceived disruption to exercise training/performance and fatigue, but these relationships were independent of iron status. Iron repletion improved exercise and aerobic capacity, but only in those more severely iron deficient (serum ferritin $<15 \mu g \cdot L^{-1}$), with wide individual variation, unrelated to baseline serum ferritin.

In conclusion, HMB is a risk factor for ID, physiological and psychological function decrements in exercising women. Intravenous iron repletion effectively restores iron status and improves functional exercise capacity when true ID exists. Serum ferritin as a biomarker for ID and its associated normative data should be re-evaluated to avoid false positive ID diagnosis.

Impact statement

UCL research has highlighted a lack of awareness and understanding of common issues experienced by exercising women that can affect both their exercise performance and general health. These findings should inform future practice amongst sports physicians and sports scientists, and a forthcoming research focus should be placed on identifying treatment options.

The menstrual cycle has the potential to affect exercise performance. However, the causes for this and optimal treatment options are largely unknown. Prior research assessing menstrual dysfunction in athletes has primarily focussed on amenorrhea and oligomenorrhea. However, here, heavy menstrual bleeding (HMB) was shown to be a prevalent issue in both recreational and elite level athletes, with more than 1 in 3 reporting a history of this condition. Menstruation is the leading cause of iron deficiency in developed countries, likelihood is inevitably exacerbated in those with HMB. HMB was found to have a detrimental effect on physiological and psychological function.

Those who exercise have an increased risk of iron deficiency, and supplementation amongst this populous is common. Accordingly, nearly 80% of elite athletes reported use of iron supplementation. However, many were found to supplement without prior knowledge of iron status. Unnecessary iron supplementation is not beneficial to exercise or aerobic capacity, it can cause several unwanted side effects and is a primary risk factor in those with genetic abnormalities that predispose them to iron overload.

There has been a previous lack of clarity surrounding the diagnosis of iron deficiency, particularly amongst those who exercise. Serum ferritin is the primary marker used for diagnosis, and cut-offs typically range from 12 - 40 $\mu g \cdot L^{-1}$. However, applying the principle that a positive response to iron repletion indicates iron deficiency, the results from a recent clinical trial suggest that existing diagnostic guidelines and markers need to be re-evaluated. Diagnosing iron deficiency using a serum ferritin cut-off of 30 $\mu g \cdot L^{-1}$, an overall improvement in physiological and psychological function was seen when exercising women had their iron stores repleted. However, when broken down into groups based on severity of iron deficiency, as defined using serum ferritin, endurance and aerobic capacity only increased in those with more severe iron deficiency (serum ferritin < 15 $\mu g \cdot L^{-1}$). However, amongst this group there was a range of individual variation in response, not predicted by baseline serum ferritin. This trial firstly highlighted that existing serum ferritin diagnostic cut-offs may be too lenient, and secondly suggests that while serum ferritin can be used to identify those with an increased iron deficiency risk, measurement of other iron

status markers alongside this is necessary to enhance diagnostic ability. For best practice, iron status should be monitored longitudinally, with intervention only when a significant decline is observed.

Sports physicians should assess for HMB on presentation in athletes and need to be aware of the potential impacts it can have. Other markers in addition to serum ferritin should be used for iron deficiency diagnosis, and both athletes and athlete support personnel need to ensure that appropriate advice is sought prior to supplementation.

Table of contents

Acknowledgements **2**

Abstract **3**

Impact statement **4**

List of abbreviations **11**

List of tables **13**

List of figures **16**

List of publications **18**

1 Chapter 1 ..... **21**
1.1 General introduction **21**

2 Review of the literature **26**
2.1 What is iron? **26**
2.1.1 Chemistry of iron 26
2.2 Distribution of iron in the body **27**
2.3 Functions of iron **28**
2.3.1 Iron-containing haem compounds 28
2.3.2 Iron containing haem enzymes 30
2.3.3 Iron containing non-haem enzymes 30
2.3.4 Immune function 30
2.3.5 Other functions 31
2.4 A lack of iron: iron deficiency and iron deficiency anaemia **31**
2.5 Diagnosis of IDA and iron deficiency **32**
2.5.1 Serum ferritin for diagnosis of IDA in regular exercisers 32
2.5.2 Haemoglobin concentration ([Hb]) for diagnosis in those who exercise 33
2.5.3 Other candidate markers for the determination of iron status 34
2.6 Total haemoglobin mass **34**
2.7 Prevalence of IDA, anaemia and iron deficiency **36**
2.8 Types and causes of anaemia **36**
2.8.1 Macrocytic anaemia 37
2.8.2 Microcytic anaemia 37
2.8.3 Normocytic anaemia 38
2.9 Causes of iron deficiency **38**
2.10 Iron bioavailability, types of dietary iron, and essential trace elements **40**
2.10.1 Iron bioavailability and types of dietary iron 40
2.10.2 Dietary factors that can impact iron absorption 41
2.11 Susceptibility to iron deficiency in exercising women **43**
2.11.1 Iron deficiency in the exercising population 43
2.11.2 Iron deficiency specifically in exercising women 46
2.11.3 Iron deficiency in menstruating women 47
2.12 The menstrual cycle in female athletes **50**
2.13 Effect, diagnosis and treatment of iron deficiency **51**
2.13.1 Effects of iron deficiency in animal models 52
2.13.2 Effects of iron deficiency on exercise performance 52
2.13.3 Previous research evaluating the impact of iron therapy in iron deficient, exercising participants 54
2.13.4 Other relevant effects of iron deficiency 62
2.13.5 The impact of iron therapy on fatigue and mood disturbance 62
2.14 Iron deficiency and the female athlete triad **63**
2.15 Management and treatment of iron deficiency **65**
2.15.1 Dietary intervention 65
2.15.2 Oral supplementation 65
2.15.3 Intravenous injections 66

2.15.4 Intramuscular injections 67
2.16 Effects of iron supplementation in iron sufficient athletes 67
2.17 Transport, delivery and storage of iron 67
2.17.1 Iron transport within blood 67
2.17.2 Iron tissue uptake 68
2.17.3 Iron storage 70
2.17.4 Toxicity of iron 70
2.17.5 Release of free iron and haemoglobin into the circulation 71
2.18 Maintenance of iron homeostasis 72
2.18.1 Iron entry into the circulation 72
2.19 Regulation of iron homeostasis 76
2.19.1 Systemic control of iron homeostasis 76
2.19.2 Cellular control of iron homeostasis 79
2.20 Summary 82
2.21 Overall aims and hypotheses of this thesis 83
2.21.1 Thesis aims 83

3 General methodology 84
3.1 Female Health Questionnaire 84
3.1.1 Diagnosis of Heavy Menstrual Bleeding (HMB) 85
3.1.2 Accompanying questions in the Female Health Questionnaire 87
3.1.3 Limitations with the existing 'Female Health Questionnaire'. 89
3.2 University ethics approval process 94
3.3 Female Health Questionnaire v2 95
3.3.1 Limitations with the Female Health Questionnaire v2 96

4 The identified prevalence of heavy menstrual bleeding (HMB) and its association with reported menstrual cycle driven disruption to exercise training/performance in elite and non-elite athletes 100
4.1 Abstract 100
4.2 Introduction 101
4.2.1 Study aims 101
4.2.2 Study hypotheses 101
4.3 Materials and Methods 102
4.3.1 Ethics and participant consent 102
4.3.2 Inclusion criteria 102
4.3.3 Participants 102
4.3.4 Study design 102
4.3.5 Data Analysis 103
4.4 Results 105
4.4.1 Participant characteristics - stage 1 105
4.4.2 Participant characteristics - stage 2 106
4.4.3 Results from stages 1 and 2 106
4.4.4 Elite Athletes 109
4.4.5 Elite vs non- elite athletes 109
4.4.6 Association between HMB, a reported knowledge of a history of anaemia and disruptions to exercise training/performance caused by the menstrual cycle 111
4.5 Discussion 113
4.5.1 Limitations 116
4.6 Conclusions 116
4.7 Future Perspectives 117
4.8 Acknowledgements 117
4.9 Author Contributions 117

5 The association between identified heavy menstrual bleeding and iron status, and the association of these with the perception that the menstrual cycle disrupts exercise training/performance in exercising women. 119
5.1 Abstract 119

5.2 Introduction 120
5.2.1 Study aims 120
5.2.2 Study hypotheses 121
5.3 Materials and methods 121
5.3.1 Ethics and participant consent 121
5.3.2 Inclusion criteria 121
5.3.3 Exclusion criteria 121
5.3.4 Participants 121
5.3.5 Study design 122
5.3.6 Measurements and definitions 124
5.3.7 Data analysis 124
5.4 Results 125
5.4.1 Participant Characteristics 125
5.4.2 Identified prevalence of HMB, iron deficiency and IDA 127
5.4.3 Level of awareness of HMB and iron status 128
5.4.4 Association between identified HMB, iron deficiency and IDA with reported menstrual cycle disruptions to everyday lifestyle/exercise performance 130
5.5 Discussion 134
5.5.1 Limitations 137
5.6 Conclusions 138
5.7 Future perspectives 139
5.8 Acknowledgements 139
5.9 Author Contributions 139

6 The impact of identified heavy menstrual bleeding and iron status on fatigue in exercising and menstruating women 140
6.1 Abstract 140
6.2 Introduction 141
6.2.1 Study aims 142
6.2.2 Study hypotheses 142
6.3 Materials and Methods 142
6.3.1 Ethics and participant consent 142
6.3.2 Inclusion criteria 142
6.3.3 Exclusion criteria 142
6.3.4 Participants 143
6.3.5 Study design 143
6.3.6 Measurements and definitions 144
6.3.7 Data handling and statistical analysis 145
6.4 Results 147
6.4.1 Participant Characteristics 147
6.4.2 Prevalence and impact of identified HMB on IDA, anaemia and iron deficiency 147
6.4.3 Impact of HMB and iron status independently on fatigue 148
6.5 Reliability of the MFI-20 152
6.5.1 Relationship between fatigue and markers of iron status 152
6.5.2 Relationship between identified HMB, anaemia, IDA and fatigue 153
6.5.3 Relationship between HMB and fatigue 155
6.6 Discussion 157
6.6.1 Limitations 159
6.7 Conclusions 160
6.8 Future perspectives 161
6.9 Acknowledgements 161
6.10 Author Contributions 161

7 IRONWOMAN trial: the impact of intravenous iron on exercise and aerobic capacity, fatigue and mood disturbance in iron deficient, non-elite exercising women 162
7.1 Abstract 162

7.2 Introduction 163
7.2.1 Study aims 164
7.2.2 Study hypotheses 164
7.3 Materials and methods 164
7.3.1 Ethics and participant consent 164
7.3.2 Inclusion criteria 164
7.3.3 Exclusion criteria 165
7.3.4 Participants 165
7.3.5 Study design 165
7.3.6 Measurements 166
7.3.7 Measurement of Exercise and aerobic capacity: Exercise test 166
7.3.8 Measurement of Exercise and aerobic capacity: Total haemoglobin mass test 168
7.3.9 Haematology and biochemistry 172
7.3.10 Fatigue 173
7.3.11 Mood disturbance 173
7.3.12 Iron administration 174
7.3.13 Data analysis 174
7.4 Results 176
7.4.1 Participant characteristics 176
7.4.2 Exercise and aerobic capacity 176
7.4.3 Haematology and biochemistry 182
7.4.4 The effect of baseline serum ferritin 182
7.4.5 Relationship between baseline iron status and change in end points. 184
7.4.6 Relationship between $\dot{V}O_{2max}$ and total haemoglobin mass 190
7.4.7 Other markers that are potentially related to change in endpoints. 191
7.4.8 Training and menstrual cycle questionnaire 193
7.4.9 Fatigue 194
7.4.10 Mood disturbance 199
7.5 Discussion 201
7.5.1 Limitations 210
7.6 Conclusions 211
7.7 Future perspectives 212
7.7.1 Future studies 212
7.8 Author Contributions 213

8 General Discussion, overall conclusions and future research 214
8.1 Prevalence and impact of HMB in the exercising population 214
8.2 HMB, iron status and fatigue 217
8.3 Prevalence of iron deficiency 218
8.4 Impact of iron deficiency 218
8.4.1 Effect on exercise and aerobic capacity 218
8.4.2 Effect on fatigue and mood disturbance 219
8.4.3 Diagnosis 219
8.4.4 Mechanism of iron deficiency 220
8.4.5 Awareness and supplementation 221
8.5 Limitations 223
8.6 Future studies and directions 224
8.6.1 Future derivation of a Female Health Questionnaire 224
8.6.2 Other future directions 224
8.7 Conclusions 225
8.8 Key recommendations 227

9 References 229

10 Appendices 262
10.1 Publication: The prevalence and impact of heavy menstrual bleeding among athletes and mass start runners of the 2015 London Marathon. (Letter to the Editor) 262

10.2 Publication: The Prevalence and Impact of Heavy Menstrual Bleeding (Menorrhagia) in Elite and Non-Elite Athletes. (Research Article) 264
10.3 Publication: Sport exercise and the menstrual cycle: where is the research? (Editorial).....273
10.4 Other publications.....274
10.5 Oral presentations 274
10.6 Abstracts and Posters.....275
10.7 Grants.....276
10.8 Selected media exposure.....276
10.9 Other work 279
10.10 IRONWOMAN Trial: Participant Information Sheet 280
10.11 IRONWOMAN Trial: NHS Ethics Approval letter.....284
10.12 IRONWOMAN Trial: St Mary's Ethics Approval.....288
10.13 IRONWOMAN Trial: Informed consent 289
10.14 IRONWOMAN Trial: Female athlete monitoring questionnaire.....290
10.15 Questionnaires 294

List of abbreviations

BMI	Body mass index
BRUMS	Brunel mood scale
CaO_2	Arterial oxygen content
CH	Corpuscular haemoglobin content
CHCM	Corpuscular haemoglobin concentration mean
CI	Confidence interval
CO	Cardiac output
COHb	Carboxyhaemoglobin
%COHb	Percentage carboxyhaemoglobin
CO_2	Carbon dioxide
CvO_2	Venous oxygen content
EPO	Erythropoiesis
GDF-15	Growth differentiation factor - 15
Hb	Haemoglobin
[Hb]	Haemoglobin concentration
Hct	Haematocrit
HDW	Haemoglobin distribution width
HMB	Heavy menstrual bleeding
HR	Heart rate
ID	Iron deficiency
IDA	Iron deficiency anaemia
IDNA	Iron deficiency non-anaemia
IL	Interleukin
IV	Intravenous
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MFI-20	Multidimensional Fatigue Inventory-20
MPC	Mean platelet component
MPM	Mean platelet mass
MPV	Mean platelet volume
NICE	National Institute for Care Excellence
O_2	Oxygen
OR	Odds ratio
PCT	Platelet haematocrit
PDW	Platelet distribution width
PV	Plasma volume

RCV	Red cell volume
RDW	Red cell distribution width
RBC	Red blood cell
SATA	Singapore Anti-Tuberculosis Association
SD	Standard deviation
sFer	Serum ferritin
tHbmass	Total haemoglobin mass
TIBC	Total iron binding capacity
TSAT	Transferrin saturation
TTE	Time to exhaustion
$\dot{V}O_2$	Exertional oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen consumption
$\dot{V}CO_2$	Carbon dioxide output
WBC	White blood cell
WHO	World Health Organisation

List of tables

Table 2.1 A summary of the effects of iron repletion on physical performance in iron deficient but not anaemic exercising participants.

Table 4.1 Participant characteristics, including identified prevalence of heavy menstrual bleeding (HMB).

Table 4.2 The perception that the menstrual cycle disrupts training/performance, seeking of help/advice for heavy periods, knowledge of a history of anaemia and iron supplementation in those who have and have not met the heavy menstrual bleeding HMB criteria, when identified using the outlined criteria.

Table 4.3 A comparison between elite and non-elite characteristics.

Table 4.4 The unadjusted and adjusted results from the logistic regression of the perception that the menstrual cycle disrupts exercise training/performance.

Table 5.1 Clinical characteristics of participants.

Table 5.2 A comparison of characteristics between those with and without heavy menstrual bleeding (HMB), when identified using the outlined criteria.

Table 5.3 Reported historical knowledge of anaemia, iron deficiency and use of supplementation in those with iron deficiency anaemia and severe (serum ferritin < 15 $\mu g \cdot L^{-1}$) and moderate (serum ferritin < 30 $\mu g \cdot L^{-1}$) iron deficiency.

Table 5.4 The likelihood of having IDA, or severe (serum ferritin < 15 $\mu g \cdot L^{-1}$) or moderate (serum ferritin < 30 $\mu g \cdot L^{-1}$) iron deficiency based on whether those identified with HMB have or have not sought advice/help for heavy periods.

Table 5.5 Likelihood of reporting that the menstrual cycle disrupts everday lifestyle/exercise performance in those identified with heavy menstrual bleeding (HMB), iron deficiency anaemia (IDA) and severe and moderate iron deficiency (ID).

Table 5.6 The effect of iron status on the perception of whether the menstrual cycle disrupts everday lifestyle/exercise performance

Table 5.7 The unadjusted and adjusted results from the logistic regression of the perception of disruptions of the menstrual cycle to everday lifestyle/exercise performance .

Table 6.1 The difference between the prevalence of IDA, anaemia and iron deficiency in those who did or did not meet the outlined HMB criteria.

Table 6.2 A comparison in fatigue scores (MFI-20) between those identified with and without heavy menstrual bleeding (HMB).

Table 6.3 A comparison between different fatigue scores (MFI-20) across the total sample between those with and without anaemia, IDA or ID. Anaemia ([Hb] < 12.0 $g \cdot dL^{-1}$), IDA ([Hb] < 12.0 $g \cdot dL^{-1}$ and serum ferritin < 15 $\mu g \cdot L^{-1}$), and iron deficiency (ID; serum ferritin < 15 $\mu g \cdot L^{-1}$).

Table 6.4 A comparison between different fatigue scores (MFI-20) in those identified with HMB (n=112) between those with and without anaemia, IDA or ID. Anaemia ([Hb < 12.0 $g \cdot dL^{-1}$), IDA ([Hb] < 12.0 $g \cdot dL^{-1}$ and serum ferritin < 15 $\mu g \cdot L^{-1}$), and iron deficiency (ID; serum ferritin < 15 $\mu g \cdot L^{-1}$).

Table 6.5 Mean and standard deviations for each aspect of fatigue, alongside inter-item correlations, Cronbach's alpha if the item is deleted, and the corrected item-total correlation.

Table 6.6 The association between haemoglobin concentration ([Hb]) and serum ferritin and the five subscales of the MFI-20 and total fatigue.

Table 6.7 The unadjusted and adjusted results from the logistic regression of anaemia.

Table 6.8 The unadjusted and adjusted results from the logistic regression of IDA.

Table 6.9 The unadjusted and adjusted relationship between identified heavy menstrual bleeding (HMB) and general fatigue.

Table 7.1 Baseline participant characteristics.

Table 7.2 A comparison between exercise and aerobic capacity, and haematology and biochemistry measures at baseline and post intravenous iron injection.

Table 7.3 Changes in endurance and aerobic capacity when comparing those with severe (sFer < 15 $\mu g \cdot L^{-1}$) and moderate (sFer > 15 $\mu g \cdot L^{-1}$ but ≤ 30 $\mu g \cdot L^{-1}$) iron deficiency.

Table 7.4 The relationship between baseline haemoglobin ([Hb]) and serum ferritin (sFer) and the changes in $\dot{V}O_{2max}$ and total haemoglobin mass.

Table 7.5 The unadjusted and adjusted relationship between the combination of haemoglobin ([Hb]) and serum ferritin and change in total haemoglobin mass (g).

Table 7.6 The unadjusted and adjusted relationship between haemoglobin ([Hb]) and serum ferritin and change in total haemoglobin mass ($g \cdot kg^{-1}$).

Table 7.7 The unadjusted and adjusted relationship between haemoglobin ([Hb]) and serum ferritin and change in $\dot{V}O_{2max}$ ($mL \cdot min^{-1}$).

Table 7.8 The unadjusted and adjusted relationship between haemoglobin ([Hb]) and serum ferritin and change in $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$).

Table 7.9 The relationship between both change in haemoglobin ([Hb]) and change in serum ferritin (sFer) and the changes in $\dot{V}O_{2max}$ and total haemoglobin mass.

Table 7.10 The impact of day of the menstrual cycle on the relationship between baseline haemoglobin ([Hb]) and serum ferritin (sFer) and changes in $\dot{V}O_{2max}$ and total haemoglobin mass.

Table 7.11 Mean and standard deviations for each aspect of fatigue, alongside inter-item correlations, Cronbach's $\propto$ if the item is deleted and the corrected item-total correlation for tests at baseline.

Table 7.12 Mean and standard deviations for each aspect of fatigue, alongside inter-item correlations, Cronbach's alpha if the item is deleted and the corrected item-total correlation for the post-injection tests.

Table 7.13 The unadjusted relationship between MFI subscales and baseline haemoglobin concentration and level of serum ferritin.

Table 7.14 Effects of intravenous iron on feelings of health on the day of testing using the EQ-5D-5L questionnaire. The percentage of participants who reported no problem compared to those who reported any problem (from slight to extreme).

List of figures

Figure 1.1 An overview of the hypotheses. i. There is an association between HMB and IDA and IDNA; ii. IDA and IDNA are on the causal pathway between HMB and perceived reported effects of the menstrual cycle on exercise training and performance. iii. The relationship between HMB and fatigue is mediated by IDA and IDNA; and iv. IDNA causes a reduction in aerobic and exercise capacity, while also being associated with increased fatigue.

Figure 2.1 The association between haemoglobin concentration and maximal oxygen uptake ($\dot{V}O_{2max}$). Figure adapted from (1).

Figure 2.2 The association between total haemoglobin mass and maximal oxygen uptake ($\dot{V}O_{2max}$). Figure adapted from (1).

Figure 2.3 Factors that contribute to increasing the susceptibility to iron deficiency in exercising women.

Figure 2.4 Potential relationship between iron deficiency and the female athlete triad in exercising women as outlined in a recent review (2).

Figure 2.5 The mechanism by which iron enters enterocytes, and is then either stored within the cell or is exported from the basolateral membrane into the circulation. The mechanism for haem iron entry into the cell is not yet understood, however it is thought to involve the haem carrier protein 1. Non-haem iron enters the cell via the DMT1 iron transport protein after being reduced by Dcytb. It is then either stored as ferritin or transported through the cell for export via ferroportin. Figure adapted from (3).

Figure 4.1 Reported number of symptoms of in the outlined heavy menstrual bleeding (HMB) diagnostic series across participants when ranked based on 5 km personal best times. Q1 represent those with the fastest times, Q4 the slowest.

Figure 5.1 Derivation of study sample.

Figure 6.1 Derivation of study sample.

Figure 7.1 Study design.

Figure 7.2 $\dot{V}O_{2max}$ test in the St Mary's University performance lab.

Figure 7.3 Haemoglobin mass test equipment - glass spirometer (A), with bag (B) and mouthpiece attached (C), gas syringe (D), oxygen valve (E). Figure adapted from (4).

Figure 7.4 Effect of intravenous iron on primary and secondary endpoints. Individual changes in $\dot{V}O_{2max}$, time to exhaustion (TTE), total haemoglobin mass (tHbmass), haemoglobin concentration ([Hb]), corpuscular haemoglobin content (CH) and ferritin (sFer) from baseline to 2 weeks post intravenous

iron. Data are presented as a percent change from baseline with individual changes shown in grey, and mean change in black.

Figure 7.5 Percentage change in $\dot{V}O_{2max}$ from baseline in response to intravenous iron when participants were grouped by baseline serum ferritin. Severe iron deficiency – sFer < 15 $\mu g \cdot L^{-1}$ and moderate iron deficiency - sFer > 15 $\mu g \cdot L^{-1}$ but ≤ 30 $\mu g \cdot L^{-1}$.

Figure 7.6 The relationship between total haemoglobin mass and $\dot{V}O_{2max}$ at a. baseline and b. post injection.

Figure 7.7 The relationship between change in total haemoglobin mass and change in total $\dot{V}O_{2max}$ in response to the intravenous iron injection.

Figure 7.8 Relationship between change in total haemoglobin mass and a. 5 km personal best time; b. weekly exercise volume; c. baseline $\dot{V}O_{2max}$; d. CHCM, and haematocrit in response to intravenous iron, $p < 0.05$; f. Relationship between change in haemoglobin concentration ([Hb]) and change in $\dot{V}O_{2max}$ in response to intravenous iron.

Figure 7.9 Relationship between change in total haemoglobin mass and baseline platelet biomarkers. a. association between change in haemoglobin mass and baseline mean platelet component; b. association between change in haemoglobin mass and platelet distribution width.

Figure 7.10 Effects of intravenous iron on fatigue (MFI). A comparison between subscale Multidimensional Fatigue Inventory scores (MFI; median ± IQR) at baseline and post-injection.

Figure 7.11 Effects of intravenous iron on fatigue (Piper fatigue score). A comparison between subscale Piper fatigue scores (PFS; median ± IQR) at baseline and post-injection.

Figure 7.12 Effect of intravenous iron on mood disturbance using the BRUMS mood scale. A comparison between mood disturbance at baseline and post-injection.

Figure 8.1 An overview of the conclusions. i. There is an association between HMB and IDA and IDNA; ii. The association between HMB and perceived reported effects of the menstrual cycle on exercise training and performance is independent of IDA and IDNA; iii. The relationship between HMB and fatigue is not mediated by IDA and IDNA; and iv. There was an overall increase in exercise and aerobic capacity in response to intravenous iron in iron deficient (serum ferritin < 15), non-elite exercising women, however this response was not universal and independent of serum ferritin. However, a reduction in fatigue was observed in all. There is potential for IDA and possibly IDNA to actually be responsible for HMB.

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1 Chapter 1

1.1 General introduction

In the words of Nelson Mandela “Education is the most powerful weapon which you can use to change the world..”

Athletes are always striving for the best. Pushing their bodies to their physiological limit. But while the positive health effects of regular exercise are clearly established (5), it is also important to ‘first do no harm’ (6). Research shows that athletes respond differently to the general population (7). Sports medicine and sports science researchers are constantly trying to address how we can help athletes to perform better; run faster, recover better, reduce risk of injury and illness. However, in the past, the vast majority of research has been conducted in male athletes (8).

Historical context

Historically, medical trials were solely conducted in men. This gender bias in research dates back to before World War II where women were labelled as ‘protected subjects’ due to fear that any clinical testing could potentially cause harm to unborn foetuses (9). The fluctuations in hormones through the menstrual cycle also caused concern, increasing the complexity of studies resulting in a need for greater sample sizes, adding to the expense. Despite observations that women and men are physiologically different, men were deemed to be adequate proxies for women (10,11).

In 1990, the National Institutes of Health, a part of the United States health agency, formed the Office of Research on Women’s Health in an attempt to overcome this research gender gap and to ensure adequate research into women’s health was conducted (10). Despite clear advances in research through the publication of resources (12), development of centres of excellence and the formation of multiple independent bodies (10), in 2010 a report by the Institute of Medicine concluded that research findings were not being effectively translated into general practice (13). A recent review looking at female representation in 1,382 sport and exercise research studies involving over six million participants between 2011 - 2013 found the representation of women to be only 39% (8). Further, within studies, where both genders were included in research, the relative representation of females across three key sports science journals was found to be 33 - 37%. There were even fewer female only studies (14). Evidently, this is a significant cause for concern, and must be addressed.

Research has increasingly attempted to assess how athletic performance may be affected by the changes in levels of ovarian hormones (15,16). For example, Sung *et al*. (2014) found resistance training in the follicular phase to be more effective in the development of muscular strength when compared to training in the luteal phase, suggesting that for maximal benefit strength training could be based on time in the menstrual cycle (17). There is a need to investigate this further in the future as it could have significant implications for optimal performance.

Female physiology and the menstrual cycle

The ovarian hormones fluctuate in a cyclical pattern over an average of 28-days (15). As previously highlighted, this hormonal cycle can be a confounding factor to research in women, therefore where studies are conducted in women, the impact of the menstrual cycle is often ignored altogether, alternatively, women are only tested in the follicular phase, where hormone levels are at their lowest, or only women using a contraceptive device to stabilise hormone levels are included (16). Evidently, this has resulted in distinct knowledge gaps.

A number of elite athletes have recently attributed sub-optimal sports performance to their menstrual cycle (18-20), but scientific knowledge surrounding female physiology is yet to elucidate conclusive reasons for this (15). Dependent on sport type, training volume and performance level, 6 - 79% of exercising women experience reproductive system irregularities (21). The principal menstrual cycle dysfunctions studied in exercising women are primary and secondary amenorrhea, anovulation, luteal phase deficiency and oligomenorrhea (22-24). Accordingly, pre-pubertal participation in intensive exercise is associated with a three-fold increase in susceptibility to amenorrhea (25). Yet, other menstrual cycle dysfunctions are seldom researched (24).

Heavy menstrual bleeding

Heavy menstrual bleeding (HMB) is defined by the National Institute for Care Excellence (NICE) in the United Kingdom, as “excessive menstrual blood loss which interferes with a woman’s physical, social, emotional and/or material quality of life” (26), while historically defined as 80 mL blood loss or more (27). However, as will be explained further in this thesis, neither of these are universally used or recognised, and are clearly difficult to evaluate. Therefore, other surrogates for diagnosis have been applied. But there is yet to be a recognised and validated means for HMB diagnosis. This is likely in part due to a lack of clarity regarding the desired outcome or impact of HMB, be it psychological or physiological impact. Is it the sheer psychological impact of perceived large amounts of blood loss or is it the repercussions or the associated factors that occur from excessive

blood loss, such as iron deficiency. Where there is an increase in menstrual blood loss, risk of iron deficiency is clearly enhanced, which can ultimately progress to iron deficiency anaemia (IDA). Despite being very common, IDA is slow to manifest and often goes unnoticed. It can significantly impact both quality of life and exercise performance. Identification of HMB could both highlight IDA risk and also enable earlier detection of a potentially debilitating issue (28,29).

Despite this, there is a lack of research evaluating whether excessive blood loss is indeed an issue amongst those who exercise. Consequently, the potential for HMB to affect exercise performance is unknown, in addition to the reasons for this. This will be addressed in Chapter 4, where, using a combination of a number of existing definitions, the prevalence of HMB in both marathon runners and elite athletes will be evaluated (15).

Historically, the primary treatment option for menstrual cycle dysfunctions has been through hormonal intervention, for example use of the oral contraceptive pill (OCP) or intrauterine devices (15,30,31). The use of hormonal intervention will provide a more stable hormonal milieu and is often used as a treatment option for HMB (15,31). However, recent research has identified negative outcomes of the use of hormonal contraceptives, such as the OCP, for example, OCP use has been associated with increased inflammation, decreased aerobic ability and a reduction in maximal power, while significantly, also potentially masking an underlying energy deficiency (31-34). The effects on performance of other possible forms of hormonal contraception such as intrauterine systems, the depot injection, the vaginal ring and the hormonal implant need further research.

In summary, there is an evident need to assess prevalence of other menstrual cycle dysfunctions that could be an issue in athletes to try and elucidate reasons for reported effects on performance. Identification of HMB could highlight potential iron deficiency risk. Given the highlighted repercussions associated with use of hormonal contraception methods, alternative treatment options are also necessary.

Menstruation and iron status

Iron is an essential element, required by all cells, and vital for physiological function and physical performance (35,36). As a result of menstrual blood loss, women are particularly susceptible to iron deficiency and IDA (37). Due to the increased menstrual blood loss associated with HMB, it is recognised as an important risk factor for iron deficiency (28,29,38). However, as a result of the highlighted lack of research surrounding HMB in exercising women, it is unknown how this may affect performance and whether iron is causative. This will be addressed in chapter 5, where the mediating effect of iron status in

the association between identified HMB, and a reported disruption of the menstrual cycle to exercise training/performance will be evaluated. HMB and iron deficiency are both associated with increased fatigue (28,38), however to my knowledge, the role of iron in the causal pathway between HMB and fatigue has not been assessed. Excess fatigue will clearly impact exercise performance, and needs consideration if indeed HMB is found to be prevalent amongst those who exercise, therefore this will be addressed in chapter 6.

Iron status and exercise capacity

The impacts of IDA on exercise performance are well established (36), however, the consequences of iron deficiency without anaemia are less clear (39). The World Health Organisation (WHO) define IDA in non-pregnant woman as a haemoglobin concentration ([Hb]) < 12.0 $g \cdot dL^{-1}$ and a serum ferritin < 15 $\mu g \cdot L^{-1}$ (40). But, there is much ambiguity surrounding the diagnosis of iron deficiency without anaemia, particularly amongst the exercising population. The WHO indicate that iron depletion occurs in both men and women when serum ferritin < 15 $\mu g \cdot L^{-1}$ (41), however a lack of iron in bone marrow has been observed when serum ferritin is 30 $\mu g \cdot L^{-1}$ (42). As a result of the haemoconcentration that can ensue post exercise and its acute phase reactant property, serum ferritin is subject to transient increases post exercise, potentially masking an underlying deficiency (43,44). Accordingly, it is estimated that day-to-day variation in serum ferritin in endurance athletes is 13 - 75% (45). Therefore, especially in elite athletes, cut-off values for serum ferritin used by sports medicine doctors to indicate iron deficiency in women, and therefore the point at which to intervene with either oral or intravenous iron supplementation are typically high, and may be as high as 40 $\mu g \cdot L^{-1}$ (46).

Applying the principal that that a positive response to iron therapy indicates iron deficiency (47), studies evaluating the impact of iron repletion in iron deficient but not anaemic athletes when using a an inclusion criteria for serum ferritin ranging from 12 - 40 $\mu g \cdot L^{-1}$ have produced conflicting results (48-54). This is likely due to inconsistencies in study protocols, the varying serum ferritin cut-off values, small sample sizes, and differing administration routes (48-54). Further, previous studies, using intravenous iron repletion have only been conducted in elite and well-trained populations (51,52), who, as previously highlighted, may respond differently to non-elite athletes (7). Since intravenous injections bypass gut absorption (55), they offer a much faster and more efficient way for repleting iron stores and assessing the effect. This will be addressed in chapter 7, where the impact of iron repletion will be evaluated in non-elite exercising women, with a serum ferritin ≤ 30 $\mu g \cdot L^{-1}$.

It has been suggested that iron repletion may afford some other non-haematological effects in those who are iron deficient. In a non-athletic population, reductions in fatigue and improved cognition and quality of life have been observed in response to iron repletion (56,57). In fact, a recent study hypothesises that cognitive function is impaired prior to physical function when iron stores are sub-optimal (58). However, previous studies in iron deficient, exercising women have only evaluated the effect of iron repletion on physiological and haematological outcomes in isolation, subjective markers of fatigue and mood have not previously been considered in conjunction. Therefore, chapter 7 will also incorporate measures of fatigue and mood disturbance alongside markers of aerobic and exercise capacity.

There are numerous side effects to oral supplementation including gastrointestinal distress and nausea (59), while supplementation when iron levels are sufficient is not associated with an improved performance (60). In fact, excessive supplementation can increase oxidative stress and inflammation (61). Further, in light of the transient increases in hepcidin, the peptide hormone responsible for control of iron absorption (62), that occur in response to iron, unnecessary supplementation has the potential to worsen iron status, due to windows of limited potential for absorption (51). Therefore, there is an evident need for better clarification about when intervention is necessary.

This thesis will first assess whether HMB, when identified using a diagnostic series, is indeed a problem in those who exercise. It will then identify the prevalence and establish whether it is associated with reported disruption to exercise training/performance caused by the menstrual cycle. This will then be considered in relation to iron status. Finally, when using serum ferritin for diagnosis, the impact of iron deficiency on aerobic and exercise capacity in conjunction with fatigue and mood disturbance will be evaluated. Existing criteria and markers used for iron deficiency diagnosis will be questioned, alongside highlighting the impact that a deficiency has. This will also enable the assessment of whether iron deficiency, possibly caused by HMB, could be a cause for some of the reported detrimental effects on performance attributed to the menstrual cycle in women.

2 Review of the literature

Exercising women are prone to a number of health issues, some caused or exacerbated by their participation in sport (7). These have the potential to impact on their exercise performance and general state of health and well-being. Results from a number of different studies have found risk of iron deficiency to be increased in those who exercise, with evidence suggesting 24 – 47% of exercising women to be iron deficient (59). In fact, the IOC recommends regular screening for iron deficiency (6). This section firstly explains what iron is and its role in the body. The diagnosis, causes of, and susceptibility to iron deficiency will then be discussed in addition to the possible effects it may have, particularly in those who exercise. Treatment options and optimal dietary sources will then be highlighted. The second half of this review will provide an overview of cellular and systemic iron homeostasis, describing the impact of exercise on iron metabolism.

2.1 What is iron?

2.1.1 Chemistry of iron

Iron the most abundant element in the earth's core, and the fourth most abundant in the earth's crust (63). It has an atomic number of 26 and is a d-block element in the periodic table with an electron configuration of $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6 4s^2$. As a transition metal, it can exist in many oxidation states from -2 to +6, however it is most commonly found in either its ferrous (Fe(II); Fe^{2+}) or ferric (Fe(III); Fe^{3+}) ionic form. Since the electrons in the 3s and 4d outer subshells are close in energy, iron is very reactive, being able to change oxidation states and exchange electrons readily, therefore having good redox potential.

The physical state of iron is dependent on the surrounding environment (64). It reacts readily with oxygen, and is influenced by the temperature and pH of its surroundings (64). The reactive nature of iron ions, with unoccupied d orbitals, means that it does not tend to exist alone in the environment, coordinating reversibly with various organic and inorganic ligands alongside a concomitant transfer of electrons (35). The primary ligands to which iron binds are oxygen, sulphur and nitrogen, for example, it is commonly found bound to oxygen as haematite (Fe_2O_3) or magnetite (Fe_3O_4) (35). It's ability to adjust redox potential and electronic spin state (1000mV to -550mV) means that it is highly useful in human physiology and has evolved to become an invaluable element for biochemical reactions (35,65). It is therefore evident that a lack of iron is likely to have a significant impact on physiological function.

Despite iron being an essential micronutrient for all living organisms (66,67), excess free iron within tissues under aerobic conditions can be very harmful. With very good redox potential, iron is highly toxic, readily reacting with oxygen, to produce reactive oxygen species (ROS) based on Fenton and Harber-Weiss reactions (68). Therefore, regulatory protection mechanisms have evolved, as will be discussed further in this thesis, significantly, in part as a means for protection against the harmful effects of free iron, in aerobic conditions, and at a neutral pH, iron is most commonly found in its ferric form (69). The insoluble nature of ferric (Fe^{3+}) ions reduces iron availability in this environment. This means that despite the physical abundance of iron, acquisition at a neutral pH, in aerobic conditions, is problematic (69).

2.2 Distribution of iron in the body

Typically, the human body contains 3 - 4 g of iron (70). Very specific transport processes and mechanisms have evolved to enable the controlled uptake of the insoluble ferric iron into the bloodstream (69). The majority of body iron is intracellular, and approximately two thirds (~2 – 3 g) is incorporated into haemoglobin within erythrocytes, and around a tenth is stored as myoglobin within muscle (71). The rest is located to macrophages (in the liver, spleen and bone marrow), iron containing proteins and enzymes (such as transferrin), with any excess iron being stored as either ferritin or haemosiderin (typically 0 - 1g) (71). Ferritin and haemosiderin are iron storage proteins and are primarily found in the liver and spleen, but can also be found in the duodenum, bone marrow, skeletal muscle and other anatomical areas (72). Since iron containing proteins are essential for ATP production, iron is present in all cells to some extent. Despite the toxic nature of iron, there is no specific iron excretion mechanism, therefore regulation of iron metabolism, transport and storage occurs in a tightly controlled process, and control of total body iron is maintained through the regulation of iron absorption which will be discussed in the second part of this section (73).

On a daily basis, very little iron is lost, amounting to approximately 1 - 2 mg each day, and this is primarily from epithelial shedding, particularly from skin and the gastrointestinal and genitourinary tracts, in addition to sweat (74,75). Menstruating women inevitably experience increased blood loss, and, on average this amounts to another 1 – 2 mg each day when bleeding (74).

2.3 Functions of iron

Iron is required for many biological functions, including; oxygen transport and storage, cellular and mitochondrial respiration, electron transfer reactions, cell growth and differentiation, while also vital for immune function (35). The majority of iron exists in complexes bound to proteins, either coordinated to amino acid side chains or forming part of a prosthetic group (73). Being necessary for their synthesis and function, iron-containing proteins can either be haem compounds, such as haemoglobin and myoglobin, haem enzymes, such as haem oxidase, or non-haem compounds, such as metalloflavoproteins (67). A summary of the different types of iron-containing proteins and examples of their key roles of iron are described below:

2.3.1 Iron-containing haem compounds

2.3.1.1 Oxygen transport in blood

Oxygen is transported in the blood in two forms, approximately 98% is reversibly bound to haemoglobin in erythrocytes, and the remaining 2% is dissolved in the plasma. Within each erythrocyte there are approximately 270 million haemoglobin molecules. On a daily basis, erythropoiesis typically requires 20 - 30 mg of iron as more than two million erythrocytes are produced and cleared each second (76). Therefore, the majority of plasma iron is directed to the bone marrow for this process (76).

Haemoglobin is a tetrameric protein made up of two pairs of interconnecting polypeptide globulin chains. One pair of chains is alpha-like and the other is beta-like. Each chain has a haem prosthetic group comprised of a protoporphyrin IX tetrapyrrole ring and a central iron atom, which is typically in its ferrous (Fe^{2+}) form (35). Dioxygen can reversibly bind to the haem prosthetic groups, facilitating oxygen transport in the circulation (35,77). Since each haemoglobin molecule has four haem groups, there are four oxygen binding sites, and therefore it can bind to and transport four oxygen molecules. Haem synthesis is a complex process involving eight different reactions and enzymes. Half of the reactions take place in the cytoplasm and half in the mitochondrion. Iron is essential for functioning of the enzyme 5-aminolevulinic acid synthase, which controls the first step in haem synthesis, in the mitochondrion (78). Therefore, iron availability in part dictates the initial rate-limiting step in haem synthesis (35). The following four steps in the haem synthesis pathway occur in the cytoplasm. Then the final three reactions take place in the mitochondrion, with the process ultimately involving the insertion of ferrous iron into the protoporphyrin ring (78). This final step is catalysed by ferrochelatase, an enzyme that also contains iron as part of an iron-sulphur cluster (79,80). Iron regulatory proteins and iron regulatory elements are involved in iron sensing to ensure enough iron is present to enable synthesis of erythroid

haem. Therefore, iron is both a vital part of haem and the erythropoiesis process (81). Iron is essential for the process by which oxygen is transported from the lungs to tissues for oxidative phosphorylation.

Maximal oxygen uptake, which can also be termed $\dot{V}O_{2max}$ is determined by both the amount of oxygen in the blood and the oxygen consumption of skeletal muscle (1). As the transport of oxygen in blood is dependent on haemoglobin, this is clearly dependent on sufficient iron availability. The unloading of oxygen at target tissues from haemoglobin is dictated by the surrounding environment. The affinity of haemoglobin for oxygen varies dependent on pH, pCO2, the presence of phosphates and the temperature. Changes in these conditions alter the haemoglobin-iron interaction, which in turns reduces or increases oxygen affinity (35).

2.3.1.2 Oxygen transport in muscle

Iron is also a component of myoglobin, a protein that functions to transport oxygen from erythrocytes to muscle myocytes. Myoglobin consists of a single polypeptide chain, containing eight alpha helices (82). Similarly to haemoglobin, it has a porphyrin ring containing a central ferrous (Fe^{2+}) ion. The ferrous ion interacts with six ligands in a myoglobin molecule, one of which is the binding site for oxygen, to which it can bind reversibly (82). Myoglobin will reduce in states of iron deficiency, decreasing oxygen availability for myocyte function (83).

2.3.1.3 Electron transfer reactions and iron containing enzymes

There are a number of iron-containing proteins that are involved in oxidative phosphorylation in the inner mitochondrial membrane, where a series of redox reactions occur as proteins donate and accept electrons in the electron transfer respiratory chain (84). This action creates a pH electrochemical gradient across the membrane, which is then used by protons as they enter the mitochondrion through ATP synthase, driving ATP synthesis from ADP. A total of 40 different proteins are involved in the respiratory chain including both haem-containing proteins and non-haem iron-sulphur proteins (35).

ATP functions to store and transport chemical energy within cells for metabolism. ATP is required by all cells, and when the body is placed under increased physiological strain, for example, during exercise, demand for ATP is increased. Hence, there are large numbers of mitochondria in muscle tissue to meet the increased ATP requirements that typically occur in this tissue. Energy is released from ATP as it is broken down into ADP.

2.3.2 Iron containing haem enzymes

Iron-containing haem enzymes are a group of enzymes which have a haem prosthetic group typically complexed to another enzyme (84). Cytochoromes are a type of haem-containing enzymes, and are involved in oxidative phosphorylation. Haem is located at the active site, and electrons are simultaneously accepted or donated by the iron ion, as it changes oxidation state, transferring between its reduced ferrous (Fe^{2+}) state and its ferric (Fe^{3+}) oxidised state (35). Specific examples include cytochromes b, bc1 and c, cytochrome c oxidase and succinate dehydrogenase (85). Cytochrome bc1 also has an iron-sulphur protein with a 2Fe-2S centre, which is called a Rieske Centre (86).

2.3.3 Iron containing non-haem enzymes

Iron-sulphur proteins form a group of non-haem enzymes, where iron is bound to either two or four sulphur atoms (86). Iron-sulphur cluster biogenesis takes place in mitochondria (84). The iron-sulphur groups are typically ligated to cysteine residues on proteins (86). Ferredoxins (e.g. NADH dehydrogenase) are an example of iron-sulphur proteins which are involved in electron transfer reactions in mitochondria. Again, here an iron ion simultaneously loses or gains an electron transferring between its reduced ferrous (Fe^{2+}) state and its ferric (Fe^{3+}) oxidised state, without a significant change in structure. Unlike cytochromes, iron-sulphur proteins are non-haem, and they specifically accept or donate a single electron. They are also involved in regulatory iron sensing and the ligation of certain substrate ligands (87). Many cytosolic and mitochondrial types of iron-sulphur proteins have been identified and more are likely to be discovered as technology advances (88).

2.3.4 Immune function

Iron is required for the immune response, however invading pathogens also require iron, so internal iron sequestering is necessary to prevent pathogenic availability and therefore survival (35,89). Inevitably, pathogens have evolved, finding ways to leach iron for survival. However, protection mechanisms are in place to reduce iron availability; firstly, the majority of iron is intracellular, located to haemoglobin and myoglobin reducing availability; and secondly any free iron in the circulation is primarily sequestered by high affinity proteins, namely haptoglobin and haemopexin (35,89).

The iron-regulatory peptide hormone hepcidin is also involved when an infection is present, in a process that will be described further subsequently, its expression is upregulated, increasing release from the liver, and limiting iron release into the circulation, promoting cellular storage (90), and potentially preventing uptake into enterocytes through control of

divalent metal transporter 1 expression (91). As part of the immune response, extra hepcidin is also released from some immune cells, including macrophages and neutrophils (92). There are a number of further mechanisms which restrict iron availability, limiting intracellular iron availability, sequestering iron (89).

2.3.5 Other functions

In addition to the above, iron is necessary for many other physiological processes, for example iron-sulphur clusters as cofactors for many processes (93). Indeed, it is thought that more necessary functions of iron will be discovered in time (93). DNA synthesis for example is iron dependent, with iron being an essential cofactor for the production of the rate limiting enzyme, ribonucleotide reductase (71). It is also required for DNA replication, steroid synthesis, gene regulation and cell proliferation and differentiation (71).

2.4 A lack of iron: iron deficiency and iron deficiency anaemia

Iron deficiency can be defined as a reduction in total body iron, typically occurring when iron losses exceed iron absorption over extended periods of time. Iron deficiency is the most common nutritional deficiency globally (94). Given the many essential roles of iron, a lack of iron has the potential to fundamentally impact upon physical, physiological and psychological function. Accordingly, there is much scope for impairments to exercise capacity and functional performance as will be discussed in more detail subsequently.

Iron deficiency can occur in isolation or in conjunction with anaemia. Anaemia can be defined as a reduction in erythrocytes or a reduction in haemoglobin (95). Accordingly, iron deficiency anaemia (IDA) occurs when the cause for the reduction in erythrocytes and haemoglobin is a lack of iron. Where iron deficiency occurs in isolation, and therefore body iron stores are either reduced or depleted, typically defined by a decrease in serum ferritin, but haemoglobin ([Hb]) is still within the clinical reference range used for the diagnosis of IDA ([Hb] 12.0 – 16.0 $g \cdot dL^{-1}$ in women), iron deficiency non-anaemia (IDNA) is diagnosed. In IDNA it is assumed that iron supply for erythropoiesis is suffice, however if left untreated, IDNA can progress to IDA.

Both iron deficiency and IDA can manifest in many ways including fatigue, muscle weakness, dizziness, pallor, often with non-specific signs and symptoms that are slow to develop so can often go unnoticed with the establishment of a new sense of ‘normal’. As a result, the impacts are often not appreciated until iron levels are repleted (96). There are also some neurological sequelae that can occur as a result of IDA including pica (a compulsive need for non-food materials including ice, paper and dirt), restless leg

syndrome, reduced neurotransmitter synthesis, hypomyelination and neurocognitive effects (97).

2.5 Diagnosis of IDA and iron deficiency

The World Health Organisation (WHO) define anaemia as a [Hb] < 13.0 $g \cdot dL^{-1}$ in men, [Hb] < 12.0 $g \cdot dL^{-1}$ in non-pregnant women, and [Hb] < 11.0 $g \cdot dL^{-1}$ in pregnant women (95). The most accurate means for diagnosing iron deficiency is through bone marrow aspiration, however due to the invasive nature of this technique, it is seldom used (98). Since a good correlation has been found between serum ferritin and total body iron stores under steady state conditions, this is typically used as the primary marker of iron status, and therefore for the diagnosis of iron deficiency (99). The WHO define iron depletion as serum ferritin < 15 $\mu g \cdot L^{-1}$ (41). Iron deficiency anaemia (IDA) is therefore defined as [Hb] < 13.0 $g \cdot dL^{-1}$ alongside a serum ferritin < 15 $\mu g \cdot L^{-1}$ in men, and as [Hb] < 12.0 $g \cdot dL^{-1}$ alongside a serum ferritin < 15 $\mu g \cdot L^{-1}$ in non-pregnant women (100).

However, the efficacy and reliability of both [Hb] and serum ferritin have been questioned, particularly in certain populations, including in those who exercise (45). Accordingly, the clinical ranges may be of limited significance in this group.

2.5.1 Serum ferritin for diagnosis of IDA in regular exercisers

It is widely acknowledged that serum ferritin is the primary means for assessing iron stores in healthy individuals (101). However, the suitability and reliability of serum ferritin as a means for indicating iron levels in those who exercise has been questioned. In endurance athletes specifically it is estimated that day-to-day variation is 13 - 75% (45). Levels have been shown to increase directly after exercise (102). One study found a 22% increase in serum ferritin in response to a maximal rowing ergometer test (102). The cause is not fully understood, but several factors have been suggested including: a.) a haemoconcentration, as a result of exercise-induced plasma volume shifts; b.) the effect of an inflammatory response, where since ferritin is an acute phase protein, dependent on intensity, exercise may trigger a transient increase in inflammatory cytokines, acutely increasing levels of serum ferritin; and c.) an exercise-induced red blood cell lysis (43,44). Accordingly, there is evident potential for underlying iron deficiency to be masked, and for there to be a disconnect between serum ferritin levels and iron stores in bone marrow. It is therefore important to appreciate that serum ferritin only reflects body iron stores in the absence of inflammation.

The specific serum ferritin cut-off for iron deficiency diagnosis is also unclear. When compared to a bone marrow aspiration, a diagnosis of iron deficiency using serum ferritin < 16 $\mu g \cdot L^{-1}$ has a specificity of 98% and a sensitivity of 75% (98). Further, the absence of bone marrow iron stores have been demonstrated when ferritin ≤ 12 $\mu g \cdot L^{-1}$ (103). However, there has been much debate over the point at which low serum ferritin levels may have a detrimental impact on physiological function, and therefore athletic performance. The typical reference range in women is extremely broad, from 10 – 200 $\mu g \cdot L^{-1}$ (104,105). Yet, in 1993, Hallberg *et al.* suggested a lack of iron stores and signs of iron deficient erythropoiesis when serum ferritin was 25 - 40 $\mu g \cdot L^{-1}$ (98). Others also question the lower end of the reference range, feeling that despite being the point at which the WHO specify iron depletion, an iron deficient state will have already been reached by this point, likely affecting physiological function (98,106). Another study showed that when using a serum ferritin cut-off of 30 $\mu g \cdot L^{-1}$, the sensitivity and specificity for iron deficiency diagnosis improved to 92% and 96% (42). While Heinrich *et al.* (1970) identified that a cut-off of 30 $\mu g \cdot L^{-1}$, through the assessment of the absorption rate of ^{59}Fe, could conclusively be used to identify iron deficiency, while the possibility of pre-latent iron deficiency needs to be considered in those with a serum ferritin in the range of 30 – 99 $\mu g \cdot L^{-1}$ (107). As a result, there is a lack of consensus over the required serum ferritin level to prevent a negative impact on performance in sports medicine, where the pursuit for optimal performance is paramount (46). As will be discussed further in Section 2.13, diagnostic criteria used in studies assessing the impact of iron deficiency have used a range of cut-offs for iron deficiency diagnosis, and this will be addressed further in this thesis.

2.5.2 Haemoglobin concentration ([Hb]) for diagnosis in those who exercise

[Hb] is dependent on the total circulating mass of haemoglobin and plasma volume. Therefore, [Hb] is susceptible to distinct variation through intravascular fluid shifts, so exercise, posture and hydration status can all impact on the result, potentially causing an inaccurate representation (108,109). For example, an acute haemoconcentration can occur post exercise, while a haemodilution, a pseudoanaemia, can also result in response to an expansion in plasma volume in response to chronic training (44,110). In a measurement which will be discussed subsequently, the use of total haemoglobin mass provides a more reliable alternative (Section 2.5.3). Since the total amount of oxygen transported in plasma is minimal, this provides a much more accurate means for assessing oxygen carrying capacity (1).

The typical female [Hb] reference range is broad, (12.0 - 16.0 $g \cdot dL^{-1}$), therefore there is potential for significant decreases in [Hb] to occur before IDA is diagnosed, effectively creating a relative state of IDA. This was again highlighted as an issue by Hallberg *et al.*

in 1993, he proposed that the relative decrease in [Hb] from the individual's habitual optimal value should be interpreted as opposed to the individual value itself (98). This suggests that [Hb] should be longitudinally monitored, however determination of a baseline 'normal' value may be problematic.

Clinically, both [Hb] and serum ferritin measurements are required for the diagnosis of IDA. Transferrin saturation and serum iron may also be considered. However, despite the evident questions of the suitability of serum ferritin and [Hb], and ambiguity over cut-off values for diagnosis, the use of serum ferritin and [Hb] are currently acknowledged to be the most efficient and cost effective means for diagnosing IDA and iron deficiency, so these are frequently measured in isolation (111,112).

2.5.3 Other candidate markers for the determination of iron status

In addition to transferrin saturation and serum iron, other biomarkers that may be more effective for analysis of iron status and warrant future investigation include transferrin and soluble transferrin receptor. Soluble transferrin receptor is less biologically variable than serum ferritin, and is not an acute phase reactant (113). It is also a marker of increased erythropoiesis (114). Interestingly, the serum transferrin receptor/log ferritin index has also shown significant potential for the indication of IDA and iron deficiency, with indication improving when combined with the individual readings of serum ferritin and serum transferrin receptor (115). However, particularly in a sports medicine context, serum ferritin is currently the primary measure used (111,112).

Red cell indices including mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) can also be used for the diagnosis of pre-latent anaemia and anaemia. These are often measured as part of the full blood count alongside [Hb], and can particularly be used to aid diagnosis of the specific type of anaemia as will be explained further in Section 2.7.

2.6 Total haemoglobin mass

Total haemoglobin mass offers a different means for indirectly assessing iron status. Total haemoglobin mass represents the total circulating mass of haemoglobin in the body. By definition, anaemia indicates a deficiency in [Hb], which will cause a reduction in maximal oxygen uptake (1). Maximal oxygen uptake, also termed $\dot{V}O_{2max}$, is determined by both the amount of oxygen in the blood and the oxygen consumption of skeletal muscle, with iron being essential for both. Clinically, [Hb] is typically used to determine the amount of oxygen in that can be carried in the blood, hence this is used for IDA diagnosis. Therefore, in states

of IDA, $\dot{V}O_{2max}$ will be compromised. However as demonstrated in Figure 2.1, the correlation between $\dot{V}O_{2max}$ and [Hb] is small, reflecting the confounding influence of blood volume on [Hb], again questioning the suitability of [Hb] for IDA diagnosis (1). There is a much stronger relationship between total haemoglobin mass and $\dot{V}O_{2max}$ (Figure 2.2), suggesting this to be a more reliable means for indication of a reduction in maximal oxygen transport uptake than [Hb], and for the indication of when IDA is present (1).

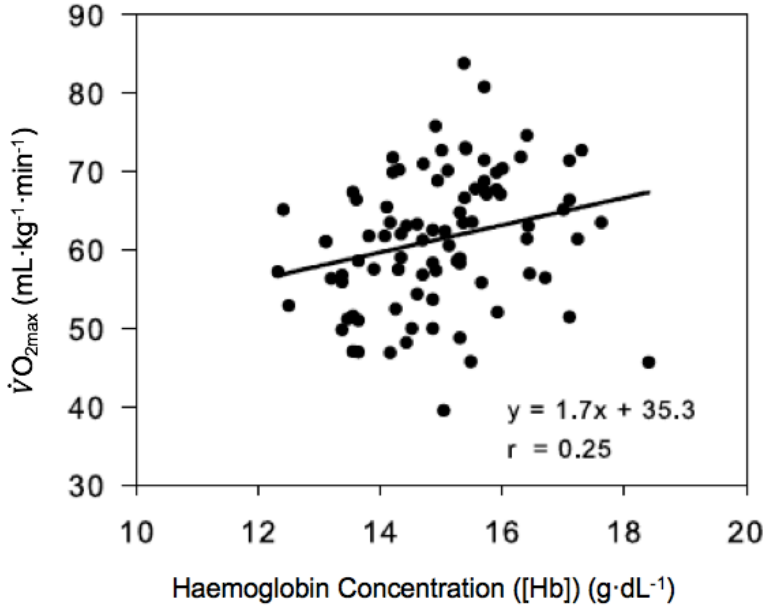

Figure 2.1 – The association between haemoglobin concentration and maximal oxygen uptake ($\dot{V}O_{2max}$). Figure adapted from (1).

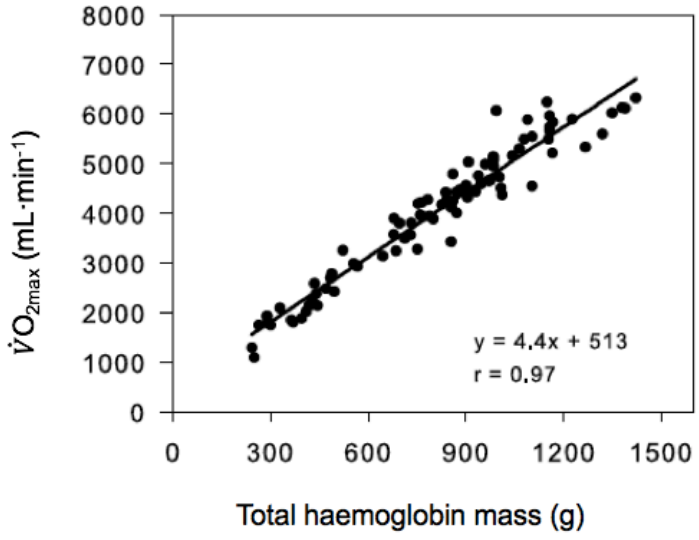

Figure 2.2 – The association between total haemoglobin mass and maximal oxygen uptake ($\dot{V}O_{2max}$). Figure adapted from (1).

2.7 Prevalence of IDA, anaemia and iron deficiency

In 2011 the World Health Organisation (WHO) estimated that globally, anaemia is present in 2 billion individuals, equating to 29.0% of non-pregnant women of a reproductive age (aged 15 - 49), increasing to 38.0% of pregnant women (aged 15 - 49), with the greatest prevalence being in infants aged 5 - 59 months, where 42.6% were found to be anaemic (273.2 million) (116). This research did not include men or the elderly, however earlier research conducted from 1993 - 2005 found prevalence in men to be 12.7%, equating to 260 million, while prevalence in the elderly was 23.9% (164 million) (37). Therefore, pregnant women and young infants are at the greatest risk of anaemia, while risk is lowest in adult males. The increased prevalence in the elderly is likely due to a greater likelihood of inflammatory conditions or illnesses which compromise iron absorption.

The most common cause of anaemia across the world is iron deficiency, being the cause for approximately half of all cases of anaemia (117). While more common in less developed countries, IDA, anaemia and iron deficiency are prevalent conditions across the total population (116).

Despite relatively cheap and effective treatment options, iron deficiency is the most common nutritional deficiency globally, thought to affect approximately 50% of the total population (94,111). According to the National Diet and Nutrition Survey in the general population in the United Kingdom, the prevalence of iron deficiency (serum ferritin < 15 $\mu g \cdot L^{-1}$) in adult men aged 19 - 49 is 0 - 2.5 %, whereas in non-pregnant women this is ranges from 8.2 – 16.0 % (118). As will be described subsequently, there are certain groups with an increased susceptibility to iron deficiency, and this thesis will specifically focus on those who exercise (59). This risk is clearly greater in menstruating women, and exacerbated further as volume of menstrual blood loss increases. There is however significant variation in statistics defining the prevalence of both iron deficiency alone and IDA in exercising women in the literature. In exercising women specifically, prevalence of IDNA varies from 24 – 47% (59), while IDA prevalence in exercising women varies from 8 – 14% (119-121). Part of this ambiguity is likely to be caused by the effects of different sport, intensity and volume of training in addition to other factors which will be explained further in this thesis.

2.8 Types and causes of anaemia

There are different types of anaemia, with a variety of different associated aetiologies and characteristics. These are identified by assessing the characteristics of red blood cells, including their size and [Hb]. Historically, this classification system, as outline outlined by Maxwell Wintrobe in the 1930s, used MCV and MCHC for anaemia-type identification

(122,123). More recently red cell distribution width (RDW) was added to improve diagnostic ability (124), however due to variation in the technology used for assessment and therefore reference intervals there is a need for a variety of algorithms and calculation to aid diagnosis (125,126). With the advance of technology, new techniques and tools are emerging designed to increase the reliability and accuracy of the diagnosis of anaemia, producing specific percentages of cells that are hypochromic, macrocytic and microcytic (126). The different types of anaemia and possible aetiology and characteristics associated with them are explained below:

2.8.1 Macrocytic anaemia

In macrocytic anaemia, erythrocytes are larger than normal, this is typically diagnosed by an increased mean corpuscular volume (MCV) or a greater percentage of macrocytic cells (126). There are two types of macrocytic anaemia; megaloblastic or non-megaloblastic, which are caused by a number of different factors including; a nutritional deficiency (primarily vitamin B12 or folate), drug use, the presence of a chronic illness (e.g. hypothyroidism, reticulocytosis) or a disordered bone marrow (127,128). These will either result in a defect in DNA synthesis, or a defect to the erythrocyte membrane (127,128). Typically, where there is a DNA defect, erythrocytes are oval in shape, whereas where the membrane is defected, cells are round (125,128). The most likely cause of megaloblastic macrocytic anaemia in exercising women is a vitamin B12 or folate deficiency, and a lack of either of these nutrients causes a defect in DNA synthesis, resulting in oval shaped erythrocytes (127,128). Under these circumstances, RNA synthesis continues regardless, and dyspoietic cells are produced which are larger than normal in size, with a bigger nucleus (127). The most common cause of non-megaloblastic macrocytic anaemia is excessive alcohol intake (129).

2.8.2 Microcytic anaemia

In microcytic anaemia, haemoglobin synthesis is affected, and as a result erythrocytes are smaller with a lower haemoglobin content than normal and therefore mean corpuscular volume (MCV) is low and there are an increased number of hypochromic cells (126). There are four different causes of microcytic anaemia including thalassaemia, iron deficiency, anaemia of inflammation and sideroblastic anaemia (130).

Thalassaemia

This is a hereditary condition where there is a mutation to either the $\propto$- or β- globin gene, resulting in either a reduction in or a lack of synthesis of the respective haemoglobin chain (130). As a result, a reticulocytosis occurs.

Iron deficiency anaemia

This is the most common type of anaemia and occurs because there is insufficient iron for the erythropoietic process, causing cells to be microcytic and hypochromic. Senescent erythrocytes will therefore have less iron impacting upon the essential functions for which iron is required. IDA can be multifactorial and the causes will be explained in further detail in Section 2.9.

Anaemia of inflammation

An increased production of inflammatory cytokines causes renal erythropoietin release to be supressed, while also resulting in increased hepcidin release, reducing iron uptake and entry into the circulation (130,131). As a result, erythropoiesis is reduced.

Sideroblastic anaemia

This can be either acquired or hereditary. Iron accumulates in the mitochondria of atypical developing erythroblasts. Iron is not available for haem synthesis, resulting in the development of ringed sideroblasts as opposed to erythrocytes (130). One relatively rare cause of sideroblastic anaemia is a vitamin B6 deficiency (132). Pyridoxal phosphate, which is a catabolically active form of vitamin B6, acts as a cofactor for 5–aminolevulinate synthase, which catalyses the initial step in haem synthesis, where coenzyme A, 5-aminolevulinic acid and carbon dioxide and produced from the reaction between succinyl-CoA and glycine (133). Therefore, a lack of vitamin B6 will inevitably affect haem synthesis.

There are a number of techniques used for identification of the aetiology of microcytic anaemia, including determining the proportion of hypochromic cells. In states of IDA, more red blood cells are hypochromic than microcytic when compared to those with a β-thalassaemia trait (134).

2.8.3 Normocytic anaemia

In some cases during early state anaemia, after acute blood loss or if there are multiple underlying anaemia causes present, countering one another (e.g. iron and vitamin B12 deficiencies), values for MCV can be within the normal range (128). This is a common form of anaemia, highlighting the need for the newer means for diagnosis (126).

2.9 Causes of iron deficiency

Iron deficiency is frequently multifactorial, and there are four primary causes including:

1. Increased loss
2. Decreased intake
3. Increased demand
4. Decreased absorption

1. Increased loss

This is most commonly caused by a large amount of blood loss. There are several ways that this can occur, examples include: a haemorrhage, major surgery, illness, childbirth, significant menstrual blood loss, blood donation, excess use of non-steroidal anti-inflammatories, bleeding defects, and athletic-related losses (135). Particularly in less developed countries, malaria is a major cause of increased blood loss through intravascular haemolysis, with a resulting haematuria. The immune response to malaria also supresses erythropoietin release.

2. Decreased intake

Women typically eat less iron rich food than men, and unsurprisingly risk of a lack of intake is increased in vegetarians and vegans (36). However, poverty and malnutrition resulting in a lack of dietary iron intake are the most common causes of iron deficiency particularly in less developed countries (135).

3. Increased demand

Infants and adolescents have an increased requirement for iron as they grow, and pregnant women, particularly in their second and third trimesters also have an increased need as foetal and maternal erythroid mass increases. Demand is also increased post-partum; however, this is likely offset by the absence of menstruation (136). Increased erythropoietic stimuli will also require increased iron, this could be as a result of a hypoxic stimulus, increased exercise training, or administration of erythropoiesis stimulating agents (135).

4. Decreased absorption

Conditions resulting in inflammation, increasing hepcidin production will reduce iron absorption, such as irritable bowel disease and chronic heart failure. A reduction in iron absorption can also occur as a result of various types of infection which may trigger an immune response such as malaria, or other conditions specifically inhibiting absorption such as Helicobacter pylori infections, coeliac disease and bariatric surgery (135-138). Alternatively, the consumption of certain foods (as will be discussed further below) can also have an impact.

Notably, there are genetic conditions that can result in loss of function or mutations to genes involved in iron metabolism which can increase susceptibility to iron deficiency.

2.10 Iron bioavailability, types of dietary iron, and essential trace elements

2.10.1 Iron bioavailability and types of dietary iron

The typical western non-vegetarian diet contains approximately 7 mg per 1000 kCal of iron, however of this, it is estimated that only 5 – 35 % is absorbed (67). On a daily basis, this equates to in the region of 1 - 2 mg. Recommended daily intake for non-pregnant premenopausal women is 18 mg, which is significantly more than the 8 mg advised for men (139). However, absorption of iron is dependent on a number of factors including the type of iron (i.e. haem or non-haem) (140). Haem iron primarily comes from the haemoglobin found in animal sources; meat and fish. Whereas non-haem iron typically comes from plant-based sources, including vegetables, fruits, legumes, fortified cereals, nuts and pulses.

The bioavailability of haem iron is superior to that of non-haem, therefore absorption of haem iron is better. Approximately 15 – 35 % of haem iron is absorbed, compared to 2 – 20 % of non-haem iron. However, typically, intake of non-haem iron is much greater than haem iron, so its relative contribution to total iron is often larger (140,141).

As will be explained further, despite being easier, research is inconclusive as to the mechanism by which haem iron is transported into enterocytes. The haem iron transporter - HCP1 has been suggested, but clarity as to the specific process is lacking (142). As will be described in further detail below, absorption of non-haem iron is more complex, hence explaining the difference in bioavailability. There is also potential for various other dietary elements to affect absorption of iron, non-haem iron in particular (140,143).

2.10.1.1 Essential trace elements for iron absorption

Alongside ensuring iron intake and uptake is suffice, it is also important to appreciate the influence of other elements which are also necessary for iron acquisition and metabolism. One such example is copper. This is an essential trace element for iron absorption, and a deficiency can also be a cause for anaemia with or without iron deficiency (144). Despite definitive research in animal models, the exact mechanism by which this manifests in humans is not conclusive (144).There are a number of copper-dependent proteins that are involved in iron uptake and utilisation, and a lack of copper has been found to result in a drop in [Hb] (145,146). It has previously been suggested that the mechanism involves a reduction in copper-dependent ferroxidases, in a process that will be described further subsequently, the copper-dependent ferroxidases ceruloplasmin (in hepatocytes and macrophages) and hephaestin (in enterocytes), are necessary for the oxidation of ferrous

iron to ferric iron for transferrin binding and transport in the circulation, in addition to preventing harmful ferrous iron from entering the circulation (144,145). However, increasing evidence suggests that this may not be the primary mechanism by which a copper deficiency impacts, and that there are other copper-dependent processes which are also involved in iron uptake and utilisation that cause erythropoiesis to be restricted when there is a lack of copper (144). Evidently, while copper uptake needs to be suffice for the maintenance of optimal iron status, further research is required to this avail.

2.10.2 Dietary factors that can impact iron absorption

There are several dietary elements that are thought to play a role in the cellular uptake of dietary iron. As previously explained, with no iron excretion mechanism, there is a tightly regulated uptake process. This is primarily controlled by an internal feedback mechanism involving iron sensing and hepcidin, however dietary factors can also have a significant influence.

Research demonstrating the inhibiting and enhancing effects of dietary elements is often inconclusive, primarily because the effects are problematic to measure. The effect of an element that enhances dietary iron uptake may be offset by an inhibitor, for example eating a kale and broccoli salad with wholegrain bread. Kale and broccoli are good sources of ascorbic acid, aiding iron uptake, while the wholegrain bread is rich in fibre and phytates, potentially countering this (147).

While there is a known association between iron absorption and iron stores (ferritin) (98), there is a dearth of conclusive research assessing the combined effect of dietary iron intake and menstruation on risk of iron deficiency. Very few have found a correlation between total dietary iron intake and iron status (148-151). Specific haem intake however has been found to correlate more effectively by most (147,149,152,153). Accordingly, some have found vegetarianism to be associated with an increased risk of low serum ferritin (154,155), including a recent meta-analysis (156). Yet interestingly, in 2005, a study was conducted in the United Kingdom comparing iron status in those following a lacto-ovo-vegetarian diet (no meat or fish for at least a year), a poultry/fish diet and a red meat diet (157). Serum ferritin in the poultry/fish diet was found to be significantly greater than the other two groups, which were very comparable (157). However, this study also established that menstrual blood loss was a much more significant predictor of serum ferritin levels than iron intake (157). With blood loss being inversely proportional to serum ferritin levels (157). In fact, dietary iron intake was not associated with serum ferritin, despite the superiority of the poultry/fish diet. While iron status in those following a lacto-oco-vegetarian diet did not appear to differ to those following a red meat diet, total iron intake was actually greater in

those in the lacto-ovo-vegetarian group, potentially explaining this finding (157). Evidently further research is required to specifically evaluate the impact of diet on iron status, including assessing the effect of other nutritional elements that could alter absorption.

2.10.2.1 Enhanced iron uptake

Ascorbic and citric acid

Ascorbic and citric acids found in foods such as fruits, vegetables and juices are the only factors that can enhance iron uptake in a vegetarian diet (158). Ascorbic acid can act in two ways to optimise iron uptake. The primary mechanism is through its ability to reduce ferric iron to ferrous iron prior to entry into enterocyte, facilitating iron absorption (159). It is also thought to be able to chelate iron, further enhancing iron uptake (159). However, the positive impact of ascorbic and citric acids on iron uptake can be reduced by cooking and processing, therefore careful handling should be applied (160). Studies assessing the effect of ascorbic acid on iron status have been equivocal, with some concluding no impact, however there are a number of possible confounding factors that should be considered, including the absence of a control group, other potentially confounding deficiencies, insufficient time for an effect to manifest, and small sample sizes (147). Further, the criteria used for iron deficiency diagnosis varied and not all participants were deficient. Conversely, a randomised controlled trial where 122 iron deficient women ([Hb] ≥ 11.0g·L^{-1} and serum ferritin ≤ 40μ·L^{-1}) were given fruit juice daily, some fortified with iron and some not, found that iron status only improved in those consuming the fortified juice (161). This suggests that iron intake may need to be increased alongside ascorbic acid.

Animal and fish products

Intake of meat, poultry and fish has also been shown to enhance non-haem iron uptake. Yet, despite the efficacy being well evidenced, the specific mechanism by which animal tissue may aid iron uptake is unsure. It has been suggested that the peptides within the animal tissue act in a similar way to ascorbic acid, reducing and chelating iron (162,163). Others have suggested a specific involvement of glycosaminoglycan's and L-a-glycerophosphocholine (164,165). Evidently further research is warranted as these factors could have a significant impact on iron status.

2.10.2.2 Inhibition of iron uptake

Factors that can specifically inhibit iron absorption include phytates (phytic acid), calcium, tannates, phosphates, carbonates, oxalates, polyphenols and peptides from partially digested proteins (140).

Phytates and polyphenols

Phytates from foods such as grains (e.g. oats and bran), vegetables, seeds, nuts and fruits, are thought to be the primary and most potent inhibitor, and phytate intake should be considered alongside meals when determining iron absorption (140,143). Phytic acid chelates iron, resulting in the formation of an insoluble complex which cannot be digested or absorbed due to a lack of phytase enzymes in the intestine (166-168). Intake of ascorbic acid can however counteract these effects (169). The processing and cooking of phytates can also reduce their inhibitory effects (143). Polyphenols, typically occurring in vegetables, fruits, cereals, tea, coffee, legumes and wine also have an inhibitory effect (170,171).

Dairy products and calcium

Calcium has been found to have a negative impact on both haem and non-haem iron absorption (172,173). This inhibition is suggested to occur at the point of initial uptake into the enterocyte (174). The extent to which calcium can have an impact, particularly when eaten in conjunction with other foods, is unsure, and again further research is required to decipher this. Inhibitory effects of other animal proteins such as egg, milk and albumin in addition to soy protein have also been demonstrated (175,176).

Future research should address the potential for iron fortified products and further evaluate the interrelation of and effect of consumption of dietary elements on the absorption of iron.

2.11 Susceptibility to iron deficiency in exercising women

There are a range of populations with an increased susceptibility to iron deficiency; for the purposes of this review, two groups will be focused on:

- Exercising population
- Menstruating women

2.11.1 Iron deficiency in the exercising population

Those who exercise have been found to have an increased susceptibility to iron deficiency, likely experiencing increased losses and a reduction in absorption as explained further below. As a result, demand is increased, and dietary intake or absorption rate is often not sufficient to meet increased requirements.

2.11.1.1 Increased loss

There are four mechanisms by which increased iron losses occur through exercise including sweating, gastrointestinal bleeding, haematuria (presence of blood in urine), and exercise-induced haemolysis.

Sweating

Iron is a physiological constituent of sweat, and it is therefore inevitable that exercise is associated with increased iron losses through increased sweat (177). Exercising in the heat, for longer durations and at a higher intensity can therefore potentially increase iron loss. Interestingly, iron loss through sweat during exercise appears to be greater in the first hour than in the second (178), so training more than once a day may cause greater iron loss than completing one longer session.

Gastrointestinal bleeding

Gastrointestinal complaints are common amongst those who exercise, with 30 – 70% reporting this (179). Iron losses can also occur as a result of gastrointestinal bleeding (180). Due to the exercise-induced vascular shunts where blood flow to the skin and muscles is increased, blood flow to the gastrointestinal tract will decrease (181,182). The reduction in blood flow can cause the cells of the gastrointestinal tract to become starved of oxygen and other essential substrates, resulting in necrosis and mucosal bleeding, and therefore iron loss (182). Gastrointestinal damage, resulting in blood loss can also be caused by use of non-steroidal anti-inflammatories and other painkillers, use of which is thought widespread by athletes (182).

Haematuria

Likely to be dependent on intensity, exercise can cause haematuria (blood in urine). The effects of the mechanical trauma that can ensue with exercise can cause microscopic legions in the glomerulus and bladder resulting in blood loss (183,184). Furthermore, the repetitive impact from exercising can also cause haemolysis in the glomerulus, further increasing blood loss (184,185).

Exercise induced haemolysis

Exercise-induced red blood cell lysis is also postulated to result in increased iron losses (186). The exact mechanism for this is not yet fully understood (187,188). But the extent to which this occurs is likely to be dependent on the type of exercise, increasing in high impact sports, however haemolysis has been reported in non-impact sports such as swimming where high intensity muscle contractions may compress blood vessels, resulting in red cell lysis (180,189). The extent of haemolysis in weight baring sports, for example running, or sports with a large amount of contact such as rugby is likely to be significantly greater, while also thought dependent on exercise intensity, biomechanics and the ground surface (180,186). Interestingly, research suggests running speed and exercise intensity are more of a determinant of haemolysis rate than ground surface (188). As red blood cells

are destroyed, haptoglobin will sequester the haemoglobin, in a process that will be described in the second part of this chapter. The magnitude of haemolysis can be identified by post-exercise free plasma haptoglobin measurements (180,190). Haptoglobin binds to the haemoglobin that is released when red cell lysis occurs, sequestering the iron (191,192). In response to a significant amount of haemolysis, haptoglobin levels will decrease (193).

2.11.1.2 Decreased intake

Typically consuming less dietary iron, women have been found to be at an increased risk of insufficient intake than male counterparts (194-196). With risk also inevitably exacerbated in vegetarians and vegans (36).

2.11.1.3 Increased demand

Exercise creates an increased erythropoietic stimulus and causes various iron-dependent pathways to be upregulated to meet increased physiological demand. Accordingly, required daily intake is suggested to be elevated by 30-70% in those who exercise, since iron is essential for erythropoiesis (194,195,197). A practice often used by elite athletes, training at altitude also accelerates erythropoiesis, further increasing demand for iron (198).

2.11.1.4 Reduced absorption

The exercising population are also at increased risk of iron deficiency through reduced absorption. As will be explained further in the second part of this review, hepcidin is the key regulator of systemic iron metabolism, and as an acute phase protein, it is also upregulated in response to particular pro-inflammatory cytokines (199). Depending on the intensity, exercise can cause an inflammatory response. For example, research has shown increases in interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor (TNF) in response to exercise (200,201). These cytokines have been shown to increase in individuals both immediately and 2 hours after a marathon (202). As a result, transient hepcidin increases can occur post exercise, which may impact upon iron absorption into the gut and subsequently, the bloodstream. Research suggests that hepcidin levels typically peak 3 – 6 hours after the exercise-induced peak in the pro-inflammatory cytokines (203). In light of this, dietary iron consumption or oral iron administration during this time is likely to be ineffective (204). Therefore athletes who are in hard training have an increased susceptibility to iron deficiency, and this is exacerbated by those who train more than once a day, reducing the window of possibility for iron absorption (204). A recent study found that even after 12 hours of rest, when comparing athletes and non-athletes, hepcidin levels were significantly greater in athletes, highlighting the significant potential for reduced absorption (205). Recent research has however found that iron status

supersedes inflammation in control of hepcidin regulation therefore in states of iron deficiency the magnitude of the hepcidin response may be attenuated (51,203). Regardless, this effect has the potential to worsen iron status, and previous studies have found both iron deficiency and IDA to be more common in exercising women when compared to sedentary controls (120,121,206). Significantly, it is well-known that athletes frequently supplement with iron, with some supplementing without prior knowledge of iron status (39). Supplementation can incur unwanted side effects, increase oxidative stress and inflammation (61), while also causing transient increases in hepcidin, reducing iron absorption and potentially worsening iron status (51). The use of non-steroidal anti-inflammatory drugs has also been shown to reduce absorption.

2.11.2 Iron deficiency specifically in exercising women

Most research suggests that the prevalence of IDA and iron deficiency is higher in athletes than in non-athletes, with risk specifically increasing in exercising and menstruating women. There are a multitude of factors that increase susceptibility, which are shown in Figure 2.1. Significantly, menstrual blood loss has been estimated to equate to iron loss of 1 – 2 mg per day during menstruation (74), and when compared to men, women typically consume less iron (194-196). Numerous studies have attempted to assess the prevalence of iron deficiency in exercising women, however, given that this is likely confounded by different serum ferritin cut-off values to indicate iron deficiency (ranging from 12 - 40 $\mu g \cdot L^{-1}$), specific prevalence has not been defined, with results suggesting that 18 – 57% of exercising women are iron deficient (106). The ambiguity may also be in part due to the level of the athlete, the type of sport they compete in, the training volume, training surface, diet, and intensity, in addition to volume of menstrual blood loss and dietary iron intake amongst other possible factors. It is also important to consider that particularly in the case of elite athletes, some sports and/or federations may undertake regular screening for the assessment of iron status, which could confound prevalence data. The prevalence of IDA in a group of elite level soccer players was found to be 29% (207). While in a mixed group of athletes from a range of different sports, both those primarily aerobic-based and anaerobic-based, iron deficiency was found in 47.4% (208). Another study in elite basketball players found IDA in 14% of female athletes, with 35% being iron deficient (119). While prevalence of IDA in a group of US recreational athletes was found to be 9.7%, and iron deficiency 39% (120). Evidently, there is significant variation in reported IDA and iron deficiency prevalence. A significant confounder to the prevalence data is the ambiguity surrounding the definition of these conditions when using [Hb] and serum ferritin as diagnostic tools.

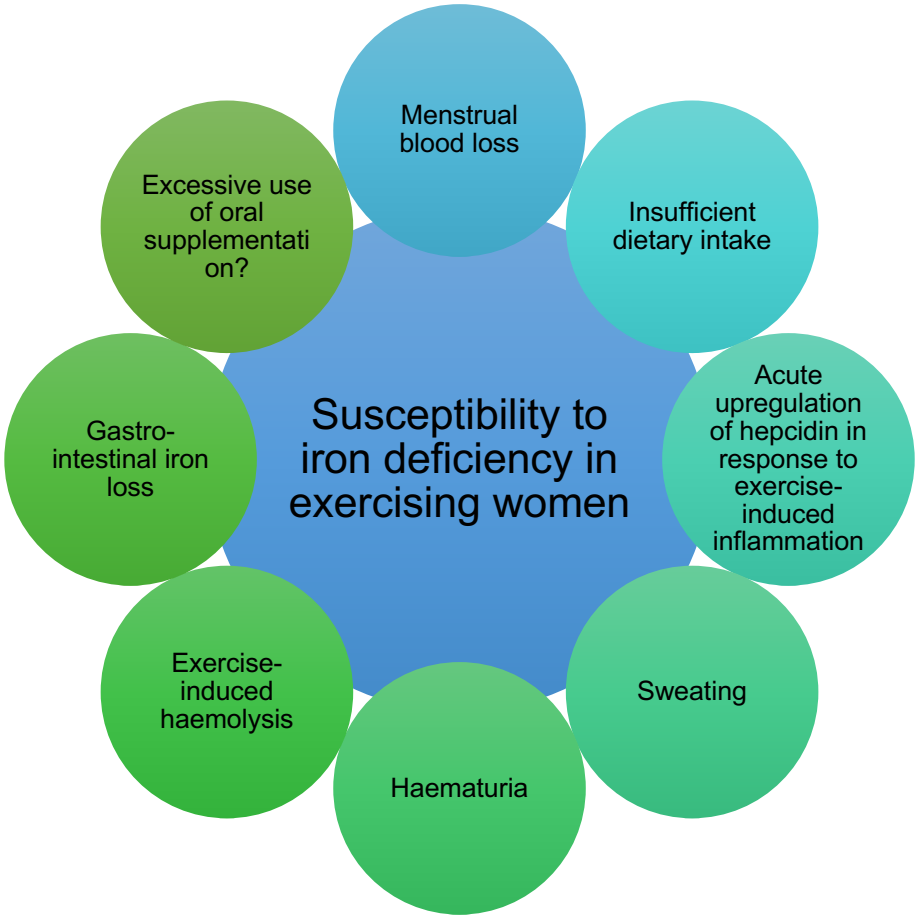

Figure 2.3 – Factors that contribute to increasing the susceptibility to iron deficiency in exercising women.

2.11.3 Iron deficiency in menstruating women

As previously highlighted, prevalence of iron deficiency in women of a reproductive age is greater than male counterparts and post-menopausal women. The primary cause of both iron deficiency and IDA in high-income countries is menstrual blood loss (74,209). It is inevitable that those with increased menstrual blood loss have a greater likelihood of being iron deficient or having IDA. Average volume of blood loss each menstrual period is estimated to be 30 - 50 mL, but this can vary significantly, but is difficult to measure (210). In 1964, Hallberg and Nilsson used an alkaline haematin method to quantify menstrual blood loss (27). In this process, haem is extracted from collected sanitary products using 5% sodium hydroxide (27). However, this process is expensive, labour intensive and deemed unhygienic by some, and so is infrequently used. It has however been refined (211), but the introduction of 'ultra-slim' sanitary products interferes with haem absorption, affecting reliability, and increasing potential for [Hb] to be underestimated (212).

Once menstrual blood loss and [Hb] are obtained, using the following formula, menstrual iron loss can be evaluated (157):

$$Menstrual\ iron\ loss = \frac{Menstrual\ blood\ loss\ \times\ [Hb] \times\ 0.00334}{Cycle\ length}$$

While found to substantially vary between individuals (213), the volume of blood loss by an individual does not tend to vary much between cycles (214). Research evaluating determinants in control of blood loss assessed non-identical and identical twins, concluding a significant genetic component, with identical twins showing much less variation in blood loss (215). Suggesting that volume of blood loss is primarily genetically controlled. It is thought that for every 1 mg of iron lost, there is a concomitant decrease of 6.9 $\mu g \cdot L^{-1}$ in serum ferritin (157). Therefore the enhanced risk of iron deficiency in this group is evident (74,157).

2.11.3.1 Heavy menstrual bleeding (HMB)

Iron losses in those with heavy menstrual bleeding (HMB) are on average 5 - 6 times greater than those without each cycle (216). The National Institute for Care Excellence (NICE) of the United Kingdom define HMB as “excessive menstrual blood loss which interferes with a woman’s physical, social, emotional and/or material quality of life” (26). Earlier research applied a more quantitative approach for the definition, classifying HMB to be present when there is at least 80 mL of blood loss each cycle (217). However, the subjectivity of the NICE definition is hard to evaluate, there are no means to quantify what equates to “interferences” with the different aspects of quality of life. While there are numerous issues associated with measuring total volume of blood loss, including those associated with hygiene, in addition to the potential for an appreciable amount of blood to be lost extraneously and therefore not collected by sanitary products (212). Also, given that total blood volume can significantly vary from person to person, a loss of 80 mL blood will be far more significant in some individuals than others. The existing means for diagnosis as specified by the NICE is through the opinion of the woman herself (26), which is inevitably problematic particularly given that this is a personal topic which is not commonly discussed, meaning that awareness is likely to be poor. It is therefore clear that there is a need for an improved means for HMB diagnosis. The American College of Obstetrics and Gynaecology go some way to providing a more objective diagnosis, recognising the presence of any of the following symptoms to equate to HMB:

1. bleeding that lasts more than 7 days;
2. bleeding that soaks through one or more tampons or pads every hour for several hours in a row;
3. needing to change pads or tampons during the night; or

4. menstrual flow with blood clots that are as big as a quarter or larger (218).

Similarly, Fraser *et al.* (2015) have recently identified a four-part diagnostic series which has been used effectively to facilitate diagnosis (28). If two or more of the symptoms are reported, a diagnosis of HMB is made (28). The criteria include;

1. Passing of large blood clots;
2. Need for double sanitary protection (both towels and tampons);
3. Need for frequent changes of tampons and towels (meaning changes every 2 hours or less, or 12 sanitary items per period); and
4. Flooding through to clothes or bedding (28).

Clearly, both of these guidelines rely on self-report and are perceptual in nature. However, they are clearly more objective than the diagnosis by the NICE, and a combination of these was applied in this thesis.

Depending on the criteria utilised, in the general population, approximately 20 - 30% of menstruating women have HMB (219,220). This may be in conjunction with, or occur as a result of other conditions, for example, endometriosis, polycystic ovary syndrome or a bleeding disorder such as von Willebrand disease (219). However, a study in university students found only 20.7% of those identified with HMB (n = 82) to have an underlying menstrual cycle-related pathology or a bleeding disorder (Gursel et al. 2014). Suggesting HMB to be a condition in its own entirety.

Using the four-part diagnostic criteria outlined above, Fraser *et al*. (2015) conducted an internet-based survey across five European countries, including France, Spain, Germany, Netherlands and Switzerland. A total of 4506 were surveyed, and overall HMB prevalence was found to be 27.2% (28), and therefore in accordance with previous indications of prevalence (219,220). More specifically the prevalence per country was: France - 30.3%, Spain - 32.9%, Germany – 32.9%, Netherlands – 22.2% and Switzerland – 25.0% (28). The results from this survey also showed that approximately half of those with HMB had not sought medical help (28). However, worryingly, half of those who reported seeking medical help did not have HMB diagnosed (28). Further, of those with HMB, 81.8% indicated that their symptoms were not under control, and the majority indicated that HMB affected their quality of life (28).

A number of studies have examined the prevalence of IDA and iron deficiency in those with HMB, finding approximately 3 in 5 to be iron deficient, and 1 in 4 to have IDA (19,20).

While highlighting the potential impact that increased menstrual blood loss can have on iron status, the relevance to this amongst those who exercise is uncertain, as no research into HMB has been conducted within this population. This will be addressed in this thesis.

Despite HMB having a significant impact on quality of life, mood, energy levels and productivity, due to the sensitivity of the condition, particularly amongst certain cultures, it frequently goes unnoticed. Significantly, the symptoms of HMB are similar to those seen in iron deficiency and IDA, so notwithstanding the likely relation between the two, there is potential for one to be missed in diagnosis (28). However as previously highlighted, the prevalence of HMB in the athletic population is unknown.

2.12 The menstrual cycle in female athletes

Athletes are more likely to have an irregular menstrual cycle than non-athletes, and inevitably dependent on sport type, training volume and performance level, 6 - 79% of exercising women experience reproductive system irregularities (21). Pre-pubertal participation in intense exercise typically delays onset of menarche by around a year, and also increases likelihood of future menstrual disorders (24). In exercising women, the most researched and commonly discussed dysfunctions include primary and secondary amenorrhea, anovulation, luteal phase deficiency and oligomenorrhea (22-24,31). The prevalence of oligomenorrhea (infrequent menstruation) and amenorrhea (absent menstruation) is increased in athletes when compared to the general population, and in particular in those competing in certain sports and events where a low body mass is desirable for performance, training volumes are large and/or where there is substantial focus on aesthetics, such as ballet and endurance running (15,31). In fact, it has been suggested that up to 69% of those who exercise have secondary amenorrhea (where individuals have had at least one menstrual cycle previously, but then absent menstrual cycle for at least 6 months), compared to 2 - 5% of the general population (31). Susceptibility to secondary amenorrhea is also increased in those with a delayed circadian rhythm due to alterations in melatonin release, e.g. in flight attendants (221). This is hypothesised to be caused by the impact of melatonin on luteinising hormone, and could have implications for athletes undergoing long haul travel, however further research is required to investigate this (222).

Amenorrhea can be caused by a number of factors, as discussed widely in the literature; one factor particularly relevant in those who exercise is energy deficiency. The female athlete triad is a term used, specifically relating to menstrual dysfunction in exercising women, describing the interrelationship between low bone density, low energy availability

and menstrual dysfunction (223). In 2014 however, the IOC created a new term – Relative Energy Deficiency in Sport (RED-S) (224). This more broadly demonstrates the impact that an energy deficiency in sport can have. The potential role of iron in the female athlete triad will be discussed further in Section 2.11 (2).

There have been a number of cases where athletes have cited their menstrual cycle to be a cause for poor athletic performance in the press (18-20). However, research defining the effects of the menstrual cycle on exercise, and suitable strategies to mitigate these is absent. With the primary research focus being on dysfunctions associated with amenorrhea (24,31), there is an evident need to consider other menstrual dysfunctions. It is outside of the scope of this thesis to look at all types of dysfunction, however the primary issue that is likely to be associated with iron deficiency; heavy menstrual bleeding, will be addressed.

It is possible that risk of IDA and iron deficiency is further increased in those who exercise and have HMB, however despite some research in the general population, there is a lack of research in the exercising population. In light of the interferences with a women's physical, social, emotional and/or material quality of life, as per the NICE definition for HMB (26), notwithstanding the known negative impacts of IDA and the potential impacts of iron deficiency it is important to address whether this may be a problem in this population, where the potential to impact exercise performance is evident. This thesis will identify the effect of HMB and iron deficiency with or without anaemia on markers of exercise performance. If indeed HMB is found to be prevalent amongst exercisers, awareness needs to be raised as it is currently not a dysfunction that is commonly considered amongst the athlete populous (31).

2.13 Effect, diagnosis and treatment of iron deficiency

IDA has a distinct impact on exercise performance, however the impact of iron deficiency without anaemia (IDNA) is less certain. Much previous research evaluating the effects of IDNA and the efficacy of treatment has focused on clinically unwell populations, where other medical issues, such as heart failure or kidney disease, are likely to confound results (225,226). These results are therefore not applicable to exercising, healthy populations in whom there exists substantial ambiguity as to the impact on performance, the clinical diagnosis and optimal treatment options. The primary reasons for this are:

- There is a lack of a universally agreed consensus on serum ferritin cut-off values for the diagnosis of iron deficiency – optimal levels of serum ferritin for those who

exercise are unknown and research addressing the possible impacts of iron deficiency have typically used cut-off values ranging from 12 - 40 $\mu g \cdot L^{-1}$ (48-54).

- Differing administration routes, which are likely to have varying efficacy – studies have used intravenous injections, oral tablets or intramuscular injections.
- Differing study protocols – studies have used different dosing strategies, i.e. a single high intravenous dose compared to smaller doses spread across a length of time, and different time courses for follow up testing, typically ranging from 24 hours – 12 weeks post iron treatment.
- Small sample sizes – the absence of a power calculation in some studies has further made the drawing of conclusions problematic.
- Differing populations – some studies use elite and well-trained athletes, whereas some use non-elite, recreational exercisers. Elite and highly trained athletes are likely to undergo increased physiological stress compared to the more general exercising population, resulting in differing responses to interventions such as iron administration, and possibly having differing optimal levels of iron stores (48-54). Further, potential for significant increases in exercise and aerobic capacity is much smaller, as these are likely to already be very high. So, despite increases being meaningful on an individual level, these may not be reflected statistically.

2.13.1 Effects of iron deficiency in animal models

The effect of IDNA in animals has been shown. Several murine studies have been conducted comparing IDA, IDNA and iron sufficient rats (227-229). IDNA was found to cause suboptimal mitochondrial function and capacity, likely through modifications to iron-containing proteins. Specifically, Finch *et al.* (1976) attributed the reduced function to the non-haem containing enzyme ∝-glycerophosphate (227), while Willis *et al.* (1987) observed a reduction in cytochrome c alongside other iron-containing enzymes (228). Evidently, IDNA has the potential to have non-haematological effects, altering oxidative enzymes and mitochondrial function and future human studies are required to assess this further specifically at a cellular level.

2.13.2 Effects of iron deficiency on exercise performance

This thesis will primarily focus on how iron deficiency may impact upon components of exercise performance in humans. Where [Hb] is reduced in states of IDA, maximal oxygen uptake, $\dot{V}O_{2max}$, as determined by the amount of oxygen in the blood and the oxygen consumption of skeletal muscle, is reduced (1). On the other hand, $\dot{V}O_{2max}$ can increase through an increase in cardiac output and [Hb]. Cardiac output is most likely to increase

through an enhanced blood volume, which can occur as a result of an increase in erythrocyte mass, again necessitating iron (1).

As explained in section 2.6, IDA is typically measured using [Hb], however as demonstrated in Figures 2.1 and 2.2, there is a minimal relationship between $\dot{V}O_{2max}$ and haemoglobin concentration, likely reflecting the influence of blood volume on this reading. Figure 2.2 demonstrates that total haemoglobin mass correlates much more closely with $\dot{V}O_{2max}$, suggesting that this may provide a more accurate means for indication (1). As previously highlighted, being an essential part of myoglobin, iron deficiency will also impact oxygen transport to muscle myocytes. Further, a lack of iron will impair the function of iron-containing oxidative enzymes, including those involved in the electron transfer process in mitochondria, affecting the production of ATP.

The reduction in oxygen carrying capacity associated with IDA evidently explains how this condition affects exercise performance. However, where absolute haemoglobin mass and therefore oxygen-carrying capacity is not affected in states of IDNA, the impact on exercise performance is less certain (46,110). *G*iven the many other essential roles of iron, there is evidently still significant potential for a negative impact on physiological function. While non-haematological effects have been shown in animals (227-229), some have demonstrated other factors that could impact either directly or indirectly on exercise performance. For example, iron deficiency has recently been associated with an increased risk of osteoporosis (230), while, in non-athletes, research suggests cognitive performance may be affected, which could impact upon exercise performance through a lack of motivation and concentration (57). This will be discussed further below. Low iron stores have also been associated with increased levels of oxidative stress (231).

Yet studies assessing the impact of iron repletion in IDNA athletes have led to inconclusive results, questioning not only the mechanism of impact of IDNA but the accuracy of the diagnosis itself. Research is hampered by a lack of human studies, in addition to studies purely looking at IDA and not IDNA and a number of other factors which will be discussed further below. Since iron deficiency can be indicated by a positive response to iron therapy (47), it could be suggested that where no improvements in functional outcome are seen, iron deficiency is either not present or did not impact on the measured outcomes. The following section will focus on previous key studies that have assessed the impact of iron repletion on functional performance.

2.13.3 Previous research evaluating the impact of iron therapy in iron deficient, exercising participants

It has been widely demonstrated that where serum ferritin is < 40 $\mu g \cdot L^{-1}$, iron therapy will result in significant elevations in serum ferritin, regardless of the supplementation route (46,232). However, research pertaining to whether this is associated with a concomitant improvement in performance is equivocal. A number of studies have been conducted in an attempt to evaluate this, and summary of the findings from these, categorised by the different routes of administration, are described below and summarised in Table 2.1. Some have shown increases in iron status to be transient, returning to initial pre-supplementation levels with no associated impact on performance (51,233). Inevitably, where the gut is bypassed, i.e. using intravenous injections, increases in serum ferritin will occur regardless of baseline values.

2.13.3.1 Oral supplementation

The majority of previous research evaluating the impact of iron deficiency on haematological markers and performance measures have used oral iron repletion. This means for iron therapy is cheap and easy to administrate. However, conclusive findings from these studies are lacking, with distinct variation in study protocol, participant type, markers of assessment, and inclusion criteria. Firstly, excluding the study Powell and Tucker, (234) oral iron supplementation has been shown to increase serum ferritin when measured at a pre-determined follow-up point (49,50,52,54,235-241). The lack of increase in serum ferritin in the study by Powell and Tucker may have been due to follow-up blood tests being only 14 days after iron supplementation was initiated (234). Normal practice is to allow a period of at least three months for repletion of iron stores using oral iron (242), and while the other studies here demonstrated a significant increase in serum ferritin levels, at the end of the predetermined trial period some participants still had low serum ferritin levels. For example, in the eight week supplementation period applied by LaManca and Haymes (1993), five participants still had a serum ferritin < 20 $\mu g \cdot L^{-1}$ at the end of the trial (236).

Improvements in $\dot{V}O_{2max}$ were only evident in the studies conducted by La Manca and Haymes (1993) (236), Friedmann *et al*. (2001), (237) and in the severely iron deficient group in the study by Wachsmuth *et al*. (2015) (241). However, while an increase in in $\dot{V}O_{2max}$ was not seen, an improvements in endurance times or energy efficiency, and a reduction in energy expenditure were evident in a number of studies (50,238,239,243). Significantly, participants in the studies conducted by Wachsmuth *et al*. (2015) (241) and Friedmann *et al*. (2001) (237) undertook the period of supplementation for amongst the longest time across all studies here, being 70 and 84 days respectively, and the baseline serum ferritin levels for inclusion were low (serum ferritin < 12 $\mu g \cdot L^{-1}$ and < 20 $\mu g \cdot L^{-1}$

respectively). While other studies applied the similar inclusion criteria, these studies were longer, therefore, it is possible that the individuals in the other studies had been taking oral supplementation for an insufficient time period for an impact to be significant. The only other study that was comparable in length was that by Matter *et al.* (1987) (235) lasting for 77 days. However, this study applied a more lenient criteria for inclusion (serum ferritin < 40 $\mu g \cdot L^{-1}$), potentially explaining the differing findings here. Conversely, while none of the participants in the study by LaManca and Haymes (1993) were diagnosed with anaemia, a significant increase in [Hb] was seen post treatment, suggesting that a relative state of anaemia was present, therefore improvements in maximal exercise capacity are likely to be due to increased oxygen transport (236).

Evidently, there is significant variation in the inclusion criteria used for iron deficiency across these studies, ranging from a serum ferritin of < 12 $\mu g \cdot L^{-1}$ used by Radjen *et al.* (2011) (240) and in the trial completed by Wachsmuth *et al.* (2015) (241) to a serum ferritin of sFer < 40 $\mu g \cdot L^{-1}$ used by Matter *et al.* (1987) (235). This could be another factor explaining some of the variation in findings, and could be enhanced further by the variation in study duration. For example, despite using an inclusion criteria of a serum ferritin of < 12 $\mu g \cdot L^{-1}$, the duration of the study conducted by Radjen *et al*. (2011) was only 56 days, so the absence of a change in exercise measures could be attributed to insufficient supplementation duration (240). Another significant factor that needs consideration is the compliance of supplementation. This is particularly significant given the negative side effects associated with oral iron supplementation, which could discourage use. Accordingly, a compliance of only 30% was reported in one study (239). While some studies did monitor compliance, this can be difficult to accurately measure.

As highlighted in a recent review, it is likely that some of these oral supplementation studies have not conducted a power or an effect size calculation, amounting to another factor pertaining to the ambiguity of results (244). While, significantly, the performance level of the participants in these studies also varied. Further, a number of studies assessed participants at the onset of a training block, therefore there was an inevitable increase in exercise performance markers, despite this being controlled for (50,239), there is still potential for this to distort findings.

Considering the known increase in hepcidin after iron administration, taking oral iron too frequently, and taking a dose that is too high for requirement has the potential to worsen iron status. Hepcidin levels have been found to increase for three hours post supplementation, therefore whenever iron is taken, iron absorption may not be possible for the subsequent three hour period, this is particularly significant if this falls at a meal time

(180,203). Further, if iron is taken in the period after exercise, the acute inflammatory response may drive hepcidin levels up, again preventing iron absorption (180,203). Therefore, despite evident increases in serum ferritin, absorption may have been limited and could have confounded study results.

2.13.3.2 Intramuscular iron therapy

Likely due to the pain associated with injection, there is a sparsity of studies assessing the impact of iron repletion using intramuscular iron therapy. Both studies that did apply this method followed very similar protocols (48,53). Trained women were administered 2 mL of iron every other day for 10 days (5 x 2 mL) into the gluteus maximus (48,53). A comparable increase in serum ferritin was seen when participants were tested 5- and 10-days after study initiation, and 20- and 24-days after study initiation respectively (48,245). To evaluate performance, Blee *et al*. (1999) assessed a variety of measures intending to indirectly test anaerobic power and endurance capacity, finding the iron administration to have no impact (48). Peeling *et al.* (2007) also concluded similar findings, seeing no improvement in submaximal or maximal exercise capacity (53). Therefore, despite effectively restoring serum ferritin, from the two studies assessing intramuscular iron therapy, it can be concluded that there was no impact on performance. Despite this, there is potential that the small sample sizes in these studies could have limited the applicability of the results, while the criteria used for diagnosis of iron deficiency was also very lenient (Table 2.1).

2.13.3.3 Intravenous iron therapy

Similar to research assessing intramuscular iron therapy, likely due to the only relatively recent development of safer formulations (55), few studies have evaluated the effect of intravenous iron therapy in exercising women. Using highly trained distance runners, both Garvican *et al.* (2014) (52), and Burden *et al*. (2015) (51) found intravenous iron injections to significantly increase serum ferritin. Garvican *et al.* (2014) (52) also found an increase in $\dot{V}O_{2max}$ and total haemoglobin mass in response to intravenous iron therapy. However, this finding was not mirrored in the study by Burden *et al*. (2015) (51) where no changes were seen in any markers of performance. The reasons for this are unsure and could be attributable to several factors. Firstly, despite the total dose of iron administered being similar (500 mg and 550 ± 171 mg, the latter calculated using the Ganzoni formula (246)), Burden *et al*. (2015) (51) administered this in a single high bolus, while Garvican *et al.* (2104) (52) spread administration out over 6 weeks (2 – 4 injections). Secondly, the criteria used for diagnosis differed, Burden *et al*. (2015) (51) used a serum ferritin < 40 $\mu g \cdot L^{-1}$ for men, and a serum ferritin < 30 $\mu g \cdot L^{-1}$ for women, while Garvican *et al.* (2014) (52) used a serum ferritin ≤ 35 $\mu g \cdot L^{-1}$ in addition to a transferrin saturation < 20%, or a serum ferritin ≤ 15 $\mu g \cdot L^{-1}$ alone. Therefore, the inclusion criteria used in the study by Garvican *et al.* (2014) (52) was stricter than that used Burden *et al*. (2015) (51) suggesting that the participants

in this trial may not have been deficient. Finally, the follow up tests were performed at different times, with Garvican *et al.* (2014) (52) testing participants around one week after study completion and Burden *et al*. (2015) 24 hours post injection and 4 weeks post injection. Therefore, it is possible that despite the significant increase in serum ferritin, the impact of iron repletion on exercise performance in the case of the latter may not have had time to manifest within 24 hours, and the positive effects of this may have been lost after four weeks. However, the fact that serum ferritin levels were still supraoptimal at this point counters this later hypothesis. Evidently, the performance level of the participants in both trials was very comparable, with the desired effect of restoring iron status being obtained. With only 7 athletes in each study receiving iron, it is possible that more participants may be required to evaluate this further.

Clearly further clarification is required here, a topic that will be addressed in this thesis.

Table 2.1 – A summary of the effects of iron repletion on physical performance in iron deficient but not anaemic exercising participants.

Study	Inclusion criteria	Subjects (treatment and placebo)	Administration route	Dosing protocol and duration	Effect
Matter *et al*. 1987 (235)	sFer < 40 μg·L^{-1}	n = 29 F	Oral	50 mg elemental iron, 1 x day, 77 days	No improvement in $\dot{V}O_{2max}$
Rowland *et al.* (1988) (243)	sFer < 20 μg·L^{-1}	n = 14 F	Oral	325 mg, 1 x day, 28 days	No improvement in $\dot{V}O_{2max}$ but improved endurance performance
Powell and Tucker (1991) (234)	sFer < 35 μg·L^{-1}	n = 10 F	Oral	Ferrous sulphate, 130 mg 1 x day, 14 days	No change in blood iron indices or metabolic parameters
Klingshirn *et al.* (1992) (54)	sFer < 20 μg·L^{-1}, [Hb] > 12.0 g·dL^{-1}	n = 18 F	Oral	50 mg, 2 x day, 56 days	No improvement in $\dot{V}O_{2max}$
Fogelholm *et al.* (1992) (49)	sFer ≤ 25 μg·L^{-1}, [Hb] > 12.0 g·dL^{-1}	n = 31 F	Oral	100 mg, 1 x day, 56 days	No change in blood lactate or $\dot{V}O_{2max}$

LaManca and Haymes (1993) (236)	sFer < 20 $\mu g \cdot L^{-1}$	n = 20 F	Oral	50 mg, 2 x day, 56 days	Improved $\dot{V}O_{2max}$ and decreased blood lactate
Hinton *et al.* (2000) (50)	sFer < 16 $\mu g \cdot L^{-1}$, [Hb] > 12.0 $g \cdot dL^{-1}$	n = 42 F	Oral	10 mg, 2 x day, 42 days	Improvement in $\dot{V}O_{2max}$
Friedmann *et al*. 2001 (237)	sFer < 20 $\mu g \cdot L^{-1}$	n = 23 F, 17 M	Oral	100 mg 2 x day, 84 days	Improvement in $\dot{V}O_{2max}$, no change in blood volume
Hinton and Sinclair (2005) (238)	sFer < 16 $\mu g \cdot L^{-1}$ IDNA	n = 20 M+F	Oral	30 mg, 1 x day, 42 days	No improvement in $\dot{V}O_{2max}$, improved energetic efficiency
Della Vale and Haas 2011 (239)	[Hb] > 12.0 g/dL, sFer < 20 µg/l	n = 40 F	Oral	50 mg, 2 x day, 42 days	No improvement in $\dot{V}O_{2max}$
Radjen *et al.* 2011 (240)	[Hb] > 12.0 g/dL, sFer < 12 µg/l, TSAT > 16%	n = 17 F	Oral	50 mg, 1 x day, 56 days	No improvement in $\dot{V}O_{2max}$
Wachsmuth *et al.* (2015) (241)	sFer < 12 $\mu g \cdot L^{-1}$, or sFer < 25 $\mu g \cdot L^{-1}$ > 12 $\mu g \cdot L^{-1}$, [Hb] > 12.0 $g \cdot dL^{-1}$	n = 22 F	Oral	100 mg, 1 x day, 70 days	Improvement in $\dot{V}O_{2max}$ in severe, not in moderate. Improved haemoglobin mass in both groups

Garvican *et al.* (2014) (52)	sFer ≤ 35 $\mu g \cdot L^{-1}$ + TSAT < 20% or sFer ≤15 $\mu g \cdot L^{-1}$, [Hb] > 12.0 $g \cdot dL^{-1}$	n = 13 F, 14 M	Oral and IV	Oral – 105 mg, 2 x day, 42 days; IV - 550 mg (ferric carboymaltose 2-4 injections), 42 days	Improvement in $\dot{V}O_{2max}$ and haemoglobin mass only in IV group
Burden *et al.* (2015) (51)	sFer < 40 $\mu g \cdot L^{-1}$ for men, sFer < 30 $\mu g \cdot L^{-1}$ for women, [Hb] > 12.0 $g \cdot dL^{-1}$	n = 9 F, 6 M	IV	500 mg, single dose	No change in performance markers or total haemoglobin mass
Blee *et al.* (1999) (48)	sFer < 40 $\mu g \cdot L^{-1}$, [Hb] > 11.5 $g \cdot dL^{-1}$	n = 15 F	IM	5 x 2 ml, 8 days	No change in performance markers
Peeling *et al.* (2007) (245)	sFer < 35 $\mu g \cdot L^{-1}$, [Hb] > 11.5 $g \cdot dL^{-1}$	n = 16 F	IM	5 x 2 ml, 10 days	No improvement in $\dot{V}O_{2max}$

Oral – oral iron, IV – intravenous iron, IM – intramuscular iron, F = female participants, M = male participants, sFer – serum ferritin, [Hb] – haemoglobin concentration, TSAT – transferrin saturation

2.13.3.4 Overall assessment of iron therapy in iron deficient individuals

It is evident that providing sufficient time is allowed, where individuals have a suboptimal baseline serum ferritin (< 40 $\mu g \cdot L^{-1}$), oral, intramuscular or intravenous iron therapy will cause this to increase. Therefore, despite evident increases in serum ferritin, in the case of the oral supplementation studies, the time course for supplementation may have been insufficient for the restoration of iron status to affect markers of exercise performance. Accordingly, it is significant to note that in the studies where oral iron supplementation was taken for a longer time period, improvements in exercise performance markers were seen (236,237,241). However, the observed effect of iron repletion in both the intramuscular studies (48,53), and the results from the intravenous study conducted by Burden *et al.* (2015) (51) conflict this, since despite the follow up serum ferritin levels being significantly greater than those seen in the oral repletion studies, no increases in markers of exercise performance were seen. Conversely, the intravenous iron therapy study by Garvican *et al.* (2014) (52) did show increases in both maximal exercise capacity and total haemoglobin mass. Significantly, the criteria for inclusion in the latter study by Garvican *et al.* (2014) (52) was much stricter than that of the other injection studies, suggesting that existing diagnoses for iron deficiency may need to be re-evaluated. Given the highlighted adverse effects of excess iron, there is an evident need to address the point at which supplementation should be applied, and this will be addressed in this thesis.

Additionally, given the wide reference range for [Hb] and the discussed potential for intravascular fluid shifts, despite the inclusion of IDNA participants only, it is possible that improvements in aerobic capacity in some studies could actually be due to increases in [Hb] (52). A state of IDA could have been masked. Significantly, a moderate effect of iron therapy on [Hb] was shown in a recent meta-analysis (46).

There was also a wide variation in performance level of the participants in these studies. The participants in both studies using intravenous iron for repletion and both studies using intramuscular iron for repletion were highly trained and elite (48,51-53). Whereas the participants in the oral supplementation studies were of a wide range of ability from the more elite to recreational level. In fact, despite the fast development of the female fitness industry, no injection studies have been conducted in recreational level athletes. This is likely to be because negative performance impacts are more likely to be appreciated and highlighted in the elite populous, with a rapid need for restoration, where both athletes and coaches are carefully monitoring performance (48,51,52,180). Additionally, due to cost implications and accessibility, the use of intravenous iron therapy for the treatment of iron deficiency is clearly more likely in this population, and accordingly, to date, repletion by

this means has only been evaluated in elite and well-trained athletes (51,52). Given that highly trained athletes may respond differently to recreational exercisers, there is clearly a need to evaluate this further. This could have a substantial impact, and will be addressed in this thesis.

Considering the conflicting results from previous research outlined here, and in light of the significant variation in measurements seen in those who exercise (45), it could be suggested that the use of serum ferritin and the criteria for diagnosis when using serum ferritin for iron status evaluation should be questioned. This is something of particular interest in this thesis, and will be evaluated further.

Additionally, the differing evidence highlighted here suggests that a more overarching review in this area is warranted. The substantial variation in study design may make a meta-analysis problematic to conduct, so a review that is more systematic in nature would be more appropriate. This should enable a better evaluation of the required need for further study. Sub-group analysis could then be conducted based on trial protocol and participant status, and could lead to more tangible outcomes.

2.13.4 Other relevant effects of iron deficiency

IDA and anaemia are leading causes of fatigue, however the association between IDNA and fatigue has been less conclusive. Similar to the assessment of exercise performance in response to iron therapy, when assessing the impact of iron repletion on fatigue there have also been conflicting results, with some finding fatigue to decrease (56,226,247,248), while others not (249). Psychology plays a significant part in sports performance, however, none of these studies were conducted in an exercising population, something that will be addressed further in this thesis.

2.13.5 The impact of iron therapy on fatigue and mood disturbance

To my knowledge, one study has assessed the effect of iron supplementation on mood disturbance and fatigue in an exercising population alongside measuring exercise performance and haemoglobin mass (58). The aim of this study was to determine whether in fact serum ferritin cut-offs for both exercise related and cognitive impacts of iron deficiency need to be higher. An inclusion criteria for serum ferritin between 30 – 100 $\mu g \cdot L^{-1}$ was applied (58). Evidently, this is outside of the typical criteria used by many for the diagnosis of iron deficiency. In response to oral supplementation, the results showed that despite not finding any significant increase in haemoglobin mass or any measureable improvements in exercise performance, there was a reduction fatigue and mood

disturbance (58). This could have been due to a placebo effect, or alternatively it could suggest that for optimal cognitive performance serum ferritin should be higher than often thought, with effects on aerobic capacity occurring secondary to cognitive impacts (58). In support of this, a study specifically looking at unexplained fatigue in women, concluded that to prevent fatigue, a ferritin cut-off of < 50 $\mu g \cdot L^{-1}$ should be applied (247). This will be addressed further in this thesis since it has the potential to impact upon sports performance in addition to everyday health.

Clearly, there is reason to suggest that iron deficiency could impact upon both exercise performance (physical and psychological) and fatigue (50,238,239,250), however there is also compelling evidence countering this (51). Given the known negative impacts of iron overload and toxicity further investigation is important in this thesis, particularly as supplementation is believed common amongst the exercising population (39).

2.14 Iron deficiency and the female athlete triad

A review article has recently been published suggesting a role for iron deficiency in the female athlete triad (2). As previously described, the female athlete triad is a syndrome that has three primary causes, including a poor energy status, impaired bone health and a compromised reproductive function (31). All three factors are frequently seen in female athletes, be it together, or in isolation, and are particularly common in those competing in endurance sports, or in events with an increased drive for thinness (31). The effects of these can be severe and detrimental to a woman's exercise performance, and significantly, to their health (31). Typically, low energy availability drives the impaired bone health and compromised reproductive system while also impacting upon numerous other non-vital biological processes, many of which are also seen in iron deficiency (2). For example, both iron deficiency and energy deficiency are associated with an increased risk of hypothyroidism, and in an energy deficient state, the presence of iron deficiency is likely to exacerbate risk of hypothyroidism (251,252).

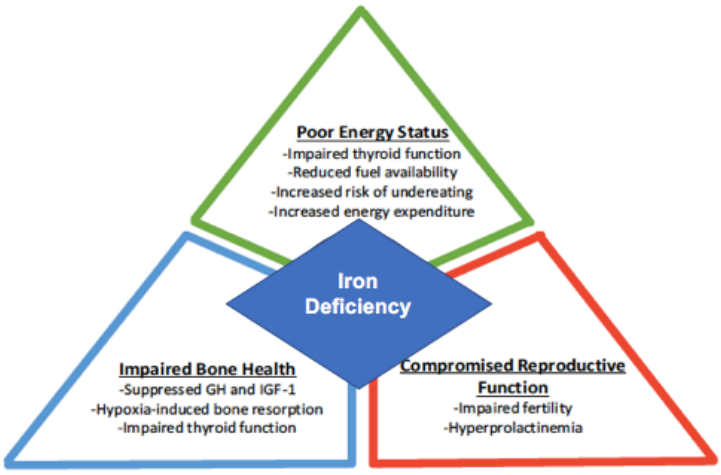

Figure 2.4 – Potential relationship between iron deficiency and the female athlete triad in exercising women as outlined in a recent review. Figure adapted from (2).

As highlighted previously, iron deficiency and IDA have been suggested to have a role in cognitive function, with research suggesting that it also decreases serotonin and noradrenaline function (253). Since the development of eating disorders is believed to be significantly driven by anxiety, the reduction in these hormones could be, in part, responsible for restrictive eating behaviours (253). Undereating inevitably increases risk of iron deficiency, and those who exercise are more susceptible to this, not least because exercise can cause appetite suppression (254). Iron deficiency itself has also been found to reduce appetite, exacerbating the issue (2).

Increases in oestrogen have been found to decrease hepatic hepcidin expression, therefore in energy deficient states, the lower levels of oestrogen may cause increased hepcidin expression, resulting in an increased susceptibility to iron deficiency (255). However, those with lower levels of oestrogen are also more likely to be amenorrhoeic so wouldn't be experiencing monthly blood loss, hence studies suggest that iron status may be balanced (2). Further research should address this relationship.

There is increasing evidence to suggest an association between iron deficiency with or without anaemia and an impairment in bone health (230), a factor that is compromised in those with endocrine suppression and a reduced energy availability. To conclude, evidently there are some significant overlaps between the biological repercussions of a lack of iron and the female athlete triad, often with one accentuating the effects of the other (2). More research is required particularly in human models, since most research to date has been conducted in rodents. Significantly the causative effects of one on the other should also be

considered i.e. does iron deficiency have an impact on oestrogen suppression, since this could help inform treatment options.

2.15 Management and treatment of iron deficiency

There are a number of treatment options that can be applied when iron deficiency is diagnosed. These are outlined below:

2.15.1 Dietary intervention

Dietary modifications should be the first method that should be considered when iron stores are deemed suboptimal (111). Appreciably however, this may not be sufficient to meet demand, meaning that other options are necessary (250). Dietary iron can either be haem (from animal sources) or non-haem (all other types of iron) as will be explained further below. Absorption of haem iron is far more efficient than non-haem, and there are several factors that can influence absorption, which will be outlined.

2.15.2 Oral supplementation

Oral iron is a common and cost-effective method used for repleting iron stores. While considered safe, the long-term effects of supplementation are unknown. As oral iron is typically poorly absorbed, complete repletion of iron stores can take three to six months (256). There are a number of common side effects associated with oral iron supplementation including abdominal discomfort, constipation, heartburn, vomiting and nausea (256). Up to 70% of patients administered oral iron are suggested to experience gastrointestinal side effects, with ferrous sulphate particularly problematic (257). In order to reduce side effects, a reduction in frequency and dosage of supplementation has been suggested, and that iron should be taken alongside food (inevitably this may impact absorption), while liquid formulations instead of oral formulations may also help (256). Several other side effects have been associated with unnecessary or excessive supplementation; excessive supplementation has been associated with increases in oxidative stress and inflammation in young and healthy individuals (61). while also increasing risk of haemochromatosis, particularly in those with the haemochromatosis HFE gene (258).

Increased intake of dietary and oral iron is also primary risk factor in those with genetic abnormalities that predispose them to iron overload, for example in those with haemochromatosis (7). Haemochromatosis is a hereditary condition, occurring where there is a mutation in the HFE gene located in the short arm of chromosome 6 (259). Most cases of haemochromatosis are caused by a HFE mutant genotype. HFE is involved in iron sensing, and is therefore involved in regulation of hepcidin, where there is a mutation

therefore, hepcidin expression may be limited, increasing iron bioavailability (259). In those who have homozygous or heterozygous mutations of the C282Y or H63D alleles, or alternatively with a compound mutation of C282Y/H63D, risk of clinically significant iron overload is increased, therefore caution should be applied with supplementation (259). Approximately 0.4% Caucasians carry a homozygous C282Y mutation, and 6% a heterozygous C282Y mutation (260). Interestingly, prevalence of HFE gene mutations seems to be particularly high in elite endurance athletes (259).

The timing of dosing is imperative since a large intake of iron will cause a transient increase in hepcidin, therefore reducing subsequent iron absorption (203). Therefore, if oral iron is taken just prior to a meal, absorption of dietary iron from the meal could be substantially reduced. Significantly, a recent study concluded lower and less frequent doses to be more effective at repleting iron stores (261). Suggesting that for optimal absorption it may be best to supplement with smaller doses of oral iron every other day (261). Caution should also be applied to ensure oral supplementation is not taken when inflammation is likely, e.g. post intense exercise, as the increased hepcidin levels will prevent absorption. Research has recently found hepcidin levels to remain elevated for 3 - 6 hours post the inflammation that ensues with exercise (203).

Oral iron usage has been found to be common in the sporting context, particularly given the known association between exercise and iron loss (39). This has been demonstrated in cycling, where a third of elite French cyclists were found to supplement excessively, greater than advised, resulting in hyperferritaemia (39). Coaches often encourage athletes to supplement in phases of hard training, particularly when fatigue is reported or observed regardless of prior knowledge of iron status. Burden *et al.* (2015) has suggested that this may be counterproductive because where iron status is sufficient, an increase in iron will elevate hepcidin, resulting in a decrease in iron absorption, possibly having a detrimental effect on performance (51).

2.15.3 Intravenous injections

There are some instances where oral iron is not an appropriate means for iron repletion. Therefore, other administration routes such as intravenous or intramuscular injections are necessary. Intravenous iron injections are particularly used when oral iron will not meet demand, for example after significant blood loss, or in circumstances where absorption is ineffective, for example in inflammatory conditions such as irritable bowel disease. Historically, there have been a number of severe risks associated with intravenous iron injections, including anaphylaxis caused by allergic reactions (262). However, new formulations of parenteral iron are much safer (263). Here, iron is delivered in a carbohydrate complex, enabling it to be released slowly (262,264). As a result, large doses

can be administered over a relatively short period (typically 30-60 minutes). Intravenous iron injections have the ability to fully restore iron levels in a one-off dose through direct injection into the bloodstream meaning that controlled absorption is bypassed. The main negative associated with intravenous iron is the expense.

2.15.4 Intramuscular injections

Although effective, use of intramuscular injections for the restoration of iron levels is not common, and accordingly little research has been conducted using this means for iron repletion (74). Injections are painful and are associated with gluteal sarcomas and skin discolouration (265).

2.16 Effects of iron supplementation in iron sufficient athletes

Notably, previous research has shown no performance benefit of iron supplementation in non-iron deficient or anaemic athletes, identified by the lack of response of iron status markers to oral iron therapy (60). Given the associated risks and side effects, unnecessary supplementation is not recommended and can result in severe health repercussions (59,61,187).

The second part of this literature review will firstly focus on the transport, delivery and storage of iron. It will then outline the control and maintenance of iron metabolism. Given the fundamental roles of iron described in the first section of this review, a biochemical understanding of these processes is essential when assessing iron status in the female athlete.

2.17 Transport, delivery and storage of iron

2.17.1 Iron transport within blood

On entry into the circulation, ferric iron is picked up and scavenged by transferrin for tissue delivery. Transferrin is a 75 - 80 k-Da glycosylated protein plasma iron carrier, primarily secreted by the liver with a half-life of eight days (71). In addition to binding to plasma iron, it also binds to iron in extracellular fluids such as cerebrospinal and lymph fluids, and is distributed equally between the extracellular fluid and plasma (71). Each transferrin protein can transport up to two ferric ions to target tissues, undergoing a conformational change in the process (71,73). The binding of iron is pH-dependent, and iron is not bound in acidic conditions (71). The process of iron scavenging followed by tissue delivery is very dynamic, and is regulated by the expression of transferrin receptors 1 and 2, which are located on target tissues (266). Despite this being a vital process, only a very small amount of iron is

bound to transferrin, amounting to approximately 3 mg, and equating to less than 0.1% of total body iron (67). However, in order to meet erythropoietic demands, transferrin is turned over more than ten times each day (67). The majority of iron making up this dynamic pool comes from recycled senescent erythrocytes, taken up by reticuloendothelial macrophages in a process which will be described in further detail below (67).

Under normal physiological conditions only approximately 30% of transferrin is saturated with iron (71). Where iron is present in plasma but is not bound to transferrin, it is termed non-transferrin-bound iron (NTBI). Transferrin has a high affinity for iron, so NTBI is typically only present where there is either a lack of transferrin (atransferrinemia) or if all transferrin is saturated. In these instances, other transporters such as albumin, citrate or acetate will bind to the NTBI (73). Ferritin within plasma also transports iron to tissues, however the mechanism for this is yet to be fully elucidated.

2.17.2 Iron tissue uptake

Since the primary means for iron delivery to cells is via transferrin, target cells express transferrin receptors. The primary transferrin receptors are transferrin receptors 1 and 2, and levels of these typically correlate with iron requirements of the cell. The iron-containing transferrin binds to the cell surface transferrin receptors, forming a holo-transferrin-transferrin receptor 1 complex (85). This will enter cells via receptor-mediated endocytosis, forming clathrin coated pits (267). The low pH environment in the formed endosome which is created by proton pumps, causes the complex to breakdown (85). Ferric iron is then reduced to ferrous iron, a reaction catalysed by the ferriductase six-transmembrane epithelial antigen of phosphate 3 (STEAP3) (85). Ferrous iron crosses the endosomal membrane via DMT1, entering the labile iron pool in the cytolsol (85). From here there are a number of different destinations for ferrous iron dependant on need, as explained below. Apotransferrin is then recycled and released. The effectiveness of iron uptake is dictated by the iron content of transferrin.

2.17.2.1 Ferrous iron in cells

There are three primary destinations for intracellular ferrous iron, as explained below:

Cellular iron utilisation

The majority of iron is transported to the mitochondria either for incorporation into haem or for incorporation into iron dependent proteins. The process by which iron transverses cells and enters mitochondria is yet to be decisively elucidated, in part confounded by a variation in different cell types (268,269). It is also likely that iron is transported to cells via several different processes. Firstly, a direct mechanism by which iron is delivered to the outer

mitochondrial membrane directly from the endosome has been demonstrated in reticulocytes (268,269). Secondly, iron in the cytosolic labile iron pool has been found to form low and high molecular weight intracellular complexes (270,271). While further research is required to gain a better understanding of these, chaperone proteins such as glutathione (GSH) (272), poly C binding proteins (e.g. poly (rC) binding protein 1 - PCRP1) (273), and monothiol glutaredoxins (e.g. glutaredoxin 3 - Grx3-type) (274), have been implicated in assisting with iron transport throughout the cell, including to mitochondria (85). Finally, it has also been postulated, and demonstrated in murine and yeast cells that the mitochondrial membrane potential can drive direct entry of iron into mitochondria (275,276). Further research is evidently required to more specifically identify the mechanisms involved in human cells, and to establish the specific intracellular organelles to which chaperones are responsible for transport.

There is also a degree of ambiguity surrounding the mechanism by which iron transverses the outer and inner mitochondrial membranes (277). Voltage dependent anion channels, which are the primary means for transport of metabolites into and out of mitochondria, exist in high abundance in the outer mitochondrial membrane, so it has been suggested that iron may enter via these (277). However, DMT1 isoforms have also been identified which present another option for iron import (278,279).

Mitoferrin 1 and 2 (MFRN1 and 2) are responsible for iron import across the intracellular mitochondrial membrane (280). A study in mouse erythroid cells found that iron was transported from transferrin receptor 1 containing endosomes through binding to the MFRN 1 importer. This is stabilised through interaction with an ATP-binding cassette transporter – Abcb10, facilitating iron entry (281).

Once in mitochondrion, iron will either be stored, used for the biosynthesis of iron-sulphur clusters or used for haem synthesis.

Cellular iron storage

In a cell, when the demand is low, and iron is in excess, it can be stored as ferritin within the cytosol. Some cell types also have iron stored as ferritin in mitochondria (MTFT) (267). When required, nuclear receptor coactivator 4 targets ferritin for iron release back into the cytosolic labile iron pool (282).

Cellular iron export

Excess iron can also be exported out of the cell via ferroportin (Section 2.18), with the aid of ceruloplasmin or hephaestin.

2.17.3 Iron storage

As previously explained, the majority of body iron is located to haemoglobin. Due to the toxicity of unbound iron, the storage of excess iron is crucial for optimal physiological functioning. Ferritin and haemosiderin are the iron-storage complexes which are responsible for this.

Ferritin is a spherical heteropolymeric, intracellular, cytosolic iron-storage protein consisting of 24 heavy (H) or light (L) type subunits that form a hollow sphere which can contain up to 4500 iron atoms (3,73). Cytoplasmic chaperones such as poly (rC)-binding protein 1 (PCBP1) transport iron in its ferrous form to ferritin (273). Here, it is oxidised back to ferric iron, a process which is catalysed by the heavy type subunits which act as ferroxidases (73).

While the majority of ferritin is intracellular, there is also some found within serum. Research suggests that a small amount of iron may be delivered to tissues by plasma ferritin (73). Serum ferritin is primarily comprised of light-type (L) subunits, and is thought to be secreted by macrophages, hepatocytes and Kupffer cells (283,284). This ferritin is less iron rich than that found within tissues (285). As previously highlighted, since serum ferritin correlates with iron stores in most physiological situations, in the clinical setting it is typically used as a means for measuring iron status (286,287). However, this is not always the case, as it is particularly susceptible to transient variation in certain situations, in states of inflammation, or post exercise, as previously highlighted (73,288). However, due to its role as an acute phase reactant, it is often used as a marker of both acute and chronic inflammation, and can be used as a non-specific marker of illness (288). Accordingly, the use of serum ferritin to evaluate iron status needs to be treated with caution.

Iron is also stored as haemosiderin. Iron from the labile pool is taken up by ferritin and is then transformed into haemosiderin for storage; haemosiderin is a complex of ferritin (289). The reverse of this happens for iron mobilisation back into the labile pool from haemosiderin via ferritin (289).

2.17.4 Toxicity of iron

As previously highlighted, there is no physiological mechanism for excreting surplus iron. Excess free iron within tissues under aerobic conditions can be very harmful, causing excessive production of reactive oxygen species (ROS) (68). Through evolution, organisms have developed proteins which can bind to iron to prevent harmful damage, while keeping it stable and accessible for physiological requirements. Based on the Fenton and Harber-Weiss reactions, iron in excess will catalyse the production of hydroxyl radicals

from hydrogen peroxide and superoxide (68,290). While production of ROS within cells is normal, excess production is harmful, and iron overload can cause cellular damage, for example, it will result in the peroxidation of lipid membranes (291,292). At physiological pH and under aerobic conditions however, extracellular iron is bound to transferrin keeping iron in its soluble state, making it available, while also preventing toxicity. In homeostatic conditions, approximately 30% of transferrin is bound to iron, however once transferrin saturation exceeds 80%, it becomes saturated resulting in an increase of NTBI (266,293). This NTBI is potentially harmful, having the ability to catalyse ROS-producing reactions. It can form chelates, which are subsequently internalised into cells in an unregulated manner, causing damage (68). The insolubility of iron at neutral pH and under aerobic conditions is therefore vital in serving as a protection mechanism (294).

2.17.5 Release of free iron and haemoglobin into the circulation

Under normal physiological conditions 10 - 20% of total erythrocytes are haemolysed early, before the end of the typical red blood cell lifespan and prior to senescence and macrophage identification for phagocytosis (295). During haemolysis, the erythrocytes release haemoglobin freely into plasma. Similarly to iron, free haemoglobin is toxic, causing an increased production of ROS (296). However, the two glycoproteins haptoglobin (Hp) and haemopexin (HPX) act as scavengers for the released haemoglobin and haem, primarily functioning to prevent their pro-inflammatory and pro-oxidant effects (297).

Both Hp and HPX are acute phase proteins, therefore their expression can be induced by inflammatory mediators. Hp, is secreted by hepatocytes, and specifically binds to the released haemoglobin, sequestering the iron (191,192). The Hp-Hb complex will then bind to the cluster of differentiation receptor 163 (CD163) on the surface of macrophages and will be endocytosed, resulting in Hb breakdown and iron release (295,298). Where there is a significant amount of haemolysis, Hp will be unable to sequester all the free haemoglobin. As a result, the haemoglobin will readily be oxidised to ferrihaemoglobin, releasing free haem (299). Ferrihaem will then be transported to HPX by albumin (297). HPX has the highest haem binding affinity of any known haem-binding protein, and typically binds haem that is released from early haemolytic erythrocyte breakdown (299). On binding, the haem-HPX complex undergoes receptor-mediated endocytosis via the LDL receptor-related protein (LRP1) (299,300). These receptors are particularly located to hepatocytes, macrophages and in splenic tissue, therefore the haem will enter these tissues, and will be broken down, freeing the iron (299). Essentially, Hp and HPX facilitate the recycling of haemolysed erythrocytes, while also protecting against the harmful effects

of free haemoglobin and haem. Hp and HPX biomarker levels can be used as a means for assessing haemolysis.

2.18 Maintenance of iron homeostasis

Maintenance of iron homeostasis is a very refined and important process which is controlled at both a systemic and cellular level.

2.18.1 Iron entry into the circulation

There are three cell types from which iron can enter the circulation, all of which are involved in the maintenance of iron homeostasis;

- Intestinal enterocytes – these are primarily located to the upper jejunum and duodenum. They are responsible for absorption of dietary iron.
- Macrophages - these are typically located to the spleen, liver or bone marrow. They are responsible for the recycling of iron from senescent erythrocytes.
- Hepatocytes – these store iron, releasing it when needed.

2.18.1.1 Iron entry into the circulation via intestinal enterocytes

Iron absorption into the circulation via intestinal enterocytes takes the form of 3 phases as shown in Figure 2.3:

1. Iron transport into enterocytes.
2. Iron transport across enterocytes.
3. Iron release from the basolateral enterocyte membrane.

1. Iron transport into enterocytes

The mechanism by which iron it taken up into enterocytes is dictated by the type of iron. As previously highlighted, there are two types of dietary iron, namely haem iron (typically animal sources) and non-haem iron (typically non-animal, plant sources), the bioavailability of which varies. Uptake of haem iron across the apical enterocyte membrane is much more efficient than uptake of non-haem iron, however the exact mechanism of haem uptake has historically been unsure. Recent research suggests a role for haem carrier protein 1 (HCP1), a hydrophobic protein consisting of nine transmembrane domains (301). Expression of HCP1 can be controlled both pre- and post- translationally (142,302). However some have found this protein to have a role in folate transport as opposed to haem transport, so further research is required (303).

The process by which non-haem iron is absorbed is much more complex (Figure 2.5). At the typical physiological pH (approximately 7.4), ferrous iron (Fe^{2+}) is oxidised to ferric iron

(Fe^{3+}), which is insoluble, and therefore direct entry into cells is not possible. However, the gastric acid and ferric reductase enzymes (such as duodenal cytochrome B) in the proximal duodenum lower the pH, enabling the reduction of ferric iron back to ferrous iron, which can then be transported across the apical membrane of enterocytes by a ferrous iron transporter such as divalent metal transporter 1 (DMT1) (67).

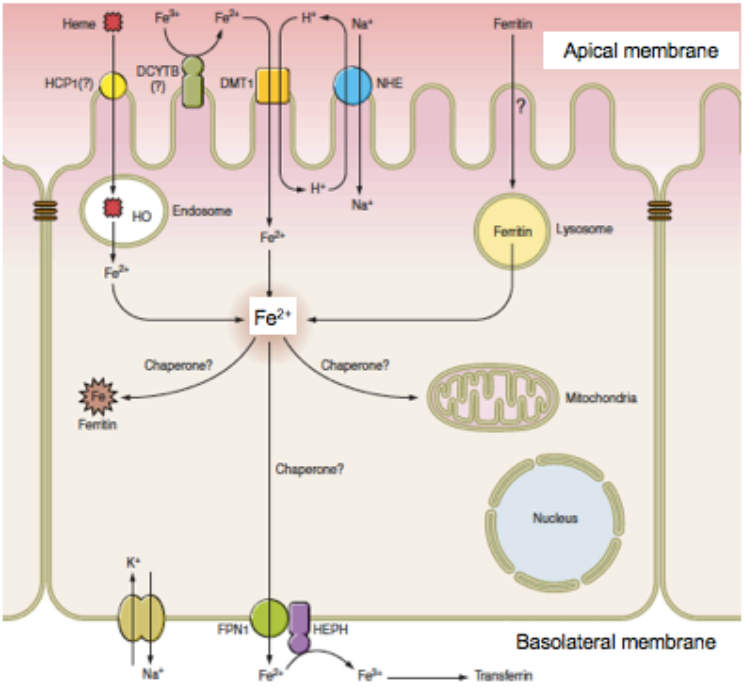

Figure 2.5 – The mechanism by which iron enters enterocytes, and is then either stored within the cell or is exported from the basolateral membrane into the circulation. The mechanism for haem iron entry into the cell is not yet understood, however it is hypothesised to involve the haem carrier protein 1 (HCP1). Non-haem iron enters the cell via divalent metal transporter 1 (DMT1), an iron transport protein, after being reduced by duodenal cytochrome B (Dcytb), a ferriductase. It is then either stored as ferritin or transported through the cell for export via ferroportin. Figure adapted from (3).

The reduction of ferric iron to ferrous iron in the presence of oxygen is catalysed by a ferrireductase such as duodenal cytochrome B (Dcytb), located on the apical enterocyte membrane (304,305). Dyctb is a di-haem transmembrane oxidoreductase which reduces ferric iron, and requires endogenous or secreted reductants to act as an electron donor in the process (304,306,307). Ascorbate is thought to be the primary reducing agent, however some research also suggests that this process could be facilitated by other reductants (308). While much research has focused on the role of ascorbate in the control of iron uptake (304), other roles for this ester have been suggested in addition to increasing the reductase activity of Dyctb (304,306).

Once reduced, ferrous iron (Fe^{2+}) enters enterocytes via a divalent mental transporter (DMT1), this is a protein that transverses the apical cell membrane. DMT1 is located on the brush border of the apical enterocyte membrane and in addition to ferrous iron, it can also transport other metals providing they have a valency of two. DMT1 is coded by the SLC11A2 gene, and has 12 transmembrane domains, terminating in the enterocyte cytoplasm (309,310). The primary function of DMT1 is to co-transport protons alongside ferrous iron into cells (310,311). The required proton gradient is thought to be driven by a sodium/hydrogen exchanger on the apical brush border membrane (3). The efflux of positive ions also assists with iron absorption by lowering the surrounding pH aiding partial release of ferric iron from its protein carriers, prior to its reduction by Dyctb to ferrous iron (304).

2. Iron transport across enterocytes

Within enterocytes, haem iron is thought to be mobilised by haem oxygenase 1 (HO-1), this frees ferrous iron ions into the labile iron pool (312). The specific transport process of both haem and non-haem derived ferrous iron within enterocytes is unsure, however it is thought that it is either chelated by small molecular weight organic acids, intracellular proteins or by amino acids (3). Poly-r(C)-binding iron-trafficking proteins have been suggested in this role (313). Depending on cellular iron status, iron will either be transported straight across the enterocyte to the basolateral membrane for entry into the blood, or will be stored. When iron is in excess, or suffice, it is stored as ferritin. In humans, enterocytes are typically sloughed every 2-5 days, so stored iron that has not been transported into the circulation will be lost (314).

3. Iron transport across the basolateral membrane

Iron is exported from enterocytes into the bloodstream via ferroportin, the only mammalian ferrous iron export protein discovered to date (315). It is coded by the SLC40A1 gene and has a 12-transmembrane domain terminating in the cytoplasm (316-318). It has been specifically localised to enterocytes, hepatocytes, macrophages and on the placental syncytiotrophoblast, clearly suggesting these as key sites for the regulation of iron homeostasis (319).

Transport of iron via ferroportin is coupled to the re-oxidation of its ferrous form to its insoluble ferric form. The process is catalysed by the multicopper ferroxidase, hephaestin, which is located on the basolateral membrane (320,321). Once in the bloodstream, ferric iron will be picked up by transferrin, the glycosylated protein plasma iron carrier. Transferrin

transports iron throughout the circulation while also removing it from ferroportin to maintain the gradient to drive cellular iron export (73).

2.18.1.2 Iron entry into the circulation via macrophages

The average lifespan of an erythrocyte is 120 days, and when the cells reach senescence macrophages remove them from the circulation by erythrophagocytosis, in a process that usually takes place in the spleen, liver or bone marrow (322). On a daily basis, it is estimated that 0.8% of iron in erythrocytes is recycled, amounting to approximately 40 - 60 mg (70,73). Within macrophages, the erythrophagocytosis occurs in the hydrolytic environment of phagolysosomes. This results in the breakdown of first the erythrocyte and then haemoglobin, freeing haem (73). The specific mechanism by which haem is broken down and iron is released has not yet been elucidated. However, the enzyme haem oxygenase-1 (HO-1) has been found to break down haem, releasing the iron (73).

Depending on current iron status, the released iron is either stored within the macrophage cytoplasm or is transported into the bloodstream. The export of iron from macrophages into the bloodstream again occurs via ferroportin, and is controlled by hepcidin, in a process described in more detail subsequently (71). However iron is oxidised by the multicopper ferroxidase protein ceruloplasmin as opposed to hephaestin as in enterocytes (320). Four of the six copper atoms in each ceruloplasmin protein are involved in this oxidation process.(323)

Significantly, on a daily basis, the iron that arises from the breakdown of senescent erythrocytes in macrophages forms the vast majority of iron that enters the plasma (approximately 20 mg per day) (324). Intestinal uptake only typically amounts to 1 - 2 mg (70).

2.18.1.3 Iron entry into the circulation via hepatocytes

Iron can also enter the circulation from hepatocytes. Again, it is released via ferroportin, and is controlled by hepcidin expression. In a similar process to iron export from macrophages and enterocytes, a copper ferroxidase is responsible for the oxidation of ferrous iron prior to transferrin binding. Formed through alternative splicing, a different form of ceruloplasmin containing a glycosylphosphatidylinositol anchor catalyses this reaction (325). Hepatocytes provide a long-term store of iron, and release is thought to be slower than from macrophages (135).

2.19 Regulation of iron homeostasis

In order to maintain homeostasis and control iron absorption and release, a number of specific proteins and hormones are involved at both a systemic and cellular level. At a systemic level this control is primarily transcriptional, while at a cellular level this is post-transcriptional.

2.19.1 Systemic control of iron homeostasis

2.19.1.1 Hepcidin

Hepcidin is a 2.7 kDa peptide hormone consisting of 25 amino acids and containing four disulphide bonds (76). Coded by the HAMP gene, it is primarily produced by hepatocytes and is responsible for the regulation of iron entry into the plasma through controlling both absorption across the basolateral membrane of enterocytes and the entry of recycled iron from macrophages and stored iron from hepatocytes (62). Other cell types have also been found to express hepcidin mRNA at a much lower level, including adipocytes, cardiomyocytes, parietal cells and cells of the immune system (76). It typically exists in the circulation as a free protein, however a small fraction is weakly bound to albumin and $\alpha 2$-macroglobulin (326).

Hepcidin controls iron plasma entry post-translationally through its effect on ferroportin (199). On binding with ferroportin, it causes it to be internalised, and ubiquitinated (327). It is then endocytosed into endosomes and degraded (76,327). As a result, plasma delivery of iron into the bloodstream is prevented, and iron accumulates in the cell (328). Therefore, when levels of hepcidin are high, iron absorption from enterocytes and release from macrophages and hepatocytes is reduced, decreasing plasma iron. When iron levels are low, and therefore hepcidin levels are low, iron uptake from enterocytes and macrophage and hepatocyte release into the circulation is increased, elevating plasma iron (329). If hepcidin levels remain high, the iron stored in enterocytes will be lost as these cells are sloughed (330). Hepcidin has also been found to alter expression of DMT1, therefore influencing iron uptake into enterocytes (91).

Being the site of hepcidin production, the liver appears to be the primary controller of HAMP expression. Interestingly, circadian rhythm also has an effect on hepcidin release, as release is thought to increase through the day (136).

HAMP gene expression is homeostatically controlled by a number of factors, including iron status, erythropoiesis, hypoxia and inflammatory mediators, and will now be discussed in further detail (331,332).

2.19.1.2 Increased hepcidin expression

There are two primary factors that result in an increase in hepcidin expression including iron overload and inflammation and infection.

Iron overload

HAMP expression increases in response to elevated iron both in the liver and in plasma through independent pathways. Firstly, elevated levels of iron in the liver cause expression of the growth factor and cytokine bone morphogenetic protein 6 (BMP6) to increase (333). As a member of the transforming growth factor beta superfamily (TGF-ß) (334), once activated, this binds to haemojuvelin, a co-receptor ligand required for activation of the BMP signalling pathway. The BMP6/haemojuvelin complex then binds to the activated BMP receptor (333,334), initiating an intracellular signalling cascade starting with the phosphorylation of SMAD (contraction of Sma and Mad) (76). This results in an increase in signalling to the proximal and distal ends of the HAMP promoter, causing HAMP expression to be upregulated, increasing hepcidin production, therefore reducing iron absorption into the bloodstream (335-337).

The mechanism by which plasma iron dictates hepcidin release is not yet fully understood, however it has been elucidated that both iron in the liver and plasma transferrin saturation are sensed by transferrin receptors 1 and 2 and the hepatic haemochromatosis protein HFE (338). When iron levels are high, HFE and transferrin receptor 2 are thought to bind together to form a signalling complex which then results in hepcidin activation and an associated reduction in iron absorption (338).

Inflammation and infection

HAMP is a type II acute phase protein (199), therefore expression increases as a result of release of particular cytokine inflammatory mediators through the Janus kinase/signal transducers and activators of transcription 3 (JAK/STAT-3) pathway (333,339). For example, interleukins such as IL-6 and IL-22 have been shown to increase HAMP gene transcription (199,340). A separate additional independent mechanism for increase in hepcidin release not involving the STAT-3 pathway has recently been suggested, which is activated in response to other TGF-ß superfamily cytokines (341). This is particularly significant for those who exercise, since these cytokines are likely to be upregulated post-exercise, and recent research has specifically focused on the association between exercise and hepcidin release. Peeling *et al*. (2017) found a 7.5 fold increase in serum hepcidin three hours post exercise (342). It was concluded that a number of factors dictate this including the release of IL-6, baseline serum ferritin and serum iron, in addition to exercise duration (342). Despite previous research showing that iron status supersedes

inflammation in control of hepcidin expression (51), it is evident that those who exercise are likely to experience transient increases in hepcidin, and attention should be applied to the timing of dietary iron intake for maximal iron absorption.

As previously highlighted, given that iron is essential for the survival of most micro-organisms, this inflammation-induced hepcidin release acts as a defence mechanism, restricting iron uptake and therefore protecting against the invasion of these foreign bodies (333). Interestingly however, cytokines involved in the type I pathways (e.g. IL-1 and TNF-α) do not have the same impact on HAMP expression (199).

2.19.1.3 Suppression of hepcidin expression

There are three primary causes for a suppression of hepcidin expression, including iron deficiency, hypoxia and an increased erythropoietic stimulus.

Iron deficiency

In iron deficient states, there are a couple of pathways by which hepcidin expression is thought to be supressed. Firstly, evidence suggests that the serine protease matriptase-2 is induced in the liver, and cleaves haemojuvelin, disrupting the BMP6/haemojuvelin complex, resulting in the cessation of HAMP expression (343). Secondly, plasma iron can also specifically block hepcidin signalling through the action of transferrin receptor 1 on HFE (344). It sequesters HFE, preventing binding to and interaction with transferrin receptor 2, and therefore stopping the resulting activation of hepcidin (344).

As a result of menstruation, resting levels of hepcidin in premenopausal women are likely to be lower than those of postmenopausal women (330).

Hypoxia

Historically, it was believed that hypoxia directly supresses hepcidin. The mechanism was thought to involve the hypoxia inducible factors (HIFs), which are stabilised under hypoxic conditions (345). Up until recently, research has suggested that these could either directly supress hepcidin expression through the HAMP promoter or indirectly through a reduction in BMP6 signalling (346). However, further more recent research suggests that an increased erythropoietic drive, and therefore increased erythropoietin (EPO) release was required alongside this to supress hepcidin (347). Under hypoxic conditions this would most likely be seen, as oxygen demand is increased.

Increased erythropoietic drive

Despite much previous uncertainty, research is increasingly suggesting that erythropoiesis alone can directly supress hepcidin expression. However, there has been much ambiguity as to the direct mechanism of action. When erythropoietic demand is elevated, EPO production is increased, inevitably enhancing iron requirements. Recently, using mouse models, Kautz *et al.* (2014) have discovered erythroferrone, a hormone which has been postulated as an erythroid regulator of hepcidin (347). This is encoded by the Fam132b gene, and is a member of the Clq/tumour necrosis factor family (348). It is hypothesised to be released by erythrocytes in response to increased erythropoietin (EPO), and has been found to supress hepcidin via the JAK/STAT pathway (347). However, to date only very limited and inconclusive human research has been conducted, the vast majority has been conducted in mice (81). Erythroblasts have been found to express the erythroferrone gene (81), however function and mechanism of action is yet to be elucidated.

Both hypoxia and increases in EPO are also thought to induce matriptase-2 production in a similar mechanism to that in iron deficiency (76).

2.19.2 Cellular control of iron homeostasis

Since all cells require iron, yet an excess of iron is toxic and harmful, tight regulation and control at a both a cellular level and a systemic level is necessary. Cellular iron homeostasis is controlled by iron regulatory proteins (IRPs) and iron response elements (IREs). With regard to iron uptake, they function to control the regulation and expression of DMT1 and Dcytb, and therefore can dictate non-haem iron uptake. In states of iron deficiency, DMT1 and Dcytb expression is increased, when iron levels are optimal, expression is decreased.

2.19.2.1 Iron regulatory proteins (IRPs) and Iron response elements (IREs)

The function of iron regulatory proteins 1 and 2 (IRP 1 and IRP 2) is to sense cytosolic iron levels and then to either influence mRNA stability or alter translation of key proteins involved in the regulation of intracellular iron homeostasis (349). IRPs also regulate many other genes, demonstrating the wide range of tissues and proteins that are iron dependent, while also essential for mitochondrial function (349). IRPs post-transcriptionally regulate the expression of IREs. IREs are conserved stem-loop structures. In the case of the control of iron uptake into enterocytes and therefore the control of DMT1 expression, IREs are found on the DMT1 mRNA. They are also found on the mRNA of transferrin receptor 1, ferroportin, HIF-2α and ferritin, as will be discussed subsequently (349).

There is tissue specific variation in the response to IRP-IRE binding, largely dependent on the location of mRNA binding (93). For example, in conditions of iron overload IRPs bind to the 5'UTR of specific target mRNAs such as L- and H- ferritin, ferroportin and HIF-2α to reduce translation, preventing iron uptake and storage (71). While in iron deficient states, IRPs can bind to the 3'UTR of DMT1 and transferrin receptor 1 mRNA to increase iron absorption (349). Significantly, much of these processes have been conducted in murine models, therefore further mammalian human research is required to ascertain the exact roles of IRPs in iron homeostasis.

2.19.2.2 Ferroportin

As previously highlighted, ferroportin is the only known iron export protein. Its expression can be regulated at different levels, including transcriptionally, translationally and post-translationally.

1. Transcriptional control of ferroportin

Research evaluating the control of ferroportin at a transcriptional level is not as conclusive as translational and posttranslational control. Two primary mechanisms have been hypothesised.

Within macrophages specifically, it is thought that both haem and iron are key in regulation of ferroportin transcription. This regulation is believed to be via the transcriptional repressor Btb And Cnc Homology 1 (Bach1) (350), which is suggested to be able to sense cellular levels of both haem and iron (351). On activation, it will have a number of downstream effects resulting in increased haem-oxygenase 1, ferritin and ferroportin transcription, while also triggering iron release from haem (350). Haem activation of haem-oxygenase 1 alone further stimulates iron release (352). The released iron will then bind to IRPs, preventing IRP binding to 5'UTR ferroportin and ferritin IREs, and as a result transcription is increased (353).

Expression of ferroportin is also thought to be regulated at a transcriptional level by hypoxia inducible factor 2 alpha (HIF-2α) (354). Since HIF-2α requires oxygen for breakdown and prolyl hydroxylase 2 requires iron for function, in hypoxic conditions or iron deficiency, HIF-2α is not broken down by prolyl hydroxylase 2, and it instead binds to HIF-1ß. Increased expression of HIF-2α results in increased transcription of genes which specifically result in increased iron uptake, including ferroportin, Dcytb and DMT1 (354,355). Therefore, in hypoxia or iron deficiency, iron uptake and entry into the bloodstream is increased.

2. Translational control of ferroportin

Translation of ferroportin is controlled by IRP 1 and IRP 2. To inhibit translation, these bind to the 5'UTR of IRE's in ferroportin mRNA (356).

3. Posttranslational control of ferroportin

Posttranslational ferroportin expression is controlled by the peptide hormone hepcidin, as previously explained (327). This is believed to be the primary and most important mechanism for controlling plasma iron entry and maintaining homeostasis (357).

Non-hepcidin internalisation of ferroportin

While hepcidin is the only ligand that can cause ferroportin internalisation and degradation, ceruloplasmin is also essential for iron export (358). In the absence of ceruloplasmin, ferroportin is internalised and degraded (358).

Cellular iron is primarily homeostatically controlled by iron status and hypoxia.

2.19.2.3 Adequate iron or iron overload impact on cellular activity

When cellular iron stores are optimal or too high, activity of IRP 1 and IRP 2 is reduced. IRP 1 binds to an iron-sulphur cluster, where it functions as a cytosolic aconitase, stopping activity, and IRP 2 is degraded by ubiquitin ligase (312). A lack of IRP activity causes transferrin receptor mRNA to be degraded, therefore preventing extra cellular iron entry (312). This also allows translation of ferritin and ferroportin mRNA to enable storage and export of iron.

2.19.2.4 Iron deficiency and hypoxia impact on cellular activity

When there is a cellular iron deficiency, IRP 1 is released from the iron-sulphur cluster, and IRP 2 is stabilised. Both IRPs will then bind to IREs located in the UTRs in the mRNA of key iron uptake, storage and export proteins to influence expression. As a result, iron uptake is increased, and storage and export are decreased (71).

In addition to the previously discussed impact of hypoxia on hepcidin expression, it has been suggested that the hypoxic stimulus also drives hepcidin-independent responses at a cellular level, increasing iron absorption by increasing expression of iron transporters (81). In murine models, in addition to increasing expression of DMT1 and DcytB (359), HIF-2α has been shown to upregulate ferroportin expression by binding to the promoter on ferroportin response elements in states of both hypoxia and iron deficiency (81,354,360-

362). However, since these conclusions were drawn from murine studies, and future mammalian research is required.

2.20 Summary

Exercise clearly has the potential to increase susceptibility to iron losses, and therefore menstruating women who exercise are inevitably at increased risk of deficiency. Given the likely impact on iron status, research is required first examining the prevalence of HMB in those who exercise. The effects that this has on outcomes which have the potential to affect performance then need to be considered. The importance of iron is evident, and while the repercussions of IDA are clear, an increased understanding surrounding the impact and diagnosis of IDNA is evidently required. This will enable a more accurate assessment of prevalence and when intervention is required. Existing ambiguity may in part be due to the suitability of markers used for its diagnosis. Iron homeostasis is a very tightly controlled process, and exercise can cause necessary modification in homeostatic mechanisms, which are essential given the adverse impacts of excess free iron.

2.21 Overall aims and hypotheses of this thesis

2.21.1 Thesis aims

i. To establish the prevalence of HMB amongst exercising women of different performance levels when HMB was identified using a self-created, non-validated criteria. Then to identify whether this was associated with the perception that the menstrual cycle disrupts exercise training/performance in this population and with a history of self-reported anaemia.

ii. Using the same criteria for HMB, firstly to identify whether identified HMB presence increased likelihood of iron deficiency and IDA and secondly to evaluate whether iron status is involved in any relationship between identified HMB and the perception that the menstrual cycle disrupts exercise training/performance in an exercising population.

iii. Again, applying the same criteria for HMB identification, to first identify any association between HMB, iron status and fatigue in an exercising population, and second to evaluate whether an association between identified HMB and fatigue is mediated by IDA.

iv. Finally, to evaluate the impact of iron repletion, using a single intravenous iron injection, on exercise and aerobic capacity, haematological markers and subjective measures of fatigue and mood disturbance in iron deficient (serum ferritin ≤ 30 $\mu g \cdot L^{-1}$), non-elite, exercising women.

As outlined in Figure 1.1, the primary hypotheses for this thesis are:

i. When identified using a four-part series (outlined in chapter 3), HMB is common in exercising women, being more common in those who are recreational. and associated with both the perception that the menstrual cycle disrupts exercise training/performance and a history of self-reported anaemia.

ii. Identified HMB increases risk of iron deficiency and IDA, and iron deficiency is on the pathway between identified HMB and the perception that the menstrual cycle disrupts exercise training/performance in exercising women.

iii. The presence of identified HMB and iron deficiency, IDA or anaemia all increase fatigue in exercising women. The relationship between identified HMB and fatigue is mediated by IDA.

iv. Iron repletion in non-elite, iron deficient (serum ferritin ≤ 30 $\mu g \cdot L^{-1}$) exercising women improves exercising and aerobic capacity and reduces fatigue and mood disturbance.

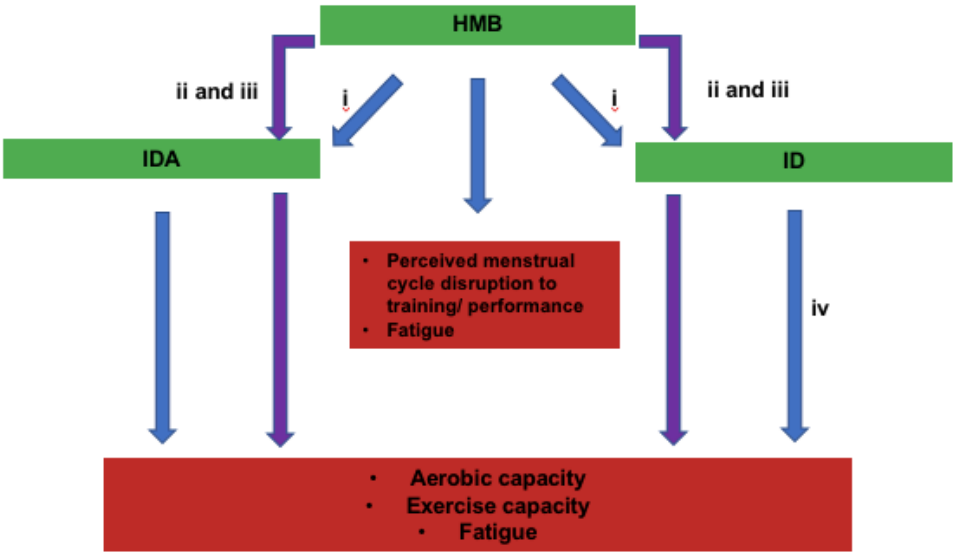

Figure 1.1 - An overview of the hypotheses. i. There is an association between HMB (when identified using a self-report, four-part series) and IDA and ID; ii. IDA and IDNA are on the causal pathway between identified HMB and perceived disruption to exercise training/performance caused by the menstrual cycle. iii. The relationship between HMB and fatigue is mediated by IDA and ID; and iv. ID causes a reduction in aerobic and exercise capacity, while also being associated with increased fatigue.

3 General methodology

Chapter 3 outlines the methods that have been used in more than one study in this thesis. Where methodologies have been applied uniquely to a chapter, these will be expanded upon within the relevant individual chapter. Therefore, here the development of the 'Female Health Questionnaire' will be detailed, including how and why the specific questions were included, in addition to the university ethics approval process.

3.1 Female Health Questionnaire

The 'Female Health Questionnaire' was developed with the primary aim of identifying the presence of heavy menstrual bleeding (HMB) when using the outlined means for diagnosis

in this thesis. Other questions were then asked alongside it so that these findings could be put into context through the consideration of typical exercise training, association of HMB with iron status, awareness of both HMB and iron status and the potential impacts that HMB may have on ability in exercise. The questionnaire was developed by the author of this thesis, and was evaluated by the primary supervisors involved in this PhD. Excluding the criteria used for the diagnosis of HMB, all questions were novel, and included for the reasons outlined in this chapter.

3.1.1 Diagnosis of Heavy Menstrual Bleeding (HMB)

As previously highlighted, the identification of HMB both in research and when patients present in the clinical setting is challenging with no validated criteria. Both the criteria applied by the National Institute for Health and Care Excellence in the UK (NICE) of 'excessive menstrual blood loss which interferes with a woman's physical, social, emotional and/or material quality of life' (26,363), and the objective definition of a loss of 80 mL of blood or more each period (213), are very difficult to assess. There is no validated guidance for the measure and interpretation of impacts on physical, social, emotional and/or material quality of life. The use of quality of life questionnaires have been proposed, however the subjective nature of these can be challenging. While the assessment of blood loss is inevitably difficult, with significant issues surrounding hygiene. Further, blood volumes can vary significantly between individuals, and it could be argued that a specific value can equate to a considerably different proportion of total blood volume in different individuals, affecting the severity of the increased loss. Further, not all menstrual loss is blood, enhancing the potential for measurement inaccuracies (212). Clearly there is a lack of clarity as to whether it is the physical blood loss that should be reflected in the definition or whether it is more perceptual, relating to the impact that it has on the individual. It is also significant to note that the aetiology of HMB is often unknown. Using the pictorial blood assessment chart, an assessment of cause for HMB in university students found only 20.7% of those with HMB (n = 82) had an underlying bleeding disorder or menstrual cycle-related pathology such as polycystic ovary syndrome (Gursel et al. 2014). Suggesting HMB to be a condition in its own entirety.

As a result, more recently, a number of different methods have been suggested to try and provide a more robust means for HMB diagnosis, as recently explained in a Special Report published by Women's Health (364). Yet there is still a lack of agreement. Therefore, it is important to acknowledge that throughout this thesis the criteria applied for identified HMB is not validated.

In this thesis the following criteria was applied for HMB diagnosis, where HMB was identified if a history of two or more symptoms was reported:

1. Flooding through to clothes or bedding
2. Need of frequent changes of sanitary towels or tampons (meaning changes every 2 hours or less, or 12 sanitary items per period
3. Need of double sanitary protection (tampons and towels)
4. Pass large blood clots

This criteria applied a combination of the diagnostic series created by Fraser *et al.* (2015) (28), and the criteria applied by the American College of Obstetrics and Gynaecology (218). Both provide a novel option and are based on the symptoms commonly experienced by individuals reporting HMB in primary care. The criteria created by Fraser *et al.* (2015) includes the following symptoms, and individuals are told to indicate a history of any of the symptoms in the previous year. If two or more are indicated, HMB is diagnosed: (28)

1. Flooding through to clothes or bedding
2. Need of frequent changes of sanitary towels or tampons (meaning changes every 2 hours or less, or 12 sanitary items per period
3. Need of double sanitary protection (tampons and towels)
4. Pass large blood clots

While the criteria utilised by the American College of Obstetrics and Gynaecology (218) includes the following symptoms. The identification of a history of any one of these at any time indicates HMB (218):

1. Bleeding that lasts more than 7 days.
2. Bleeding that soaks through one or more tampons or pads every hour for several hours in a row.
3. Needing to wear more than one pad at a time to control menstrual flow.
4. Needing to change pads or tampons during the night.
5. Menstrual flow with blood clots that are as big as a quarter or larger.

While these symptoms are inevitably subject to individual perception, they go some way to providing a more objective means for the diagnosis of HMB, while also capturing the perceptual elements highlighted by the NICE.

Previous research has found a correlation between the number of sanitary towels and tampons used with total blood loss (365). However, not all research is in agreement with this as the changing of tampons and towels may also be reflective of a women's state of

hygiene, as previously been found (366). Further, this does not capture the size or type of tampon or towel used. Despite being less likely to have an effect in developed countries, the socio-economic status of an individual may also impact upon this and still needs to be considered. The passing of large blood clots, need for double sanitary protection and flooding through to clothes or bedding, while still subjective in nature, are more objective than the number of tampon and towel changes. The need for double protection and flooding through to clothes and bedding indicates excess flow alone.

Limitations with the HMB diagnosis applied in this thesis

Evidently the diagnosis of HMB throughout this thesis and globally has to be interpreted with caution. Essentially, the end goal of HMB identification needs to be established – be it purely the need for further gynaecological assessments, the effects on quality of life or alternatively the potential increased susceptibility to other potential medical conditions or issues such as iron deficiency. For the purposes of this thesis, the key aims are to establish whether symptoms associated with HMB are something experienced by those who exercise, while also considering both the potential impact on iron status in this population and an association between this and the perception that the menstrual cycle disrupts training/performance. These have not previously been considered. If indeed deemed to be an issue amongst this population, and found to be associated with a compromised iron status and self-reported menstrual cycle disruption to exercise training/performance, this tool could be utilised by sports physicians to provide an indication of risk, suggesting when gynaecological and/or haematological input should be advised.

Evidently, it must be acknowledged that the fact that this criteria is novel, means that comparison between prevalence here and previous research is subject to inaccuracy.

Notable other methods for the quantification of blood loss have been tested including the Pictorial Blood Assessment Chart (367), however again this has a subjective element, making effectiveness questionable.

3.1.2 Accompanying questions in the Female Health Questionnaire

Questions 1 and 11 - Age

Firstly, to ensure participants were 18 years or older, there was a requirement to ask for both age and date of birth. On analysis, both were checked for inconsistencies. Age was also asked to enable identification of any associations between this and HMB presence.

Questions 2 and 3 - Performance level and exercise volume of the participants

The total exercise time and personal best times (in the last year) were requested to determine whether there was an association between the performance level of the athlete, in addition to determining whether those who exercised more were less likely to suffer HMB. As highlighted previously, typically amenorrhea and oligomenorrhea are the principle menstrual cycle dysfunctions experienced in those who exercise, other menstrual dysfunctions are often ignored, and perceived to be less prevalent.

Question 4 - Frequency of periods

Participants were asked to report the number of periods they have had in the past year to enable assessment of whether this was associated with HMB presence, in addition to gaining an indication of menstrual cycle patterns within athletes. Again, questioning the existing perceptions of amenorrhea and oligomenorrhea.

Question 5 – Advice for heavy periods

Previous research suggests that HMB awareness is poor, while also often prone to misdiagnosis. Given the association between HMB and negative impacts on quality of life, in addition to the financial burden that is associated with HMB, it is important to assess this. While not directly asking whether an individual is aware about whether they have heavy periods, this question enabled identification of willingness to seek help if perception is that an individual has heavy periods. Significantly this information could be used to help inform future practice, including highlighting the need to put in place interventions to improve education and discussion.

Question 6 - Awareness of anaemia

HMB is associated with an increased risk of anaemia, therefore the capturing of this information will provide an assessment of whether appropriate tests are being performed in clinical care, in addition to explanation of outcome. Further, this will also enable assessment of iron status awareness amongst athletes, in addition to gaining an indication of whether supplementation use is informed.

Question 7 – Use of iron supplementation

Given the increased risk of iron deficiency both in those with HMB and in those who exercise, use of iron supplementation was relevant here. Further, since supplementation in athletes is thought common, this will also provide an indication of usage.

Question 9 - Use of the oral contraceptive pill (OCP)

The oral contraceptive pill (OCP) is used by exercising women for a number of different reasons other than birth control. It is often used as a means to regulate their menstrual

cycle, or to treat amenorrhea, dysmenorrhea, HMB or to relieve other symptoms (226). However, there is little research looking at any other physiological effects of the OCP in this population that could potentially be detrimental to performance. A number of studies have recently been published suggesting that it could result in increased inflammation (368,369), oxidative stress (370), and a reduction in aerobic capacity (371). For the purposes of this research we wanted to capture general prevalence of OCP usage, while also determining if this seems to be a common treatment option used by those with HMB.

Question 10 – perception of whether an individual feels that their menstrual cycle disrupts their exercise training/performance

Given the hypothesised effects that the menstrual cycle can have on performance, it was important to establish whether individuals perceived their menstrual cycle to be disruptive. Any relationship between the indication of this and identified HMB (when identified using the criteria in this thesis) with or without an associated compromised iron status, can then be investigated.

3.1.3 Limitations with the existing 'Female Health Questionnaire'.

There are a number of limitations to the Female Health Questionnaire used in this thesis that will now be explained.

Time frames - there is a disparity in time frames used in this questionnaire. Both question 3 (performance times) and question 4 (number of periods) ask in context of the previous year, where as question 9 asks for current use, whereas the rest of the questions (2, 5, 6, 7, 8 and 10) ask or imply experiences 'ever'. Inevitably this will confound results, making conclusive evaluation problematic, therefore results must be interpreted with caution.

Question 5 – this only asks whether any 'help/advice' was sought, it does not specifically ask from whom this was sought. To gain a better indication of medical understanding and awareness it would be advisable to have directly asked whether medical help/advice had been sought.

Question 6 – this asked whether participants had ever 'had' anaemia, to their knowledge. This is perceptual and does not suggest diagnosis. Therefore, to evaluate this more accurately it would have been better to specifically ask whether they had ever been 'diagnosed' with anaemia.

Question 7 – this question asked whether an individual has supplemented with iron. It does not capture whether supplementation was prescribed. While it is significant to note supplementation use without knowledge of anaemia, and in chapters 5 and 6, supplementation use without a current iron deficiency, it is difficult to evaluate medical practice because it is based on adherence to treatment and perception around need for supplementation (which as previously highlighted is deemed common in the athletic population).

Question 9 – this only captures OCP use, and does not factor in other forms of contraception. It is also important to appreciate that use of hormonal contraception can cause altered bleeding, which could for example induce episodes of heavy bleeding. These are actually withdrawal bleeds and not specific episodes of menstruation, thus confounding study results.

Subjectivity – clearly the subjective nature of these questions must be appreciated and is acknowledged in each chapter.

St Mary's University Twickenham London

UCL

Female Health Questionnaire

The research group at St Mary's University, London in collaboration with University College London are investigating iron status in endurance athletes. One of the key factors considered to impact upon iron status in female athletes is blood loss during the menstrual cycle. This is further exacerbated in those who experience larger blood losses (heavy menstrual bleeders). Currently little research has been conducted in this area, therefore we are conducting a survey-based study firstly to look at the prevalence of menstrual issues amongst female athletes and secondly to investigate how the menstrual cycle affects training and performance. Could all women who read this please answer the survey – not just those who feel they might have an issue with their menstrual cycle.

In order to participate in this study you must be:

- Female
- 18 years or older
- Pre-menopausal

By completing this survey you are giving consent for the information you provide to be included in this study. Your participation is voluntary and is specific to this study, and shall not be taken to imply consent to participate in any subsequent experiment or deviation from that detailed. All information will remain confidential as to your identity, and you may withdraw from the study at any time without reason.

If there is anything you do not understand or wish to ask questions about, please feel free to ask.

Thank you for participating

Do you agree to these terms? YES ☐ NO ☐

1. Age ☐

2. How much time do you spend exercising each week?

	Time (minutes)
Running	
Cycling	
Swimming	
Other	

3. Please specify your personal best times for all/any of the below in the last year:

	Time (minutes)
5km run (inc Parkrun)	
10km run	
Half marathon	
10M TT cycle	
25M TT cycle	
2km row	

4. Approximately how many periods have you had in the last year? ☐

5. Have you ever sought advice/help for heavy periods?

YES ☐ NO ☐

6. To your knowledge, have you ever had anaemia?

YES ☐ NO ☐ DON'T KNOW ☐

7. Have you ever supplemented with iron?

YES ☐ NO ☐ DON'T KNOW ☐

8. Have you ever experienced any of the following? (*tick all that apply)*

Flooding through to clothes or bedding	☐
Need of frequent changes of sanitary towels or tampons *(meaning changes every 2 hours or less, or 12 sanitary items per period)*	☐
Need of double sanitary protection *(tampons and towels)*	☐
Pass large blood clots	☐

9. Do you currently use the oral contraceptive pill?

YES ☐ NO ☐

10. Do you feel that your menstrual cycle disrupts your training/performance?

YES ☐ NO ☐

11. Date of birth: *(dd/mm/yyyy)* ☐

12. We will be conducting more research in this area. If you are happy to be contacted further please provide your email address:

☐

3.2 University ethics approval process

Since the IRONWOMAN trial was to be conducted in the performance laboratory at St Mary's University, Twickenham, all university ethical approval sought in this PhD was through this committee.

St Mary's University have a three-tiered Ethical Application System, as follows:

Level 1 – Low risk research, can be signed off by supervisor and is logged by the ethics rep. and reported to the ethics sub-committee.
Level 2 – Medium risk research, where there is greater potential for harm to participants. This is signed-off by supervisor and sent to the school ethics representative for review. This will then either be approved or will be deemed high risk and will undergo a Level 3 review.
Level 3 – This involves research that is associated with physical or psychological risk. This will be reviewed by the University Ethics Sub-Committee, and the student and supervisor will be invited to attend a review meeting. Research will either be approved, rejected or amendments will be advised. In instances where further ethics approval is required (e.g. NHS ethics approval), this will need to be presented in addition to the application.

The 'Female Health Questionnaire' was approved for use in the studies in this thesis via Level 3 approval. In Chapter 7, the IRONWOMAN Trial was firstly approved via the NHS Ethics Committee (approval number: 15/LO/1570), and it was then approved via the Level 3 route. The clinical trials registration number for this trial was ISRCTN14032359.

In Chapters 5 and 6, while the 'Female Health Questionnaire' was approved via the St Mary's University ethics committee, the blood tests were conducted as part of routine 'well-woman' health appointments. Despite all participants providing written informed consent, the analysis of this data was not initially meant to be for a research study, it was meant to form a retrospective audit, therefore IRB ethical approval was not sought. However more laterally it was decided to analyse this due to the impact that these findings could have.

3.3 Female Health Questionnaire v2

This questionnaire was specifically derived for chapters 5 and 6, and with an opportunity to ask a few more questions, this was slightly longer than version 1. The alterations from version 1 and reasons for asking these are outlined below:

Questions 2 and 3 - height and weight
Information about height and weight were added firstly in order to determine whether these were risk factors for HMB or a compromised iron status, and secondly to determine whether these were confounders. This data was already due to be collected.

Question 4 - Presence of co-morbidities
The presence of co-morbidities have the potential to confound the results increasing risk of HMB and/or iron deficiency so it is important to capture and control for this information if necessary.

Question 5 - premenopausal
Given the association between age and fatigue, participants were required to be premenopausal. Further, in order to investigate the association between HMB and current iron status, it was important to only include premenopausal women.

Addition of question 7 (competition level of participants) and removal of question 3 (exercise performance times) from v.1
In the first version of the questionnaire this question was hard to quantify. The primary aim of question 3 was to gain a better indication of the performance level of the participants, however, with the possibility of there being a language barrier, and given the potential for different sports involvement, and focusing on the end goal of identifying competition level, this question was simplified.

Question 11 – knowledge of iron deficiency
Due to the limitations on total question number in version 1, a history of iron deficiency was not requested. To gain a better understanding of knowledge of iron status, this question was included.

Questions 11, 12 and 13 – knowledge of anaemia and iron deficiency and iron supplementation in the previous 3 months
As current iron status was being measured it was important to gain an understanding of awareness of this through asking this question. Additionally, by capturing current supplementation use it enabled evaluation of unwarranted iron use (particularly if no

knowledge of iron deficiency or anaemia is indicated), in addition to factoring this into analysis as a potential confounder.

Removal of question asking about OCP use
Since this was not factored into analysis in version 1, this was removed.

Question 14 - Do you feel that your menstrual cycle disrupts your everyday lifestyle/exercise performance?
This was slightly altered from the question used in the first version of the questionnaire. The main reason for this was that the group we collaborated with in Singapore wanted to gain a better generic understanding of potential menstrual cycle impact and the initial cohort did not just comprise exercisers. Therefore it is difficult to compare this question to that in the previous version, however, regardless this can still be used to ascertain whether iron status is involved in any association identified.

3.3.1 Limitations with the Female Health Questionnaire v2

A number of the limitations of version 1 of this questionnaire still apply for version 2. Similarly to version 1, the time scales in this questionnaire are inconsistent making causal inferences problematic. The majority of the questions here however are ‘ever’.

St Mary's University Twickenham London

UCL

Female Health Questionnaire

The research group at St Mary's University, London in collaboration with University College London and Vifor Pharma are investigating iron status in endurance athletes. One of the key factors considered to impact upon iron status in female athletes is blood loss during the menstrual cycle. This is further exacerbated in those who experience larger blood losses (heavy menstrual bleeders). Currently little research has been conducted in this area, therefore we are conducting a survey-based study firstly to look at the prevalence of menstrual issues amongst female athletes and secondly to investigate how the menstrual cycle affects training and performance. Could all women who read this please answer the survey – not just those who feel they might have an issue with their menstrual cycle.

In order to participate in this study you must be:

- Female
- 18 years or older
- Pre-menopausal

By completing this survey you are giving consent for the information you provide to be included in this study. Your participation is voluntary and is specific to this study, and shall not be taken to imply consent to participate in any subsequent experiment or deviation from that detailed. All information will remain confidential as to your identity, and you may withdraw from the study at anytime without reason.

If there is anything you do not understand or wish to ask questions about, please feel free to ask.

Thank you for participating

Do you agree to these terms? YES ☐ NO ☐

1. Age ☐
2. Height _____metres
3. Weight _____kg
4. Please state any co-morbidities (e.g. diabetes, heart failure, kidney disease)
   _____
5. Are you premenopausal?

YES ☐ NO ☐

6. How much time do you spend exercising each week?

	Time (minutes)
Running	
Cycling	
Swimming	
Other (please detail exercise type and time)	

7. Competition level (if more than one form of exercise is performed please select the answer to reflect the highest level):

Do not exercise	
Recreational, just for fun	
Take part in races, in a relatively non-competitive manner	
Regional level	
National level	
International level	

8. Approximately how many periods have you had in the last year? ☐
9. Have you ever sought advice/help for heavy periods?

YES ☐ NO ☐

10. To your knowledge, have you ever had anaemia?

YES ☐ NO ☐ DON'T KNOW ☐

If YES, have you experienced this in the last 3 months?

YES ☐ NO ☐ DON'T KNOW ☐

11. To your knowledge, have you ever been deficient in iron?

YES ☐ NO ☐ DON'T KNOW ☐

If YES, have you been deficient in the last 3 months?

YES ☐ NO ☐ DON'T KNOW ☐

12. Have you ever supplemented with iron?

YES ☐ NO ☐ DON'T KNOW ☐

If YES, have you supplemented in the last 3 months?

YES ☐ NO ☐ DON'T KNOW ☐

13. Have you ever experienced any of the following? (*tick all that apply)*

Flooding through to clothes or bedding ☐
Need of frequent changes of sanitary towels or tampons ☐
(meaning changes every 2 hours or less, or 12 sanitary items per period)
Need of double sanitary protection *(tampons and towels)* ☐
Pass large blood clots ☐

14. Do you feel that your menstrual cycle disrupts your everyday lifestyle/exercise performance?

YES ☐ NO ☐

15. Date of birth: *(dd/mm/yyy)* ☐

16. We will be conducting more research in this area. If you are happy to be contacted further please provide your email address:

☐

4 The identified prevalence of heavy menstrual bleeding (HMB) and its association with reported menstrual cycle driven disruption to exercise training/performance in elite and non-elite athletes

4.1 Abstract

Background: Despite the use of a number of different means for diagnosis, HMB has been found to be common in the general population, yet the prevalence of heavy menstrual bleeding (HMB) in exercising women is unknown. HMB is associated with an increased incidence of iron deficiency and iron deficiency anaemia, both of which may have a significant effect on exercise training and performance.

Methods*:* A 'Female Health Questionnaire' was designed incorporating a diagnostic HMB series, demographics, exercise ability data, training status, knowledge of anaemia, iron supplementation and perception of whether the menstrual cycle disrupts exercise training/performance. The survey was conducted in two stages; initially online, advertised via social media, and then repeated via face-to-face interviews with runners registered for the 2015 London Marathon.

Results*:* 789 participants responded to the online survey (median age: 30 years (24 – 37 years), and 1073 completed the survey at the marathon (median age: 33 years (27 – 40 years). Utilising a diagnostic series, HMB was identified in half of those online (54%), and by more than a third of the marathon runners (36%). Surprisingly, HMB was also identified to be prevalent amongst elite athletes (37%). Overall, 32% of exercising women reported a history of anaemia, and 50% had previously supplemented with iron. Only a minority (22%) had sought help/advice for heavy periods.

Conclusions*:* When using this diagnostic series, HMB was found to be highly prevalent in exercising women, associated with self-reported anaemia, increased use of iron supplementation and a perceived disruption to exercise training/performance caused by the menstrual cycle. Further research is needed to investigate the impact of HMB, iron deficiency and anaemia in exercising women.

4.2 Introduction

Heavy menstrual bleeding (HMB) is thought common, affecting approximately a quarter of the general female population (28). By definition, HMB can negatively impact physical, emotional and social quality of life and reduce work capacity (26,372), but individuals are reluctant to seek help, with only a small minority (6%) of women seeking medical advice annually (373). It was estimated in 2010 that HMB cost the National Health Service of the UK more than £50 million (374). Yet, as highlighted in both chapters 2 and 3, the absence of a unified and validated identification criteria has made historical diagnosis and comparison between different populations problematic. The recent advent of more objective criteria, such as the four-part diagnostic criteria by Fraser *et al.* (28), and the criteria outlined by the American College of Obstetrics and Gynaecology (218), makes evaluation significantly easier.

With the primary research focus on menstrual dysfunction in exercising women being on amenorrhoea and oligomenorrhea, other dysfunctions are seldom considered (49,54). The prevalence of HMB, and the impact it may have on performance in those who exercise is unknown. This group already have an increased susceptibility to iron deficiency through a greater erythropoietic drive, increased iron losses, the post-exercise inflammatory response and often a limited dietary iron intake (178,180,186,375-379). Considering that menstrual blood loss in those with HMB is typically five to six times greater than those without (216), it would seem prudent to suggest that iron deficiency risk may be exacerbated in those who both exercise and have HMB. This clearly has potential to inhibit exercise performance.

Despite increasing anecdotal reports (18-20), the causes for the impact of the menstrual cycle on exercise performance are unknown. HMB could provide one explanation.

4.2.1 Study aims

1. The primary aim was to identify the prevalence of HMB amongst exercising women when adopting a self-created criteria (outlined in chapter 3), and to establish whether it differs according to exercise performance level (i.e. between elite athletes and recreational exercisers);
2. The secondary aim was to determine whether HMB was related to a perceived disruption to exercise training/performance caused by the menstrual cycle.

4.2.2 Study hypotheses

1. HMB, when identified using the outlined criteria (chapter 3) is common in those who exercise.

2. Identified HMB is more common in recreational than elite athletes.
3. Identified HMB is associated with the reporting that the menstrual cycle disrupts exercise training/performance

4.3 Materials and Methods

4.3.1 Ethics and participant consent

This research was approved by the St Mary's University Ethics Committee. The participants were informed that by indicating that they agree to the terms and completing the survey they have provided written informed consent for their information to be used in this study.

4.3.2 Inclusion criteria

The inclusion criteria were:

- Female;
- Aged ≥ 18 years;
- Pre-menopausal;
- Undertake regular exercise (≥ 90 minutes/week).

4.3.3 Participants

Stage 1 - Participants were exercising women either recruited online, and through social media, including Twitter, Facebook, online blogs and forums, university newsletters, websites, or recruited by word of mouth.

Stage 2 – Participants were women attending the 2015 Virgin Money London Marathon Exhibition prior to competing in the marathon.

4.3.4 Study design

The 12-item 'Female Health Questionnaire' as described in Chapter 3, including free-text and yes-no polar questions was used and designed to take 2-3 minutes to complete. The four-part criteria was used to identify those with a self-reported history of the identified HMB symptoms and information was collected on age, recent 'personal best' sports performance times (running, cycling, swimming and rowing), training volume, previous self-reported knowledge of a history of anaemia and iron supplementation, the menstrual cycle and disruptions caused by it, and current oral contraceptive pill (OCP) use.

4.3.4.1 Stage 1 - online questionnaire

Stage 1 aimed to identify whether HMB, when using this series for diagnosis, was a problem in exercising women, and therefore whether to continue further investigation. The questionnaire was administered online between 22 January 2015 and 19 May 2015. A link

was provided to the internet-based survey in addition to some brief information about the research.

4.3.4.2 Stage 2 - marathon exhibition questionnaire

Stage 2 aimed to establish unbiased prevalence of HMB. Women registering for the 2015 London Marathon at the pre-event exhibition were surveyed using paper versions of the 'Female Health Questionnaire' as used in stage 1. No bias was applied when selecting women to question and to avoid a response bias a scripted standardised introduction was made providing no specific information about the context of the survey. To ensure maximum response yield, surveys were completed at the time of asking. The questions and format of the paper copies used at the Exhibition were identical to the online survey to maintain equivalency and reliability of this mixed mode strategy (380).

4.3.5 Data Analysis

All stage 1 data were downloaded into an excel spreadsheet. stage 2 data were inputted anonymously into an excel spreadsheet. The statistical analysis was completed using a predictive analytic software statistics computer package (IBM SPSS Statistics for Macintosh, Version 21.0, Armonk, NY: IBM Corp.). Statistical significance was set at $p < 0.05$.

All data were tested for normality using a Shapiro-Wilk test. Data were analysed descriptively to summarise the prevalence of HMB (identified using the outlined criteria), known anaemia, iron supplementation, the seeking of advice/help for heavy periods, age, average total weekly exercise time, 5 km personal best time in the last year (where provided), current OCP usage, number of periods in the last 12 months and the perceived disruption of the menstrual cycle on exercise training/performance in both stages 1 and 2. Chi-squared tests were used to determine whether there was an association between HMB and self-reported menstrual cycle disruptions to training/performance, previous seeking of advice/help for heavy periods, a reported history of anaemia and iron supplementation, and OCP use in each of stages 1 and 2 and amongst elite athletes. Mann-Whitney U tests were used to determine whether age, average total weekly training volume, 5 km personal best time (where given), and the reported number of periods per year were related to identified HMB presence.

After combining both groups, a sub-analysis was conducted to separate out elite athletes using the following criteria: 5 km ≤ 18 minutes, 10 km ≤ 36 minutes, half marathon ≤ 80 minutes, 2 km row ≤ 7 minutes: 45 seconds (elite running criteria defined using the 2015 'Great Run' series definitions of 'elite', rowing criteria defined by GB Rowing physiologist).

Chi-squared tests were used to determine whether there was a difference in identified HMB, self-reported menstrual cycle disruptions to training/performance, a reported history of anaemia and iron supplementation, previous seeking of advice/help for heavy periods, a reported history of anaemia and iron deficiency, and current OCP use.

Both linear regression and a Kruskal-Wallis H test with a *post hoc* analysis and Bonferroni correction were used to determine whether 5 km personal best time was linked to the number of HMB symptoms reported, and Mann-Whitney U tests were used to determine whether participant performance level (based on 5 km personal best) was related to identified HMB incidence.

A multivariable binomial logistic regression was then used to assess the relationship between identified HMB, the reporting that the menstrual cycle disrupts training/performance and a reported knowledge of anaemia.

4.4 Results

4.4.1 Participant characteristics - stage 1

A total of 789 surveys were completed online. Participant characteristics are shown in Table 4.1. More than half (54.1%) of the participants were identified to have a history of HMB (identified using the outlined criteria - reporting experiencing two or more of the diagnostic HMB series at some point; Table 4.1). While 55.4% stated that their menstrual cycle disrupts their training/performance (Table 4.1).

Table 4.1 – Participant characteristics, including identified prevalence of heavy menstrual bleeding (HMB).

	Stage 1 (n=789)	**Stage 2** (n=1073)	**Elite athletes** (n=90)
Age (years)	30 (24 - 37)	33 (27 – 40)	27 (24 – 33)
Weekly exercise volume (min)	360 (212.5 – 530)	300 (240 – 450)	580 (420 – 820)
5 km PB (min:s) – previous 12 months	23:55 (20:00 – 27:32)	25:53 (23:00 – 28:00)	17:26 (16:31 – 17:50)
Identified HMB	54.1% (427)	35.5% (381)	36.7% (33)
Reported menstrual cycle disruption to training/performance	55.4% (437)	31.7% (340)	51.1% (46)
Seeking of advice/help for heavy periods	24.1% (190)	21.1% (226)	23.3% (21)
Knowledge of a history of anaemia	38.4% (303)	28.0% (300)	52.2% (47)
History of iron supplementation	57.2% (451)	45.3% (486)	78.9% (71)
Periods/year	12 (8 – 12)	12 (4 – 12)	11 (4 – 12)
Current OCP use	31.9% (252)	36.4% (391)	36.7% (33)

PB – personal best, HMB – heavy menstrual bleeding, OCP – oral contraceptive pill
Values are median (IQR).

4.4.2 Participant characteristics - stage 2

One thousand and ninety-one (1091) face-to-face surveys were collected and inputted into the Bristol Online Survey platform manually by the lead investigator and an assistant. Those with missing data or those completed by women who did not meet the inclusion criteria were excluded, resulting in a final sample size of 1073 for further analysis. Eight individuals declined to complete the survey once they had read the study information, and 61 declined answering the survey prior to being informed about the content typically citing a lack of time. Therefore, in stage 2, the survey was fully completed by 94% of randomly approached female marathon runners. Participant characteristics are shown in Table 4.1, which highlights the prevalence of HMB (when identified using the outlined criteria) in women undertaking the 2015 London Marathon to be 35.5% (Table 4.1). Overall nearly one third (31.7%) reported that their menstrual cycle disrupts their exercise training/performance (Table 4.1).

4.4.3 Results from stages 1 and 2

Across both stages, those identified with a history of HMB were more than three times more likely to cite that their menstrual cycle disrupts their exercise training/performance (stage 1: OR: 3.54; 95% CI: 2.64, 4.76; $p<0.0005$; stage 2: 3.21; 95% CI: 2.45 – 4.20; $p<0.0005$; Table 4.2). In both stages, those with a history of HMB were found to be older (stage 1: 31 (24 – 35) years vs. 29 years (24 – 39); U = 87030; $p<0.0005$; stage 2: 36 (29 – 40) years vs. 31 (27 – 38) years; U = 152120; $p<0.0005$; Table 4.2), but there was no difference in total weekly exercise volume (stage 1: 360 (240 – 540); U = 75160; $p=0.51$, stage 2: (300 (240 – 480) minutes vs 345 (240 – 420) minutes; U = 128639; $p=0.51$; Table 4.2) between those who did and did not report HMB. However, those reporting HMB had slower 5 km personal best times in both stages 1 (24 min: 05s (20:40 – 28:00) vs 22 min :59s (19:10 – 26:09); U = 36714; $p=0.002$) and 2 (26min :00s (23:25 – 28:04) vs 25 min: 00s (22:18 – 28:00); U = 57633; $p=0.008$; Table 4.2).

Table 4.2 - The perception that the menstrual cycle disrupts training/performance, seeking of help/advice for heavy periods, knowledge of a history of anaemia and iron supplementation in those who have and have not met the heavy menstrual bleeding HMB criteria, when identified using the outlined criteria.

	Stage 1				**Stage 2**				**Elite athletes**			
	HMB n = 427	**No HMB** n = 362	**OR** (95% CI)	**p-value**	**HMB** n = 381	**No HMB** n = 692	**OR** (95% CI)	**p-value**	**HMB** n = 33	**No HMB** n = 57	**OR** (95% CI)	**p-value**
Reported menstrual cycle disruption to training/ performance	69.3%*** (296)	39.0% (141)	3.54 (2.64 – 4.76)	<0.0005	48.3%*** (184)	22.5% (156)	3.21 (2.45 – 4.20)	<0.0005	66.7% (22)*	42.1% (24)	2.75 (1.12 – 6.73)	0.03
Seeking of advice/help for heavy periods	37.2%*** (159)*	8.6% (31)	6.34 (4.17 – 9.61)	<0.0005	44.6%*** (170)	8.1% (58)	9.15 (6.51 – 12.85)	<0.0005	42.4% (14)***	12.3% (7)	5.26 (1.84 – 15.04)	0.001
Knowledge of a history of anaemia	43.1%** (184)	32.9% (119)	1.55 (1.16 – 2.07)	0.003	38.1%*** (145)	22.4% (155)	2.13 (1.62 – 2.80)	<0.0005	57.6% (19)	49.1% (28)	1.41 (0.59 – 3.33)	0.44
History of iron supplementation	61.4%* (262)	41.9% (189)	1.45 (1.09 – 1.93)	0.01	55.1%*** (210)*	39.9% (276)	1.85 (1.44 – 2.38)	<0.0005	81.8% (27)	77.2% (44)	1.33 (0.44 – 3.91)	0.60
Current OCP use	24.6%*** (105)	40.6% (147)	2.10 (1.55 - 2.84)	<0.0005	25.5%*** (97)	42.5% (294)	2.16 (1.64 – 2.85)	<0.0005	30.3% (10)	42.1% (24)	1.62 (0.65 - 4.03)	0.29
	Stage 1				**Stage 2**				**Elite athletes**			
	HMB n = 427	**No HMB** n = 362	**U**	**p-value**	**HMB** n = 381	**No HMB** n = 692	**U**	**p-value**	**HMB** n = 33	**No HMB** n = 57	**U**	**p-value**

Age (years)	31 (24 – 35)***	29 (24 – 39)	87030	0.002	36 (29 – 40)***	31 (27 – 38)	152120	<0.0005	25.5 (22 – 34.5)	26 (24 – 30.5)	1003	0.71
Weekly exercise volume (mins)	360 (240 – 540)	360 (240 – 525)	75160	0.51	300 (240 – 480)	345 (240 – 420)	128639	0.51	590 (465 – 720)	530 (420 – 850)	913	0.96
5 km PB (min:s) – previous 12 months	24:05*** (20:40 – 28:00)	22:59 (19:10 – 26:09)	36714	0.002	26:00 (23:25 - 28:04)***	25:00 (22:18 – 28:00)	57633	0.008	17:21 (16:34 – 17:55)	17:28 (16:31 – 17:46)	665	0.29
Periods/year	12 (9 – 12)***	12 (6.25 – 12)	84643	<0.0005	12 (6 – 12)	12 (4 – 12)	125151	0.23	11 (8 – 12)*	9 (2 – 12)	1091	0.04

HMB – heavy menstrual bleeding, OR – odds ratio, CI – 95% confidence interval for the odds, U – Mann Whitney U test statistic, PB – personal best.

Values are median (IQR). Where percentages are given, the number in the brackets is n.

Significant difference between those reporting and not reporting HMB within each group are shown as follows ***p < 0.005, **p < 0.01, *p < 0.05.

The elite athletes are represented twice in this table – both within the stage data and as a separate group.

Across both stages, a known history of anaemia was reported by 603 participants (32.4%), while 1049 (56.3%) specified that they either did not have this or were unsure whether they have had this condition. Those meeting the HMB criteria were more likely to report a historical knowledge of anaemia (stage 1: 43.1% vs 32.9%; OR: 1.55; 95% CI: 1.16 – 2.07; *p<0.0005*; stage 2: 55.1% vs 39.9%; OR: 2.13; 95% CI: 1.62 – 2.80; *p<0.0005*; Table 4.2). Use of iron supplementation was also more common in those identified with HMB (stage 1: 61.4% vs 41.9%; OR: 1.45 (1.09 – 1.93); *p=0.*01; stage 2: 55.1% vs 39.9%; OR: 1.85; 95% CI: 1.44 – 2.38; *p<0.0005*; Table 4.2). Less than a quarter of all surveyed reported having sought advice/help for heavy periods (22.3%), with this increasing in those who met the HMB criteria (stage 1: 37.2% vs 8.6%; OR: 6.34 (4.17 – 9.61); *p<0.0005*; stage 2: 44.6% vs 8.1%; OR: 9.15, 95% CI: 6.51 – 12.85; *p<0.0005*; Table 4.2). In stage 1, those identified with HMB cited more periods over the last year than those without (12 (9 - 12) vs 12 (6.25 – 12); U = 84643; *p<0.0005*; Table 4.2), however there was no difference in number of periods in the last year reported by participants with and without HMB in stage 2 (12 (6-12) vs 12 (4-12); U = 125151; *p=0.23*; Table 4.2). In both stages 1 and 2, those identified to not have a history of HMB were approximately twice as likely to use the OCP (40.6% vs 24.6%; OR: 2.10, 95% CI: 1.55 - 2.84; *p<0.0005*, stage 2: 42.5% vs 25.5%; OR: 2.16; 95% CI: 1.64 – 2.85; *p<0.0005*; Table 4.2) than those with HMB.

4.4.4 Elite Athletes

When a sub-analysis of the elite athletes identified in both groups was conducted, 36.7% met the HMB criteria, with more than half (51.1%) indicating that their menstrual cycle disrupts their training/performance. The presence of HMB increased the odds of reporting this by 2.75 times (95% CI: 1.12 – 6.73 *p=0.03*; Table 4.2).

4.4.5 Elite vs non- elite athletes

The prevalence of HMB was as common in elite athletes as the rest of the participants (*p=0.19*; Table 4.3). Elite athletes were also as likely to report that their menstrual cycle disrupts their training/performance, to have sought help for heavy periods (*p=0.83*) and to currently use the OCP (*p=0.67*). However, elite athletes were more likely to report knowledge of a history of anaemia (*p<0.0005*), and to have supplemented with iron than those who were not elite (*p<0.0005*; Table 4.3).

Table 4.3 – A comparison between elite and non-elite characteristics.

	Elite n = 90	**Non-elite** n = 1772	**Chi-squared**	**p-value**
HMB	36.7% (n=33)	43.7% (n=775)	$X_2 = 1.73$	0.19
Reported disruption to training/ performance	51.1% (n=46)	41.2% (n=731)	$X_2 = 3.44$	0.06
Knowledge of a history of anaemia	52.2% (n=47)	31.4% (n=557)	$X_2 = 16.92$	<0.0005***
History of iron supplementation	78.9% (n=71)	48.9% (n=867)	$X_2 = 30.81$	<0.0005***
Current OCP use	36.7% (n=33)	34.5% (n=611)	$X_2 = 0.18$	0.67
Seeking of advice/help for heavy periods	23.3% (n=21)	22.3% (n=173)	$X_2 = 0.049$	0.83

HMB – heavy menstrual bleeding, OCP – oral contraceptive pill

Values are percentage (n).

Significant difference between elite and non-elite athletes are shown as follows ***p < 0.005, **p < 0.01, *p < 0.05.

Association between HMB and performance outcomes

There was a relationship between 5 km personal best time in the last year and the number of HMB symptoms reported to have been experienced, each additional symptom reported was associated with a 16.9 second increase in reported personal best time (B = 16.93; 95% CI: 4.88 – 28.98; *p=0.006*).

When simply comparing those meeting and not meeting the criteria for identification of HMB, median 5 km times were significantly different (25 min: 0 s vs. 24 min: 24 s; U = 184312; *p=0.002*). When 5 km personal best times were divided into quartiles (Q1 being the fastest athletes), a significant difference was seen in the number of HMB symptoms reported by participants in the groups ($\chi^2(3) = 13.981$, *p=0.003*). A post-hoc analysis demonstrated that there was a significant difference between Q1 and Q4 (*p=0.001*; Figure 4.1).

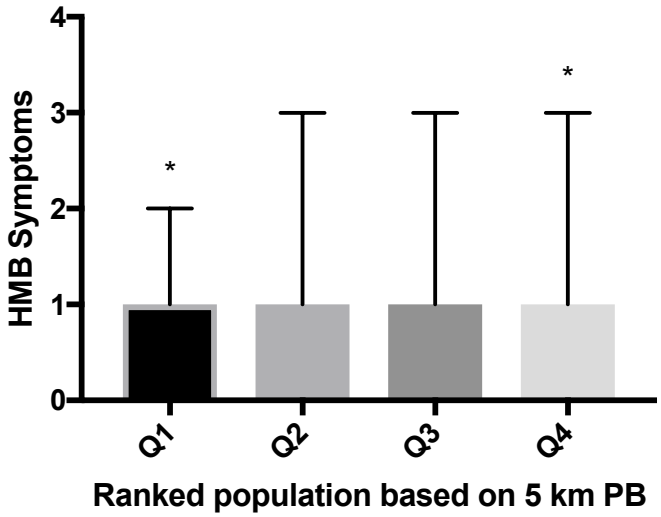

Figure 4.1 – Reported number of symptoms of in the outlined heavy menstrual bleeding (HMB) diagnostic series across participants when ranked based on 5 km personal best times. Q1 represent those with the fastest times, Q4 the slowest.

Values are median ± IQR.

Significant differences between groups are shown as follows: * p = 0.001.

Q – quartile, PB – personal best.

4.4.6 Association between HMB, a reported knowledge of a history of anaemia and disruptions to exercise training/performance caused by the menstrual cycle

In an unadjusted logistic regression model, those identified with HMB were 3.74 times more likely to report that their menstrual cycle disrupts their exercise training/performance (95% CI: 3.08 – 4.54; *p<0.0005*; Table 4.4) compared to those not identified with HMB. This relationship was slightly attenuated when adjusting for a historical knowledge of anaemia (OR: 3.53, CI: 2.90 – 4.29; *p<0.0005*; Table 4.4). Accordingly, there is evidence to suggest that those reporting historical anaemia knowledge are also more likely to report exercise training/performance disruption caused by their menstrual cycle (OR: 1.64; CI: 1.33 – 2.02; *p<0.0005*; Table 4.4). After controlling for covariates, (age, 5 km personal best (PB), typical weekly exercise volume and a history of iron supplementation), there was still a significant relationship between identified presence of HMB and reported disruptions to training/performance caused by the menstrual cycle, this was however minimally reduced (OR: 3.16; CI: 2.46 – 4.05; *p<0.0005*). However, the relationship between menstrual cycle driven disruption to exercise training/performance and knowledge of a history of anaemia was abolished (OR:1.08, CI: 0.79 – 1.48; *p=0.62*; Table 4.4).

Table 4.4 - The unadjusted and adjusted results from the logistic regression of the perception that the menstrual cycle disrupts exercise training/performance.

	Unadjusted model			Adjusted for anaemia history only			Adjusted for multiple covariates		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
HMB	3.74	3.08 – 4.54	<0.0005***	3.53	2.90 – 4.29	<0.0005***	3.16	2.46 – 4.05	<0.0005***
Knowledge of a history of anaemia				1.64	1.33 – 2.02	<0.0005***	1.08	0. 79 – 1.48	0.62
Age							1.00	0.98 – 1.01	0.57
5 km PB (last 12 months)							1.00	1.00 – 1.00	0.59
Typical weekly exercise volume							1.00	1.00 – 1.00	0.65
History of iron supplementation							1.59	1.17-2.15	0.003***

HMB – heavy menstrual bleeding, PB – personal best time, OR – odds ratio, CI – 95% confidence interval for the odds

Significant interaction of the variable in the model are shown as follows: ***p < 0.005, **p < 0.01, *p < 0.05.

Anaemia and iron supplementation

Across the total population, 50.3% (937 participants) reported having previously supplemented with iron, while 32.4% (603 participants) reported knowledge of having had anaemia. A total of 20.6% (384 participants) reported supplementing with iron without knowledge of a history of anaemia. This increased in elite athletes to 26.7% (24 participants). Further, of those who said they were unaware of whether they had ever had anaemia (210 participants), 50.5% (106 participants) reported that they have taken iron supplementation.

4.5 Discussion

This is the first study to identify that HMB when defined using self-reported responses to the outlined criteria, is a common problem amongst exercising women. Stage 1 of this research was used to ascertain whether HMB, when identified using this means, was a prevalent issue experienced by exercising women. It is acknowledged that this online survey was likely to be biased because women with menstrual cycle issues were more likely to complete the questionnaire, however with 54.1% meeting the HMB criteria this demonstrated that this is a significant problem within this populace when diagnosed by this means. To obtain unbiased prevalence data a large study, (stage 2) incorporating a number of controls to prevent bias, was conducted at the 2015 London Marathon Exhibition. This found that 35.5% of marathon runners met the outlined criteria for a history of HMB, therefore confirming the outcome in stage 1 that this is a common problem amongst exercising women when identified using this means. Utilising a different rule for diagnosis, HMB has previously been shown to affect more than a quarter of the general female population (28), but this is the first study to investigate prevalence amongst exercising women, regardless of diagnostic tool.

Only 43.1% and 38.1% of those with HMB from stages 1 and 2 had sought help/advice for heavy periods. HMB and discussion around the menstrual cycle is deemed a sensitive subject by some, and women may not feel comfortable discussing it with medical professionals or amongst peers, meaning that they may not know that their situation is abnormal. In the case of von Willebrand disease for example, a condition characterised by heavy bleeding, women typically report normal blood flow (381,382). Being a genetic condition, family members are also likely to have von Willebrand disease, and therefore a similar excess of blood loss, so it is thought normal. Alternatively, others may have just adjusted to living with it, feel that there is no solution, or that they don't have time to seek advice. Some also feel it is a normal cleansing process (383). This highlights the need for increased HMB awareness, but also highlights the need for a universally recognised means for diagnosis. However, this does support previous research which has demonstrated that almost half of those with HMB who have sought help did not have HMB confirmed (28), and the results of an audit conducted by the Royal College of Obstetricians showed that once diagnosed one third were not given treatment in primary care (384). This suggests that an increased awareness of HMB prevalence, associated symptoms and treatment options is required amongst medical professionals. Further, a review of treatment methods has shown there to be considerable variation in the treatment procedures and medications used, highlighting the need for further research and again, improved awareness (385). This is particularly significant given the large economic burden associated with HMB, identified both in the UK and the USA (386). Significantly, an earlier

study only found 20.7% of the participants identified with HMB to have an underlying bleeding disorder or menstrual cycle related pathologies (Gursel et al. 2014). In light of this, an increased awareness and future research into treatment options and underlying aetiology could result in significant beneficial financial implications.

The finding in this study that those meeting the HMB criteria were more likely to report knowledge of a previous diagnosis of anaemia than those who did not meet the HMB criteria is consistent with previous research, highlighting the increased iron deficiency and anaemia risk associated with HMB (19,20). There is potential for this to be greater as more than half of all respondents said that they were unaware of whether they have been anaemic. In the general population IDA has been shown to affect two thirds of women with HMB (26). Considering the increased iron losses (178,186,387,388), and the potential for reduced iron absorption (199) in those who exercise, the prevalence of iron deficiency and IDA in those identified with HMB in this group may be even greater.

Approximately one in three participants in stage 2, and more than half of those in both stage 1 and within those defined as 'elite' reported that their menstrual cycle disrupts their exercise training/performance. While this result in stage 1 is inevitably subject to bias, the results in stage 2 highlight that this is a common perception amongst exercisers. The identified presence of HMB increased the likelihood of reporting this, and those identified with HMB in stage 2 were more than three times more likely to cite this than those not identified with HMB. It could be hypothesised that an underlying iron deficiency with or without anaemia could be driving this perceived effect. When assessing the total population, and looking at both identified HMB and knowledge of a history of anaemia together, those who identified anaemia knowledge were also more likely to report that their menstrual cycle disrupts their training/performance, supporting this hypothesis. However, the addition of this to the relationship between HMB and menstrual cycle-related disruptions to training/performance marginally attenuated this association, potentially countering this. Further, when additional confounders were added, the relationship between knowledge of a history of anaemia and citations that the menstrual cycle disrupts training/performance became insignificant ($p=0.62$). However, in light of the potential for confusion regarding anaemia and iron deficiency, the lack of historical awareness of iron status, and the absence of haematological measures, this clearly warrants further research. It is also important to gain a better understanding of how iron deficiency without anaemia can have an impact on performance, as research is currently conflicting as will be addressed later in this thesis.

Interestingly, more than half of the population reported supplementing with iron, with this being substantially higher amongst those who were defined as elite ($p<0.0005$). Many elite athletes routinely supplement with iron, and this was shown here with 78.9% reporting supplementation. Further, more than half of those who reported they were unaware of whether they have ever had anaemia, indicated that they have supplemented with iron. Coaches often encourage supplementation without prior knowledge of iron status due to the unfounded but common belief that iron deficiency is rife and supplementation may benefit performance. Indeed, one in five of the total population and one in four of the elite population who took iron supplementation did not report a historical knowledge of anaemia. While it must be appreciated that they could have had iron deficiency alone, meaning that it is difficult to draw definitive conclusions from this observation, typically many have a limited understanding of the difference between anaemia and iron deficiency causing them to be grouped together. Significantly here, less than half of those identified with HMB have sought help/advice for heavy periods, therefore it is necessary to raise awareness as while the effects of iron deficiency are yet to be conclusively elucidated, the impacts of IDA on both general wellness and exercise performance are well established as previously highlighted (386).

The sub-analysis from the elite athlete sample identified more than one third to have a history of HMB, with prevalence being no different to the non-elite population here and actually being greater than or similar to that identified in the general population – diagnosed using a different diagnostic series (28). This is somewhat surprising given the historical research focus on the female athlete triad and the new term 'Relative Energy Deficiency in Sport' – RED-S, particularly in elite athletes (224,389). However, it must be appreciated that some of this group were from stage 1, potentially overstating prevalence, and that different criteria were used to identify prevalence in the general population. It is well documented that elite female athletes, particularly endurance athletes are susceptible to amenorrhea and oligomenorrhea often as a result of a relative energy deficiency associated with a high training volume (9,31,390). It could therefore be hypothesised that increases in training volume would equate to increased risk of amenorrhea or oligomenorrhea, potentially decreasing HMB incidence, but this was not the case in this study with no identified relationship between total number of minutes exercised per week and identified HMB presence. However, the lack of a timescale for the presence of HMB symptoms makes conclusions to this avail problematic. Those with faster 5 km personal best times were however less likely to have a HMB history. The slower times seen in Q4 where HMB prevalence was higher when compared to Q1 could be caused by an increased incidence of IDA in Q4, which is impacting upon performance, alternatively increased rates of amenorrhea in Q1 could reflect the lower HMB incidence seen here.

However, these differences were only marginal, and further research is required before forming a definitive conclusion. This study suggests that other menstrual cycle issues in athletes across all levels may be commonplace, highlighting the need for further and more general research across other menstrual cycle dysfunctions in exercising women as they clearly have potential to negatively impact performance.

4.5.1 Limitations

There are a number of limitations of this study. Firstly, the self-reported nature of this questionnaire could have resulted in inaccurate data, however the HMB diagnostic criteria does not lean itself to comparison bias, but it must be appreciated that there is no universally recognised diagnosis for HMB. Secondly, stage 2 data was only collected in marathon runners, which may not be representative of other running events and sports. It has been shown that exercise increases susceptibility to iron deficiency (36), however blood parameters including markers of iron status have not been measured therefore associations between HMB identification and awareness of anaemia are subject to inaccuracy. There are a number of limitations associated with the Female Health Questionnaire as outlined in chapter 3 that need to be considered. Essentially, there are inconsistencies in the time periods used for assessment, performance times are within the last year, OCP use is current, where as the majority of other questions are based on any time in history. Additionally, any underlying causes for HMB presence were not captured, and the presence of illnesses (e.g. endometriosis) or the use of medication was not recorded, these could increase the likelihood of participants meeting the criteria for HMB diagnosis. Further, only OCP use was asked, use of other types of hormonal contraceptives were not requested. To obtain standardised performance comparisons a means for knowing finishing time in the marathon would strengthen ability to determine any relationships between participant performance level and HMB presence. Relationship between BMI and HMB presence could also be explored. Further studies are required to address these limitations.

4.6 Conclusions

This study has demonstrated that utilising the pre-defined diagnostic series, HMB, when identified by the self-report of symptoms, is common in the exercising population. Surprisingly, when utilising this diagnostic means it was as common amongst elite athletes as recreational level exercisers. HMB was associated with a known history of anaemia, iron supplementation, disruptions of the menstrual cycle to exercise training/performance and slower performance times. Further research is however needed to explore the association between HMB, iron deficiency and exercise performance, firstly to confirm this

association with haematological markers, and secondly to determine whether the disruption to exercise training/performance could be due to a deficiency of iron. The lack of advice/help for heavy periods sought by the participants in this study suggests that women may not feel or appreciate that this is a problem, have learnt to cope with it or do not want to discuss it, suggesting that more research, education and awareness is needed. Iron supplementation amongst athletes is very common, particularly amongst those categorised as elite, often despite prior knowledge of iron status.

To conclude, regarding the outlined hypotheses for this chapter, when identified using the outlined criteria, HMB was found to be prevalent in those who exercise, therefore the first hypothesis can be accepted. However, the second hypothesis that identified HMB is more common in recreational athletes was not found. Finally, the third hypothesis can also be accepted as those identified with HMB were more likely to report that their menstrual cycle disrupts their training/performance.

4.7 Future Perspectives

This study clearly highlights that when identified using this means, HMB is a common problem in exercising women. Due to the potentially significant poor awareness of this menstrual cycle dysfunction, and the evident potential it has to affect performance, education needs to be improved. Medical professionals need to be made aware of its perhaps somewhat surprising prevalence in this population, and athletes should be alerted to the typical symptoms. Treatment options also need careful consideration. Since the analysis of elite athletes was from both study groups, future research must find an elite population in its own entity to gain another indication of prevalence.

4.8 Acknowledgements

The author would like to thank Dr Courtney Kipps and the London Marathon Medical Committee for their cooperation in facilitating access to the London Marathon Exhibition.

4.9 Author Contributions

The author wrote the ‘Female Health Questionnaire’. The questionnaire was checked by the primary supervisors prior to use. In stage 1 of this study, the author advertised an online link to the questionnaire via Twitter and Facebook and wrote on relevant exercise and training specific forums and websites. The author was also granted access to have it publicised in the university newsletter. In stage 2 of this study, the author surveyed 1091

women across four days at the London Marathon Exhibition in 2015, getting them to complete a paper survey. The author then entered this data onto the Bristol Online Survey platform, providing a second check for errors. The author conducted the full statistical analysis.

5 The association between identified heavy menstrual bleeding and iron status, and the association of these with the perception that the menstrual cycle disrupts exercise training/performance in exercising women.

5.1 Abstract

Background: In chapter 4, when utilising an outlined diagnostic series for diagnosis (chapter 3), HMB was identified to be common in exercisers in the United Kingdom, while also associated with the perception that the menstrual cycle disrupts exercise training/performance and a historical knowledge of anaemia. The mechanism by which HMB may disrupt exercise is unknown, but could be caused by a compromised iron status. Iron status, alongside HMB presence has not previously been established in exercising women, where the menstrual cycle is frequently deemed to disrupt exercise training/performance. With the previous chapter significantly also highlighted a poor awareness of iron status, with few identified with HMB reporting to have sought help/advice for heavy periods.

Methods: Healthy, exercising and regularly menstruating women in Singapore (n=271; median age: 38 years (29 – 43 years), weight: 57.0 kg (50.2 - 63.0 kg), height: 1.60 m (1.56 - 1.64 m), BMI: 22.1 $kg \cdot m^{-2}$ (20.0 - 24.3 $kg \cdot m^{-2}$)) completed a 'Female Health Questionnaire' and had blood sampling at routine healthcare appointments.

Results: Overall, HMB was identified in 60 women (22.1%) and 40 (14.8%) were diagnosed with IDA (haemoglobin < 12 $g \cdot dL^{-1}$ and serum ferritin < 15 $\mu g \cdot L^{-1}$), while 82 (30.3%) had 'severe' iron deficiency (serum ferritin < 15 $\mu g \cdot L^{-1}$), and 130 (48.0%) 'moderate' iron deficiency (serum ferritin < 30 $\mu g \cdot L^{-1}$). Women identified with HMB were three times more likely to suffer IDA (28.3% vs 10.9%; OR: 3.23, CI: 1.59 – 6.57; *p<0.0005*) and more than twice as likely to suffer severe (48.3% vs 25.1%; OR: 2.79, CI : 1.54 - 5.05; *p=0.001*; Table 4.2) or moderate (65.0% vs 43.1%; OR: 2.45, CI: 1.35 - 4.45; *p=0.003*) iron deficiency. Among those identified as having HMB, few reported seeking advice/help for heavy periods, while awareness of IDA and ID was poor. Both HMB and IDA were associated with reporting that the menstrual cycle disrupts everyday lifestyle/exercise performance, but iron deficiency alone was not. Those with HMB were more than four times more likely to report this disruption (OR: 4.15, CI: 0.13,0.44; *p<0.0005*), and this relationship is likely to be independent of IDA.

Conclusions: When identified using the outlined criteria, HMB, iron deficiency and associated IDA are common in this population with a similar prevalence to the general population. HMB increased risk of a compromised iron status. HMB and IDA were independently associated with the perception that the menstrual cycle disrupts disrupts everyday lifestyle/exercise performance, however the effect did not appear to be

cumulative. However, awareness of iron status spears to be poor and needs to be heightened, while many identified with HMB had not sought advice/help for heavy periods.

5.2 Introduction

In chapter 4, when identified using a diagnostic series (outlined in chapter 3), HMB was identified in approximately one in three exercising women of all ability in the United Kingdom (391,392). A similar percentage also cited that their menstrual cycle disrupts their exercise training/performance, increasing to nearly half of all those identified with HMB. However, if indeed HMB does cause a disruption to exercise training/performance, the mechanism by which this manifests is not known. There was a profound association between HMB and a self-reported knowledge of a history of anaemia and iron supplementation, however this has not previously been shown in this population using haematological measures. As explained in chapter 2, given that those who exercise are likely to already have an increased susceptibility to iron deficiency (178,186,376,387), the presence of HMB could exacerbate this further.

If indeed those with HMB who exercise regularly have iron deficiency, with or without anaemia, it could be hypothesised that this could in part be the cause for women to cite detrimental effects of their menstrual cycle on their exercise training and performance. Considering the known impact of IDA on oxidative metabolism, and the potential impact that iron deficiency alone may have on other iron-dependent pathways this is plausible. Further, given the previously established lack of awareness of HMB and iron status (28), it could be further be hypothesised that the identified presence of HMB, resulting in a deficiency in iron is unknown.

Significantly, since the study conducted in chapter 4 was the first time HMB was considered in those who exercise, there was an additional need to determine whether HMB presence in a similar population subset was comparable in other countries.

5.2.1 Study aims

The aims of the present study were to:

1. The primary aim was to assess the incidence of HMB (when using an outlined criteria (chapter 3), in the absence of a universally applied verified diagnostic means), and its association with iron deficiency and IDA using haematological measures in women who undertake regular exercise.
2. The secondary aims were then to gain an indication of the level of awareness of HMB and iron status; and to identify whether there is an association between

identified HMB, iron deficiency and IDA and perceived disruption to everyday lifestyle/exercise performance caused by the menstrual cycle. If an association between HMB and a reported disruption of the menstrual cycle to exercise training/performance is identified, the role of iron status in this relationship will be evaluated.

5.2.2 Study hypotheses

HMB, when identified using the outlined criteria, is common in this population., and the identified presence of HMB is associated with an increased likelihood of iron deficiency and IDA.

1. Awareness of iron status in those with a compromised iron status and the seeking of help/advice for heavy periods in those with HMB is limited.
2. Iron status is involved in the relationship between identified HMB and individuals reporting that their menstrual cycle disrupts their everyday lifestyle/exercise performance.

5.3 Materials and methods

5.3.1 Ethics and participant consent

The 'Female Health Questionnaire' was approved by the St Mary's University Ethics Committee. Participants provided written consent at the time of clinic attendance allowing for the anonymous use of their data for research.

5.3.2 Inclusion criteria

The inclusion criteria were:

- Female;
- Aged ≥18 years;
- Pre-menopausal;
- Undertake regular exercise (≥ 60 minutes/week or indicated taking part in races).

5.3.3 Exclusion criteria

The exclusion criteria were:

- Not menstruated in the past 12 months;
- Incompletion of questionnaires.

5.3.4 Participants

As a result of a collaboration between a research group in Singapore, exercising women attending routine healthcare assessments run by The Singapore Anti-Tuberculosis Association (SATA) in Singapore were used in this research. Due to limited funds, this sample provided an opportune group to study, but as a result, this population must be defined as a 'convenience' sample.

5.3.5 Study design

A retrospective analysis of this convenience sample was performed to assess exercise and menstrual cycle characteristics, iron status and HMB, as identified using the pre-identified criteria, in all women attending clinics for routine healthcare assessments run by The Singapore Anti-Tuberculosis Association (SATA) Healthcare, Singapore (insurance screening and drop-in clinics). SATA CommHealth are a non-profit organisation and were also selected to run the health checks for the Singapore 'National Population Health Survey 2016/17' (110,393).

As part of the routine 'well woman' assessment, patients had a routine blood test and were asked to complete a short 'Female Health Questionnaire' (v2 – as outlined in chapter 3 and is abbreviated version of that used in chapter 4), to audit aspects of their health, menstrual cycle, and exercise activity (typical weekly exercise volume broken down into discipline). Since the population of Singapore is considered homogenous, with relatively little variety in types of industry and a small total area, it is assumed that this sample was largely representative of the average population, while also appreciating that this population all exercise regularly so may experience slight physiological variance as would be expected in regular exercisers. The median BMI in this study was 22.1 $kg \cdot m^{-2}$, which is in accordance with data from the 2007 National Health Surveillance Survey which found mean BMI in women between the age of 18 and 49 to range from 20.9 – 23.0 $kg \cdot m^{-2}$ (394). It is also notable that SATA CommHealth describe themselves as having a focus on community health, providing 'basic health screening packages at affordable mass-market prices for everyone'. While also frequently used by local companies, providing annual check-ups (395). SATA CommHealth are a non-profit organisation and were also selected to run the health checks for the Singapore 'National Population Health Survey 2016/17' (396).

From July 2015 to November 2015 a total of 777 women attended the healthcare assessments, 84 were excluded due to non-completion of the questionnaires, a further 206 were excluded as they had not menstruated in the previous year, a final 216 were excluded due to not meeting the specified exercise level criteria (Figure 5.1). Therefore, data from 271 regularly exercising and menstruating women were included in the study.

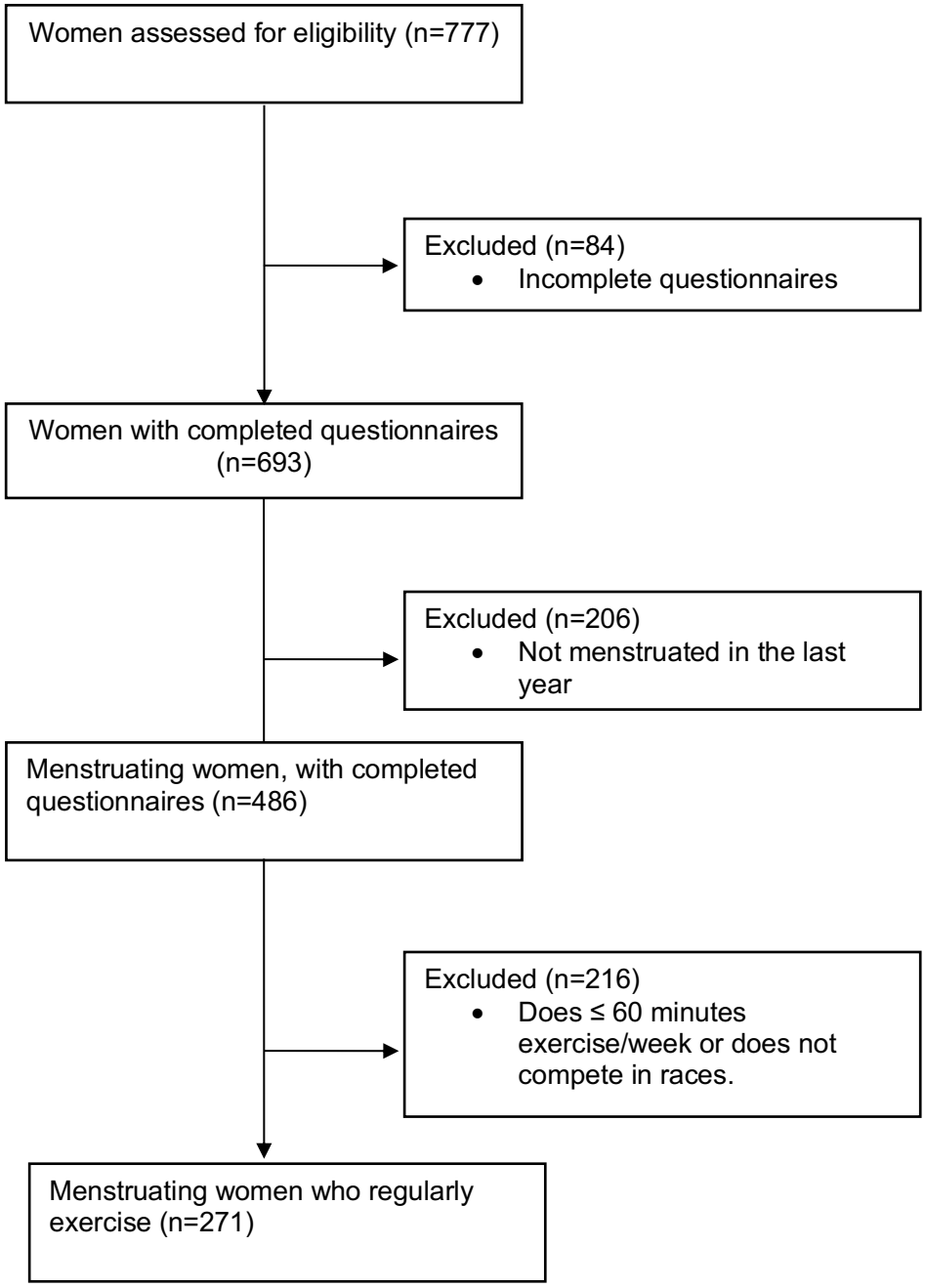

Figure 5.1 – Derivation of study sample.

5.3.6 Measurements and definitions

Female Health Questionnaire

All participants completed the 'Female Health Questionnaire', comprising the structured series of questions taking 2-3 minutes for completion including a four-part criteria for the identification of HMB (392). The questionnaire was an abbreviation of that used previously in chapter 4, and described in chapter 3 (28). This also captured additional demographics including height and weight (measured by the practitioner at the time of venepuncture).

The questionnaire also asked the participants to report their exercise competition level (If participants did more than one form of exercise they were requested to report the response that reflects the highest level), the options included:

1. Do not exercise
2. Recreational, just for fun
3. Take part in races, in a relatively non-competitive manner
4. Regional level
5. National level
6. International level.

Blood measures

As part of the SATA 'well woman' routine assessments, blood is taken to check the patient's full blood count, urea and electrolytes, and serum ferritin. Following the guidelines from the World Health Organisation (WHO), for the purpose of this study, IDA was defined as [Hb] < 12.0 $g \cdot dL^{-1}$ and serum ferritin < 15 $g \cdot dL^{-1}$. Also in accordance with the WHO guidelines, 'severe' iron deficiency was diagnosed using a serum ferritin cut-off value of < 15 $\mu g \cdot L^{-1}$ (40). This is in accordance with the WHO criteria for when iron stores are depleted (40). Since, a lack of iron in bone marrow has been observed when serum ferritin is 30 $\mu g \cdot L^{-1}$, and using this cut-off value, sensitivity and specificity for iron deficiency diagnosis has been found to be 92% and 96% respectively (42). Therefore, for the purposes of this study, 'moderate' iron deficiency will be defined implementing a cut-off for serum ferritin of < 30 $g \cdot dL^{-1}$.

5.3.7 Data analysis

Paper questionnaires were completed at the time of clinic attendance and inputted anonymously alongside blood results to a secured database.

Statistical analysis was performed using a statistics computer package (IBM SPSS Statistics for Macintosh, Version 21.0, Armonk, NK: IBM Corp.). Statistical significance was set at $p<0.05$.

All data were tested for normality using the Shapiro-Wilk test, and data were analysed descriptively to evaluate all questionnaire variables and iron status. Chi-squared tests were used to assess whether there was an association between HMB, when identified using the outline criteria (chapter 3), and both presence and historical knowledge of iron deficiency, IDA, anaemia, the use of iron supplementation and reporting whether the individual perceives that their menstrual cycle disrupts their everyday lifestyle/exercise performance. Mann Whitney U tests were used to determine whether haemoglobin concentration, serum ferritin, age, weight, height, BMI, typical exercise volume and participant competition level varied between groups. Chi-squared tests were also used to determine whether, in those identified with HMB, prevalence of IDA or iron deficiency was different based on whether or not participants reported seeking advice/help for heavy periods. Chi-squared tests were also used to determine if there was a difference in reporting disruptions of the menstrual cycle to everyday lifestyle/exercise performance between those with and without IDA or iron deficiency. Mann Whitney U tests were used to assess whether there was a difference in [Hb] and serum ferritin when comparing those who did and did not report these disruptions.

Cross-sectional predictive factors for reported disruptions to everyday lifestyle/exercise performance caused by the menstrual cycle and identified HMB were determined using binary logistic analyses. The independent variables assessed were the identified presence of HMB, anaemia, and then other relevant covariates including age, BMI, exercise volume and competition level.

5.4 Results

5.4.1 Participant Characteristics

From July 2015 – Nov 2015 a total of 271 women were included (Figure 5.1). The median age was 38 years (29 – 43 years), weight: 57.0 kg (50.3 – 63.0 kg), height: 1.60 m (1.56 – 1.63 m) (Table 5.1). Most undertook exercise recreationally (43.2%), or indicated regularly taking part in races (40.2%), while 2.6% reported being at a regional level and 1.5% at a national or international level, 12.2% did not complete this question. Overall, 41.7% reported $\leq$ 120 minutes of exercise each week, 32.5% reported $\geq$ 120 but $<$ 210 minutes, 18.8% reported $\geq$210 but $<$ 420 minutes, while 7.0% reported $\geq$ 420 minutes of exercise each week.

Table 5.1 - Clinical characteristics of participants.

	Total Sample	Range (min-max)
[Hb] ($g \cdot dL^{-1}$)	13.0 (12.2-13.6)	6.9 – 16.3
Serum ferritin ($\mu g \cdot L^{-1}$)	31.0 (12.0-56.0)	0.5 – 276
IDA	14.8% (n=40)	
Severe ID	30.3% (n=82)	
Moderate ID	48.0% (n=130)	
HMB	22.1% (n=60)	
Age (years)	38 (29-43)	17 – 60
Weight (kg)	57.0 (50.3-63.0)	40.3 – 120.0
Height (m)	1.60 (1.56-1.63)	1.40 – 1.79
Body Mass Index ($kg \cdot m^{-2}$)	22.1 (20.0-24.3)	16.2 – 53.3
Weekly exercise volume (minutes)	120 (60-210)	
Competition level	2 (2-3)	
Seeking of advice/help for heavy periods	11.4% (n=31)	
Knowledge of a history of anaemia	17.7% (n=48)	
Knowledge of a history of anaemia in last 3M	1.8% (n=5)	
Knowledge of a history of iron deficiency	22.5% (n=61)	
Knowledge of a history of iron deficiency in last 3M	3.0% (n=8)	
History of iron supplementation	31.4% (n=85)	
Reported disruption of the MC to everyday lifestyle/exercise performance	26.2% (n=71)	

[Hb] – haemoglobin concentration, IDA – iron deficiency anaemia, ID – iron deficiency, MC – menstrual cycle

Values are median (IQR). Where percentages are given, the number in the brackets is n.

5.4.2 Identified prevalence of HMB, iron deficiency and IDA

Of the 271 women, the median [Hb] was 13.0 $g \cdot dL^{-1}$, and 14.8% had IDA ([Hb] < 12.0 $g \cdot dL^{-1}$, serum ferritin < 15 $\mu g \cdot L^{-1}$; Table 5.1). The median serum ferritin was 31.0 $\mu g \cdot L^{-1}$ (Table 5.1). Iron deficiency was common; nearly one third (30.3%) had severe iron deficiency (serum ferritin < 15 $\mu g \cdot L^{-1}$; Table 5.1), and nearly half (48.0%) moderate iron deficiency (serum ferritin < 30 $\mu g \cdot L^{-1}$; Table 5.1).

Table 5.2 – A comparison of characteristics between those with and without heavy menstrual bleeding (HMB), when identified using the outlined criteria.

	HMB	Not HMB	Test statistic	p-value
n	60	211		
%	22.1%	77.9%		
[Hb] ($g \cdot dL^{-1}$)	12.9 (11.7-13.6)	13.0 (12.3-13.6)	U = 5579	0.16
Serum ferritin ($\mu g \cdot L^{-1}$)	18.5*** (7.8-35.0)	34.0 (15.5-62.0)	U = 4087	<0.0005***
IDA	28.3%* (n=17)	10.9% (n=23)	X_2 = 11.284	0.001***
Severe ID	48.3%** (n=29)	25.1% (n=53)	X_2 = 11.931	0.001***
Moderate ID	65.0%** (n=39)	43.1% (n=91)	X_2 = 8.954	0.003***
Age (years)	39 (27-43)	37 (29-44)	U = 6160	0.75
Weight (kg)	57.0 (51.3-64.0)	56.7 (50.0-62.0)	U = 4087	0.19
Height (m)	1.60 (1.57-1.63)	1.60 (1.55-1.64)	U = 6294	0.63
Body Mass Index ($kg \cdot m^{-2}$)	22.5 (20.3-25.4)	22.1 (19.9-24.1)	U = 6752	0.09
Weekly exercise volume (minutes)	120 (60-180)	120 (60-210)	U = 6328	>0.99
Competition level	2.5	2.5	X_2 = 3.44	0.63
Seeking of advice/help for heavy periods	33.3%*** (n=20)	5.2% (n=11)	X_2 = 36.464	<0.0005***
Knowledge of a history of anaemia	30.0%* (n=18)	14.2% (n=30)	X_2 = 8.87	0.01*
Knowledge of a history of anaemia in last 3M	1.7% (n=1)	1.9% (n=4)	X_2 = 0.10	0.95

Knowledge of a history of iron deficiency	30.0% (n=18)	20.4% (n=43)	X_2 = 4.08	0.13
Knowledge of a history of iron deficiency in last 3M	1.7% (n=1)	3.3% (n=7)	X_2 1.07	0.59
History of iron supplementation	43.3% (n=26)	28.0% (n=59)	X_2 = 5.13	0.08
Reported disruption of the MC to everyday lifestyle/exercise performance	50.0%*** (n=30)	19.4% (n=41)	X_2 = 22.58	<0.0005***

[Hb] – haemoglobin concentration, IDA – iron deficiency anaemia (([Hb < 12.0g·dL^{-1}, serum ferritin < 15μg·L^{-1}), severe ID – severe iron deficiency (serum ferritin < 15μg·L^{-1}), moderate ID - moderate iron deficiency (serum ferritin < 30 μg·L^{-1}), HMB – heavy menstrual bleeding, MC – menstrual cycle.

Values are median (IQR). Where percentages are given, the number in the brackets is n. Significant difference between those reporting and not reporting HMB are shown as follows ***p < 0.005, **p < 0.01, *p < 0.05.

Of the 271 women, a total of 22.1% met the outlined criteria for HMB (two or more symptoms of the outlined diagnostic series in chapter 3). Those identified with HMB were more than three times more likely to suffer IDA (28.3% vs 10.9%; OR: 3.23, CI: 1.59 – 6.57; *p<0.0005);* Table 5.2) and twice as likely to suffer severe (48.3% vs 25.1%; OR: 2.79, CI: 1.54 - 5.05; *p=0.001*; Table 5.2) or moderate (65.0% vs 43.1%; OR: 2.45, CI: 1.35 - 4.45; *p=0.003*; Table 5.2) iron deficiency compared to those without HMB. On average, women identified with HMB had a lower serum ferritin (18.5 μg·L^{-1} (7.8 – 35.0 μg·L^{-1}) vs. 34.0 μg·L^{-1} (15.5 – 62.0 μg·L^{-1}); *p<0.0005*; Table 5.2). However, median haemoglobin concentration did not vary between groups (*p=0.16*; Table 5.2). There was no difference in demographics or training and competition status between those identified with and without HMB (all *p>0.05*; Table 5.2).

5.4.3 Level of awareness of HMB and iron status

Overall, amongst those with IDA, severe iron deficiency and moderate iron deficiency awareness of iron status appeared poor (Table 5.3). Less than half of those with IDA

reported prior knowledge of anaemia (40.0%), with one in 10 (10.0%) reporting recent awareness (in the previous three months). Similar statistics were seen in those with iron deficiency, with only 40.2% of those with severe iron deficiency reporting prior knowledge, and 6.1% reporting knowledge in the previous three months (Table 5.3). Across all individuals, supplementation was reported by 31.4% at any one point in time (Table 5.1). Five percent of those with IDA and less than five percent of those with iron deficiency reported use of supplementation (Table 5.3).

Table 5.3 – Reported historical knowledge of anaemia, iron deficiency and use of supplementation in those with iron deficiency anaemia and severe (serum ferritin < 15 $\mu g \cdot L^{-1}$) and moderate (serum ferritin < 30 $\mu g \cdot L^{-1}$) iron deficiency.

	n	Anaemia history	Anaemia history – 3 months	ID history	ID history – 3 months	Iron sup – 3 months
IDA	40	40.0% (n=16)	10.0% (n=4)	52.5% (n=21)	12.5% (n=5)	5.0% (n=2)
Severe ID	82	28.0% (n=23)	3.7% (n=3)	40.2% (n=33)	6.1% (n=5)	4.9% (n=4)
Moderate ID	130	23.8% (n=31)	3.1% (n=4)	33.8% (n=44)	4.6% (n=6)	3.8% (n=5)

IDA – iron deficiency anaemia, ID – iron deficiency, ID sup – reported supplementation with iron in the previous 3 months
Values are percentage (n).

Advice/help for heavy periods had rarely been sought, with only a third of those identified with HMB reporting having sought this (Table 5.2). More than a third of those (35.0%) who were both identified to have HMB and to have sought advice/help for heavy periods had IDA (Table 5.4). But there was no difference in the prevalence of IDA or iron deficiency amongst those identified with HMB and who have and have sought advice/help for heavy periods (Table 5.4; all $p>0.05$).

Table 5.4 – The likelihood of having IDA, or severe (serum ferritin < 15 $\mu g \cdot L^{-1}$) or moderate (serum ferritin < 30 $\mu g \cdot L^{-1}$) iron deficiency based on whether those identified with HMB have or have not sought advice/help for heavy periods.

	HMB + advice/help	**HMB no advice/help**	**X_2**	**p-value**
IDA	35.0% (n=7)	25.0% (n=10)	0.657	0.42
Severe ID	55.0% (n=11)	45.0% (n=18)	0.534	0.47
Moderate ID	75.0% (n=15)	60.0% (n=24)	1.319	0.25

HMB – heavy menstrual bleeding, IDA – iron deficiency anaemia, ID – iron deficiency, X_2 – Chi-squared test statistic

Values are percentage (n).

5.4.4 Association between identified HMB, iron deficiency and IDA with reported menstrual cycle disruptions to everyday lifestyle/exercise performance

The identified presence of HMB meant that individuals were more than four times more likely to report that their menstrual cycle disrupts their everyday lifestyle/exercise performance (50.0% vs 19.4%; *p<0.0005*; Table 5.5). Similarly, women with IDA were also more likely to report a disruption of their menstrual cycle to their everyday lifestyle/exercise performance compared to those without IDA (45.0% vs 22.9%*, p=0.003*; Table 5.5*)*. This finding was not however mirrored in those with either severe or moderate iron deficiency (*p=0.*17 and *p=0.*42 respectively; Table 5.5). While there was also no difference in [Hb] or serum ferritin in those who did, and did not report this (*p=0.14* and *p=0.07* respectively; Table 5.6)

Table 5.5 – Likelihood of reporting that the menstrual cycle disrupts everyday lifestyle/exercise performance in those identified with heavy menstrual bleeding (HMB), iron deficiency anaemia (IDA) and severe and moderate iron deficiency (ID).

	Yes	No	X_2	OR	CI	p-value
HMB (n=60)	50.0%* (n=30)	19.4% (n=41)	22.58	4.15	2.52 - 7.63	<0.0005***
IDA (n=40)	45.0%* (n=18)	22.9% (n=53)	8.56	2.75	1.37 – 5.50	0.003
Severe ID (n=82)	31.7% (n=26)	23.8% (n=45)	1.84	1.49	0.84 - 2.64	0.17
Moderate ID (n=130)	28.5% (n=37)	24.1% (n=34)	0.66	1.25	0.73 - 2.15	0.42

HMB – heavy menstrual bleeding, IDA – iron deficiency anaemia, ID – iron deficiency, X_2 – Chi-squared test statistic, OR – odds ratio, CI – confidence interval for 95% confidence of the odds

A significant difference between those reporting that their menstrual does or does not affect training and performance is shown as follows: ***p<0.005.

Table 5.6 – The effect of iron status on the perception of whether the menstrual cycle disrupts everyday lifestyle/exercise performance.

	Disruption to lifestyle/performance			
	Yes	No	U	p-value
Haemoglobin ($g \cdot dL^{-1}$)	12.7 (11.9-13.6)	13.1 (12.3-13.6)	6268	0.14
Serum ferritin ($\mu g \cdot L^{-1}$)	29.0 (8.5-44.5)	32.5 (13.0-57.5)	6065	0.07

U – Mann Whitney U test statistic

Values are median (IQR).

In an unadjusted logistic regression model, those identified with HMB were 4.15 times more likely to report that their menstrual cycle disrupts everyday lifestyle/exercise performance (95% CI: 2.25 – 7.63, *p<0.0005*; Table 5.7) compared to those without HMB. Adjusting for IDA reduced the odds ratio between HMB and the reporting of this, however there was still a very strong association (*p<0.0005*). There was little evidence to suggest that an interaction between identified HMB and IDA was related to disruptions to everyday

lifestyle/exercise performance caused by the menstrual cycle, since the relationship between HMB and the citing of disruptions was marginally reduced by the addition of IDA to this model. This suggests that IDA does not have an impact on this relationship (*p=0.47*; 95% CI: 1.01 – 4.41; Table 5.7).

After controlling for other covariates (age, BMI, competition level and weekly exercise volume), the relationship between identified HMB and reported disruptions to everyday lifestyle/exercise performance caused by the menstrual cycle was only slightly attenuated (OR 3.66, 95% CI: 1.89 – 7.09; *p<0.0005*; Table 5.7).

Table 5.7 – The unadjusted and adjusted results from the logistic regression of the perception of disruptions of the menstrual cycle to everyday lifestyle/exercise performance.

	Unadjusted model			Adjusted for IDA only			Adjusted for IDA and other covariates		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
HMB	4.15	2.25 – 7.63	<0.0005***	3.71	1.99 – 6.92	<0.0005***	3.66	1.89 – 7.09	<0.0005***
IDA				2.11	1.01 – 4.41	0.047*	1.80	0.25 – 1.25	0.16
Age							0.98	0.95 – 1.01	0.27
BMI							0.98	0.91 – 1.05	0.50
Competition level							1.02	0.65 – 1.59	0.93
Weekly exercise volume							1.00	1.00- 1.00	0.34

HMB – heavy menstrual bleeding, IDA – iron deficiency anaemia, BMI – body mass index, OR – odds ratio, CI – confidence interval for 95% confidence of the odds

Significant interaction of the variable in the model are shown as follows: ***p < 0.005, **p < 0.01, *p < 0.05.

5.5 Discussion

In marathon runners in the United Kingdom in chapter 4, approximately one in three met the identified criteria for HMB (as outlined in chapter 3), with the presence of HMB being associated with both a reported historical knowledge of anaemia and a perceived disruption of the menstrual cycle to everyday lifestyle/exercise performance (392). In the present study, we firstly aimed to assess HMB prevalence, when identified using the same criteria as in chapter 4, in this 'convenience' sample – also appreciating this group likely forms a different ethnic population, and then to confirm the association between identified HMB and haematological measures of iron deficiency and IDA. We then wanted to determine whether the perception that the menstrual cycle disrupts everyday lifestyle/exercise performance, a similar factor previously found to be associated with HMB could be as a result of iron deficiency with or without anaemia be it known or unknown. Finally, we wanted to gauge the level of awareness of both HMB and iron status. We firstly identified that, when using this means for identification, HMB was common in this population, with prevalence similar to that reported in both the general population previously (28) and in the marathon runners in chapter 4 (28,392). Risk of iron deficiency and IDA was found to significantly increase in those identified with HMB. Both HMB and IDA independently increased the likelihood of individuals reporting that their menstrual cycle disrupts their everyday lifestyle/exercise performance. However, the combination of HMB and IDA did not increase this association, suggesting no cumulative effect. Further, neither iron deficiency alone, nor individual [Hb] or serum ferritin levels were associated with this. Significantly, similarly to in chapter 4 awareness of iron status appeared to be poor and a relative few identified with HMB reported the seeking of advice/help for heavy periods.

Prevalence of IDA in exercising women is thought to be between 8 – 14% (119-121), and the findings from this study are similar to this, as 14.8% were found to have IDA. The ambiguity surrounding the diagnosis of iron deficiency has meant that the prevalence of iron deficiency amongst those who exercise is unsure, as described extensively in chapter 2, but using a variety of serum ferritin cut-off values, most conclude it to be around one third (46,51,244). In the present study, using a serum ferritin cut-off of < 15 $\mu g \cdot L^{-1}$, in accordance with the WHO's criteria, nearly one third (30.3%) were iron deficient. This increased to nearly half (48.0%) when using a more lenient serum ferritin cut off-of < 30 $\mu g \cdot L^{-1}$. This is significantly greater than that observed in the general United Kingdom population (using a serum ferritin < 15 $\mu g \cdot L^{-1}$ women: 8.2 – 16.0 %; men: 0 – 2.5 %) (118). This is likely due to the effect of exercise-induced iron losses (178,186,376,387), often reduced dietary intakes in exercising women (194,195,397), and the effect of a post exercise inflammatory response, reducing absorption (377).

Similarly to in chapter 4, awareness of iron status in this cohort appeared poor. Of those with IDA, 90.0% reported no knowledge of having this within the last three months. These findings were mirrored in those with iron deficiency, and this clearly needs to be addressed. Similarly, only one third of those identified with HMB indicated that they have sought advice/help for heavy periods suggesting that awareness of HMB may also be limited. Previous studies in the general population support this, highlighting that women with HMB scarcely seek help, with estimates of only 6% consulting medical professionals about their symptoms (398). As highlighted in chapter 4, this is likely to be confounded by the sensitive nature of HMB and the menstrual cycle, meaning that it is not a subject matter typically discussed, and significantly, with some cultures in particular believing it to be a normal cleansing process (383). Significantly in this study, in those identified with HMB, the prevalence of IDA or iron deficiency was no different between those who have or have not sought advice/help for heavy periods. While the source of this help was not identified, if this means of help was from medical professionals, it could be suggested that they may not be carrying out appropriate tests when conducting an assessment. Given the negative repercussions associated with IDA and the potential impacts associated with iron deficiency, it is important that awareness amongst medical professionals of the appropriate tests and subsequent effective treatment options when an individual presents with HMB are established. Further, considering the medical checks undertaken in the present study are commonplace and typically conducted on an annual basis, the poor knowledge of iron status, and significant prevalence of IDA and iron deficiency also suggests that the appropriate screening tests, in a population subset with an increased susceptibility to iron deficiency, regardless of HMB presence, may not be being conducted, and that interpretation may not be adequate (6). This could in part be due to the historical problematic difficulties in the diagnosis of both HMB and iron deficiency. Regardless, these findings highlight the need for an improved education for both medical professionals and those who exercise (399,400).

The present study found IDA to be associated with participants reporting that their menstrual cycle disrupts their everyday lifestyle/exercise performance. Interestingly however, this finding was not observed in those with severe or moderate iron deficiency, nor was it associated with [Hb] or serum ferritin levels. As previously highlighted, the impact of IDA on exercise performance is well known, however the ambiguity surrounding the effect of iron deficiency without anaemia demonstrates the need for further research (46,51). The lack of a universal agreement on serum ferritin cut-offs for diagnosis of iron deficiency particularly amongst the exercising community, in addition to other factors confounds this as discussed in chapter 2 and as will be discussed later in this thesis

(46,244). The use of serum ferritin as a sole marker of iron status has also been questioned (51), and it is possible that optimal levels of serum ferritin are different in those who exercise. The present study suggests that the ensuing reported disruption to everyday lifestyle/exercise performance caused by the menstrual cycle may be independent from the presence of either severe or moderate iron deficiency. While inevitably making an assumption, from this it could be inferred that there is no performance detriment of having either severe or moderate iron deficiency when defined using the criteria outlined in this study. However, considering the lack of association between cited disruption to everyday lifestyle/exercise performance caused by the menstrual cycle and [Hb] and serum ferritin, it could be suggested that other markers of iron status need consideration. Alternatively,, there could be another impact of HMB that is overriding any effect of iron deficiency. Prior to forming a definitive conclusion, further investigation as to the diagnosis and impact of iron deficiency is required in addition to gaining a better understanding of the aetiology of HMB.

HMB was also associated with participants reporting that their menstrual cycle disrupts everyday lifestyle/exercise performance. Those identified with HMB were more than four times more likely to cite this. This relationship was not however enhanced when combined with IDA, and was only minimally attenuated when other potential covariates were considered. This indicates that the relationship between HMB and cited menstrual cycle related everyday lifestyle/exercise performance disruptions is independent of IDA. Given the absence of an association between iron deficiency, serum ferritin or haemoglobin and cited menstrual cycle disruptions to everyday lifestyle/exercise performance, the present study suggests that when using the outlined identification criteria, the relationship between HMB and everyday lifestyle/exercise performance disruptions is independent of iron status. Other underlying medical conditions clearly need consideration here and have the potential to confound this finding. However, previous research identified no underlying medical pathology in 61% of the 34,941 women who had recently received a HMB diagnosis (385). Evidently, there is a need to consider the aetiology of HMB, which as an inflammatory condition, results in an increase in release of proinflammatory cytokines such as tumour necrosis factor alpha (TNF-α) (401). Clearly, increased cytokine levels could be a cause for the everyday lifestyle/exercise performance disruption caused by the menstrual cycle.

Given the lack of advice/help sought for heavy periods in those with HMB, and the poor awareness of iron status, to prevent either of these from acting as a barrier to exercise participation, education surrounding both conditions needs to be improved. If indeed HMB alone has a negative effect on exercise regardless of iron status, other reasons for this need to be addressed and potential solutions evaluated. Given that a quarter of exercising

women here cite that their menstrual cycle disrupts everyday lifestyle/exercise performance this research is crucial. With dropout rates from exercise significantly greater in girls than boys, research in this area in addition to raising of awareness could go some way to addressing this problem.

This study clearly highlights that when identified using the outlined diagnostic series, those with HMB should undergo regular screening to check their iron status. Despite the relationship between HMB and the reporting that the menstrual cycle disrupts their everyday lifestyle/exercise performance being independent from iron status, the impact of iron deficiency anaemia alone on both general health and exercise capacity is well established. Future research should focus on best management and treatment strategies for HMB, the aetiology of HMB, in addition to assessing the impact of iron deficiency without anaemia on exercise performance.

5.5.1 Limitations

There are several limitations to this study. Firstly, as outlined in chapter 3 and 4, there are limitations to the 'Female Health Questionnaire' that must be considered, significantly the absence of a universally recognised diagnosis for HMB. Secondly, despite school education in Singapore being taught in English, results from the 2010 census found that only 79.9% of Singaporean residents ≥15 years are literate in English, thus there is potential for an element of confusion and misinterpretation in reading the questionnaire (402). For increased accuracy in the future the questionnaire should be written in multiple languages. Also, participants did not have their blood samples collected when fasted and were not given instructions to come in a rested state (i.e. having not exercised that day) therefore ferritin, as an acute phase protein, could have been falsely elevated. This group were assumed to be a healthy cohort, specific causes of HMB, such as von Willebrand disease were not evaluated and other underlying medical conditions were not factored in. To ameliorate this, future studies should include measurements for C reactive protein (CRP) and interleukin-6, while also capturing other medical conditions and requesting participants to be tested in a fasted and rested state. To further reduce likelihood of bias when identifying the relationship between iron status and the presence of HMB, the questionnaire could include information about dietary iron intake.

As these data were collected at a single time point, other factors could have impacted upon these results, such as the day in the menstrual cycle, current health state, or haemodilutionary impacts caused by factors such as hydration. Further, SATA CommHealth are only one health service offered in Singapore, making assessment of whether this data is reflective of only a certain type of patient in Singapore problematic.

However, the population of Singapore is fairly homogenous, with a limited range of industry and a small area. Additionally, SATA CommHealth is specifically advertised as being affordable for the mass market, and they were chosen to conduct health screens as part of the National Population Health Survey 2016/17 suggesting that their representation of an inhabitant of Singapore is likely to be relatively unbiased. Finally, as explained in the methods, the median [Hb] and BMI in this study were comparable to the mean in women of a reproductive age in Singapore, as specified by the World Health Organisation and in data from the 2007 National Health Surveillance Survey (116,394). Further suggesting that this sample is reflective of the general Singaporean population of reproductive women. It is acknowledged that the current study is observational, therefore it is not possible to make strong causal inferences about the associations we have found.

Ethnicity was also not recorded, and factors such as typical red meat consumption may vary amongst different populations (119), thus future research should include this, in addition to validating this data in a different cohort.

5.6 Conclusions

HMB, when identified using the outlined series in chapter 3, is common in exercising women and is associated with an increased risk of iron deficiency and IDA. HMB and IDA are both independently associated with the perception that the menstrual cycle disrupts everyday lifestyle/exercise performance. However, the effect of these was not cumulative, suggesting that the mechanism by which HMB may have this effect may be independent of iron status. Further, iron deficiency alone did not increase likelihood of individuals reporting these disruptions to everyday lifestyle/exercise performance. Future research is required to assess the effects of this condition. Given the lack of seeking of advice/help for heavy periods and the poor awareness of iron status in this population there is a need for an increased vigilance amongst healthcare professionals and education for women, this is particularly important given the potential for these to be detrimental to exercise performance and possibly quality of life. Medical professionals need to appreciate that HMB may occur in those who exercise regularly and will increase risk of iron deficiency. In light of its prevalence and its potential to effect exercise capabilities, future research should focus on identification of treatment options for HMB.

To conclude, regarding the outlined hypotheses for this chapter, the first hypothesis that HMB, when identified using the outlined series, was common and increases risk of iron deficiency and IDA in exercising women can be accepted. Since the seeking of advice/help for heavy periods in those identified with HMB and awareness of iron status appeared poor

the second hypothesis can also be accepted. However, the association between HMB and reported disruptions to to everyday lifestyle/exercise performance caused by the menstrual cycle was independent of iron status, so the final hypothesis suggesting that iron status was the cause must be rejected.

5.7 Future perspectives

The aetiology of HMB is thought to result in increased release of pro-inflammatory cytokines such as TNF-α. Therefore, it is possible that this could be causing the reported disruptions to everyday lifestyle/exercise performance, and this should be investigated further. Evidently there is a need to investigate the impact and diagnosis of iron deficiency without anaemia.

5.8 Acknowledgements

The author would like to thank SATA healthcare for their cooperation in facilitating access to the female participants.

5.9 Author Contributions

The 'Female Health Questionnaire' used in this study was written by the author and outlined in Section 3. Professor Toby Richards has a collaboration with a colleague in Singapore – Mr Tim Cushway, and it was agreed that they would help the Singapore Anti-Tuberculosis Association (SATA) Healthcare group in Singapore to assess whether HMB is a problem in this populace. Therefore the 'Female Health Questionnaire' was given to them for completion when patients had routine 'well-woman' health appointments. Once it became evident that HMB was common, and that the results could have significant impact on practice in this country, a formalised analysis was deemed necessary.

Therefore, all data were anonymously inputted into a secure database by researchers in Singapore, this database was then sent to the author for analysis. The author selected the criteria for definition of an 'exerciser', and in accordance with the inclusion criteria extracted the relevant data. The author performed all the statistical analysis and conducted all the data interpretation.

6 The impact of identified heavy menstrual bleeding and iron status on fatigue in exercising and menstruating women

6.1 Abstract

Background: Fatigue is particularly common in women and is frequently unexplained. This is often associated with iron deficiency and anaemia. Menstrual blood loss is the most common cause of anaemia in the developed world, with anaemia susceptibility increasing in those with heavy menstrual bleeding (HMB). The interrelationship between HMB, iron deficiency and fatigue is however unknown.

Methods:

The healthy, regularly menstruating and exercising women in Singapore attending routine clinical screening appointments used in chapter 5 were also used in this study (n = 271, median age: 38 years (29 - 43 years), weight: 57.0 kg (50.3 - 63.0 kg), height: 1.60 m (1.55-1.63 m) and BMI: 22.1 $kg \cdot m^{-2}$ (20.0 – 24.3 $kg \cdot m^{-2}$)). Participants completed the Multidimensional Fatigue Inventory (MFI-20), alongside the 'Female Health Questionnaire', and had venous blood samples taken for measurement of iron status.

Results: Increased fatigue was reported both by those identified with HMB, using the outlined criteria in chapter 3 (general fatigue; *p=0.04*), and by those identified with anaemia (mental fatigue; *p=0.047*). However, the association between HMB and fatigue was not mediated by anaemia or IDA. There was no association between iron deficiency alone or IDA and fatigue (all *p>0.05)*, nor was there a correlation between [Hb] or serum ferritin and fatigue (all *p>0.05)*. Awareness of iron status appeared poor, and those with HMB were unlikely to report having sought help/advice for heavy periods.

Conclusion: Identified HMB and anaemia are both independently associated with increased fatigue in menstruating women. However, the relationship between identified HMB and fatigue was independent of iron status, as we found no evidence that iron status mediates this relationship. Blood tests for iron status should be considered for those identified to present with HMB and/or mental fatigue. Existing markers of iron status need to be evaluated, in addition to treatment options for those identified with HMB. Awareness of HMB and iron status both amongst individuals and in medical professionals needs to be improved.

6.2 Introduction

Fatigue is more commonly reported by female patients than by their male counterparts (403). It is one of the most common reasons to seek medical advice in primary care, reported by 14 - 31% of patients (403,404). Onset of fatigue can be slow and insidious, potentially causing it to go unnoticed. Being one of the primary symptoms, fatigue is frequently attributed to iron deficiency with or without anaemia (405). In addition to many other essential roles, iron is required for neurotransmitter synthesis, activity and degradation in both the brain and central nervous system and is therefore fundamental for cognitive function (35).

As highlighted throughout this thesis, in states where iron deficiency presents without anaemia, and therefore where haemoglobin concentration ([Hb]) is not compromised, research is equivocal as to the impact on exercise capacity. Research is also inconclusive as to the effects on fatigue. Some have found iron supplementation reduces fatigue and improves mental health in iron deficient but not anaemic women (226,247), while others have found either no association between serum ferritin and fatigue, or no impact of oral or intravenous iron on either fatigue in iron deficient but not anaemic women (38,51,249). The potential reasons for this have previously been highlighted in this thesis, and may in part be due to a variation in the criteria used to diagnose iron deficiency in research studies using serum ferritin, with cut-off values ranging from 12 – 40 $\mu g \cdot L^{-1}$ (46), different supplementation protocols and routes of administration. Evidently, there is significant potential for increased levels of fatigue to impact exercise performance.

In addition to reducing physical, social, emotional and/or material quality of life, HMB is also associated with various other co-morbidities (372,406-408). In fact, a number of the symptoms of HMB also present in states of iron deficiency and IDA, including fatigue (28). A recent study in adolescents found an independent association between HMB and iron deficiency and HMB and excessive fatigue (38). However, the potential for an inter-relationship between all three has not been evaluated, it would be prudent to suggest that the fatigue associated with HMB may be due to the increased iron loss, suggesting that these are interlinked. Alternatively, as found in chapter 5, the effects of HMB may be independent from iron status.

Time for diagnosis of iron deficiency and IDA can be extensive, and an increased need to assess iron status when gynaecological conditions present has been highlighted, despite the increased susceptibility associated with HMB (409). This is exacerbated by the difficulties associated with HMB diagnosis, and the slow and insidious nature of a lack of iron.

6.2.1 Study aims

To our knowledge, the interaction between HMB, iron status and fatigue has not previously been measured in exercising women.

1. The primary aim was to assess the relationship between HMB, iron status and fatigue in exercising women. Identifying whether HMB would be associated with IDA, anaemia and iron deficiency and that this association will manifest as fatigue.
2. The secondary aims were then to assess whether a combination of HMB and fatigue will increase the risk of IDA, anaemia and iron deficiency in this population. This has high clinical relevance, since predictive measures could be used to encourage blood tests for iron status in women presenting with fatigue.

6.2.2 Study hypotheses

1. HMB and IDA, anaemia and iron deficiency all increase fatigue.
2. The relationship between HMB and fatigue is mediated by iron status.
3. The combination of HMB and fatigue will increase risk of IDA and anaemia.

6.3 Materials and Methods

6.3.1 Ethics and participant consent

The 'Female Health Questionnaire' was approved by the St Mary's University Ethics Committee. Participants provided written consent at the time of clinic attendance allowing for the anonymous use of their data for research.

6.3.2 Inclusion criteria

The inclusion criteria were:

- Female;
- Aged ≥18 years;
- Pre-menopausal;
- Undertake regular exercise (≥ 60 minutes/week or indicated taking part in races).

6.3.3 Exclusion criteria

The exclusion criteria were:

- Not menstruated in the previous 12 months;
- Incompletion of questionnaires.

6.3.4 Participants

The same initial dataset was used as in chapter 5. As previously explained, as a result of a collaboration between a research group in Singapore, in addition to limited funds, this convenience sample was utilised, and included women attending routine healthcare assessments run by The Singapore Anti-Tuberculosis Association (SATA) in Singapore.

6.3.5 Study design

A retrospective analysis utilising this convenience sample was performed to assess the prevalence of HMB, when outlined using a pre-defined diagnostic series, iron deficiency and fatigue in all exercising women attending clinics for routine healthcare assessments run by The Singapore Anti-Tuberculosis Association (SATA) Healthcare, Singapore (insurance screening and drop-in clinics). Alongside a routine blood test, patients were invited to fill in a 'Female Health Questionnaire' in addition to completing the Multidimensional Fatigue Inventory (MFI-20) (410), both in English language only. Those who met the outlined inclusion criteria and completed the questionnaires were included in the study. As highlighted in chapter 5, most employees in Singapore have yearly health checks conducted through organisations such as SATA healthcare, and it is assumed that since the population of Singapore is homogenous, with relatively little variety in types of industry and a small total area, this sample was largely representative of the average. SATA are reported to be frequently used by local companies, providing annual check-ups (395). Further, in this study, the median [Hb] of 13.0 $g \cdot dL^{-1}$, was again comparable to the mean [Hb] in women of a reproductive age in Singapore, specified by the World Health Organisation (116). The mean BMI of 22.1 $kg \cdot m^{-2}$ in this study was also in accordance with data from the 2007 Singaporean National Health Surveillance Survey which found mean BMI in women between the age of 18 and 49 to range from 20.9 – 23.0 $kg \cdot m^{-2}$ (394).

From July 2015 to November 2015 a total of 777 women attended the healthcare assessments, 84 were excluded due to incompletion of the questionnaires, 216 were excluded as they did not meet the predefined exercise criteria, and a further 207 were excluded as they had not menstruated in the previous year (Figure 6.1). Therefore, data from 271 regularly menstruating women were included in the study.

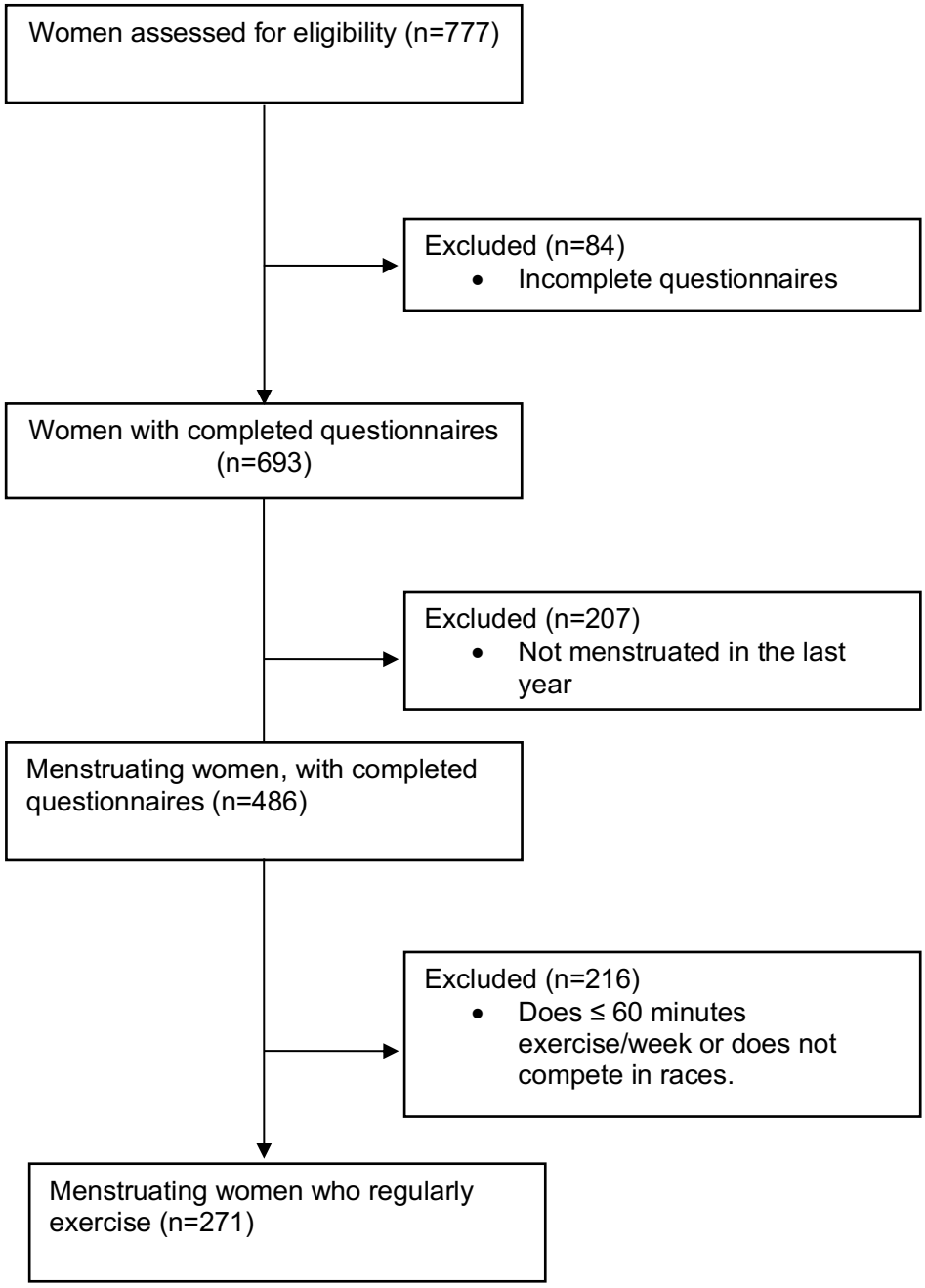

Figure 6.1 – Derivation of study sample.

6.3.6 Measurements and definitions

Female Health Questionnaire

During clinic attendance, women completed the second version of the 'Female Health Questionnaire', as described in chapter 3. This captured; a 4-part diagnostic series for the identification of HMB; the seeking of advice/help for heavy periods; awareness of historic

and current iron status (iron deficiency or anaemia), and current and historic use of iron supplementation (392).

Blood measures

As part of the 'well woman' routine SATA assessment, blood is taken to check the patient's full blood count, urea and electrolytes, and serum ferritin. Following the guidelines from the World Health Organisation (WHO), in this study, anaemia was defined as a blood concentration of haemoglobin [Hb] < 12.0 $g \cdot dL^{-1}$ (411). IDA was defined as [Hb] < 12.0 $g \cdot dL^{-1}$ with a serum ferritin < 15 $\mu g \cdot L^{-1}$ (100). Iron deficiency was defined as serum ferritin < 15 $\mu g \cdot L^{-1}$ again, the level at which the WHO specifically indicates iron depletion (100). As there was no impact of the different levels of severity of iron deficiency in chapter 5, for the purpose of this study, only the more severe cut-off was applied (serum ferritin < 15 $\mu g \cdot L^{-1}$). Given the evident ambiguity surround the use of serum ferritin, anaemia alone was also considered.

Multi-dimensional Fatigue Inventory (MFI-20) (Appendix 10.15)

Women were also asked to complete the MFI-20 which is a questionnaire designed to evaluate five different sub-categories of fatigue; general fatigue, physical fatigue, mental fatigue, reduced activity and reduced motivation (410). It is comprised of 20 questions, each with a five-point Likert scale which are broken down on analysis into the five subsections. The magnitude of the score is proportional to the extent of fatigue, i.e. a higher score means greater fatigue. It has previously been used effectively in both general and chronically unwell populations (412-414).

Both the MFI-20 and the 'Female Health Questionnaire' were designed to take 4 - 5 minutes in total for completion.

6.3.7 Data handling and statistical analysis

As per chapter 5, all data (questionnaire and blood results) were inputted anonymously into an excel spreadsheet. Statistical analysis was performed using a statistics computer package (IBM SPSS Statistics for Macintosh, Version 21.0, Armonk, New York (NY): IBM Corp.). Statistical significance was set at $p<0.05$.

All data were tested for normality using a Shapiro-Wilk test. Data were analysed descriptively to evaluate all questionnaire variables and iron status. Chi-squared tests were firstly used to determine an association between iron status, using the criteria outlined above and identified HMB, using the criteria outlined in chapter 3. Chi-squared tests were also used to determine whether the seeking of advice/help for heavy periods varied

between those with and without anaemia, IDA and iron deficiency. Mann Whitney U tests were used to determine whether there were differences in fatigue scores between women with and without anaemia, IDA, iron deficiency, and between women who were or were not identified to have HMB. Mann Whitney U tests were also used to assess if there was a difference in fatigue specific to those identified with HMB who did or did not have IDA, anaemia and iron deficiency. Spearman's correlation coefficient was used to determine whether there was an association between [Hb] and serum ferritin with all subscales of fatigue.

In order to assess the validity and reliability and to ensure internal consistency of each of the five MFI-20 subscales three reliability tests were performed; 1. inter-item correlation, 2. corrected-to-total correlation, and 3. calculation of the standardised Cronbach's alpha coefficient. Inter-item correlations ranging from 0.30 - 0.70 were kept, while a value for the corrected item-total correlation of > 0.20 was deemed adequate (415). A relatively high reliability coefficient - Cronbach's $\propto$ ranging from 0.70 - 0.95 was considered to be sufficient (416). Cronbach's alpha if item was deleted were also calculated. As the fatigue subscales were not normally distributed, correlations between each of the five MFI-20 subscales and [Hb] and ferritin independently were computed using Spearman's correlation analysis.

A multiple linear regression model was used to investigate the relationship between identified HMB and fatigue. To account for potential confounding, this was adjusted for age, BMI, number of periods in the last 12 months, and iron supplementation in the previous 3 months. Mediation analysis was performed using both the Barron and Kenny (417) and Sobel (418) methods to determine if IDA or anaemia were on a causal pathway between identified HMB and fatigue.

Cross-sectional predictive factors for IDA and anaemia were determined using binary logistic analyses. The independent variables assessed were the identified presence of HMB, the MFI-20 subscales, and then other relevant covariates including age, BMI, number of periods in the last 12 months, and iron supplementation in the previous 3 months. The Box-Tidwell (1962) procedure was applied to check for linearity between independent and the dependent variables (IDA and anaemia), and a Bonferroni correction was applied based on the number of terms (419) A multivariable binomial logistic regression was then used to assess the relationship between IDA and anaemia based on the identified presence of HMB, the responses to the MFI-20 and the other covariates.

6.4 Results

6.4.1 Participant Characteristics

As the same population was used in this study as in chapter 5, the median age was 38 years (29 - 43 years), weight: 57.0 kg (50.3 – 63.0 kg), height: 1.60 m (1.55-1.63 m) and BMI: 22.1 $kg \cdot m^{-2}$ (20.0 – 24.3 $kg \cdot m^{-2}$) (Table 5.1). Across the population (n=271), 60 women reported at least 2 of the outlined HMB criteria and were therefore identified to have HMB.

Table 6.1 – The difference between the prevalence of IDA, anaemia and iron deficiency in those who did or did not meet the outlined HMB criteria.

	Total sample	HMB	No HMB	Test statistic	p-value
n	271	60 (22.1%)	211 (77.9%)		
IDA	14.8% (n=40)	28.3%*** (n=17)	10.9% (n=23)	$X_2 = 11.284$	0.001
Anaemia	18.8% (n=51)	28.3%* (n=17)	16.1% (n=34)	$X_2 = 4.5666$	0.033
ID	30.3% (n=82)	48.3%*** (n=29)	25.1% (n=53)	$X_2 = 11.931$	0.001

BMI – body mass index, IDA – iron deficiency anaemia, ID – iron deficiency, [Hb] – haemoglobin concentration, HMB – heavy menstrual bleeding

Values are median (IQR). Where percentages are given, the number in the brackets is n. Significant differences between those with and without HMB are shown as follows: ***p < 0.005, **p < 0.01, *p < 0.05.

6.4.2 Prevalence and impact of identified HMB on IDA, anaemia and iron deficiency

As in chapter 5, the presence of HMB was reported by 22.1% of the study participants (Table 6.1). While 14.8% had IDA, 18.8% had anaemia, and 30.3% were iron deficient. Those with HMB were more likely to have IDA (28.3% vs 10.9%; *p=0.001*; Table 5.1), anaemia (28.3% vs 16.1%; *p=0.03*; Table 5.1), and iron deficiency (48.3% vs 25.1%; *p=0.001*; Table 5.1) compared to those without HMB.

6.4.3 Impact of HMB and iron status independently on fatigue

Those identified with HMB were more likely to report increased general fatigue (score: 11.50 (9.00 – 13.25) vs 11.00 (8.00 – 12.00); *p=0.04;* Table 6.2), but reported fatigue scores were not higher for total, physical or mental fatigue, while neither activity levels or motivation were affected (all *p>0.05*; Table 6.2*)*.

Those with anaemia reported greater levels of mental fatigue than those without across the total sample (10.00 (8.00 – 12.00) vs 9.00 (7.00 – 11.00); *p=0.047*,*;* Table 6.3). However, neither those with IDA nor those with iron deficiency were more likely to report any increased degree of fatigue across the measured categories when compared to those without (all *p>0.05;* Table 6.3).

Amongst those identified with HMB, those who also had IDA, anaemia or iron deficiency were no more likely to report increased fatigue (all *p>0.05*; Table 6.4).

Table 6.2 – A comparison in fatigue scores (MFI-20) between those identified with and without heavy menstrual bleeding (HMB).

	HMB n=60	**Not HMB** n=211	**U**	**p-value**
Total fatigue	48.00 (43.75 - 57.00)	47.00 (39.50 - 47.00)	7183	0.11
General fatigue	11.50 (9.00 - 13.25)*	11.00 (8.00 - 12.00)	7413.5	0.04
Physical fatigue	9.00 (8.00 - 12.00)	10.00 (7.00 - 12.00)	6766	0.41
Reduced activity	9.50 (7.00 - 11.00)	9.00 (8.00 - 12.00)	6056.5	0.61
Reduced motivation	9.00 (8.00 - 10.00)	9.00 (7.00 - 11.00)	7112.5	0.14
Mental fatigue	10.00 (8.00 - 11.00)	9.00 (7.00 - 11.00)	7012	0.20

HMB – heavy menstrual bleeding, U – Mann Whitney U test statistic

Values are median (IQR).

Significant differences between those with and without HMB are shown as follows: *p < 0.05.

Table 6.3 – A comparison between different fatigue scores (MFI-20) across the total sample between those with and without anaemia, IDA or ID. Anaemia ([Hb] < 12.0 $g \cdot dL^{-1}$), IDA ([Hb] < 12.0 $g \cdot dL^{-1}$ and serum ferritin < 15 $\mu g \cdot L^{-1}$), and iron deficiency (ID; serum ferritin < 15 $\mu g \cdot L^{-1}$).

	Anaemia n=51	**Not Anaemia n=220**	**U**	**p-value**	**IDA n=40**	**Not IDA n=231**	**U**	**p-value**	**ID n=82**	**Not ID n=189**	**U**	**p-value**
Total fatigue	50.00 (42.25-57.00)	47.00 (39.00 - 56.00)	6066	0.37	47.00 (40.75 -57.25)	47.00 (40.00 -57.00)	4851	0.61	47.00 (40.00 - 56.50)	48.00 (41.00 - 57.00)	7265	0.41
General fatigue	11.00 (9.00 - 12.00)	11.00 (8.00 -12.00)	5782.5	0.73	11.00 (8.75 -12.00)	11.00 (9.00 - 12.00)	4595.5	0.96	10.00 (8.00 - 12.00)	11.00 (9.00- 12.00)	7233	0.38
Physical fatigue	10.00 (8.00 - 11.75)	10.00 (7.00 -12.00)	6128.5	0.30	10.00 (8.00 -12.00)	10.00 (7.00 - 12.00)	4927	0.50	9.00 (7.00 - 11.00)	10.00 (7.00 - 12.00)	7123	0.29
Reduced activity	9.00 (8.00 - 12.00)	10.00 (7.00 -12.00)	5878	0.59	9.00 (7.00 - 11.25)	9.00 (7.50- 11.00)	4719.5	0.83	9.00 (7.00 - 11.00)	10.00 (8.00 - 12.00)	7064.5	0.25
Reduced motivation	9.00 (8.00 - 11.00)	9.00 (7.00 - 11.00)	5731	0.81	9.00 (8.00 -11.00)	9.00 (7.00 - 11.00)	4776.5	0.73	9.00 (7.00 - 10.00)	9.00 (7.00 - 11.00)	7345	0.49
Mental fatigue	10.00 (8.00 - 12.00)*	9.00 (7.00 - 11.00)	6603	0.047	10.00 (8.00 -12.00)	9.00 (8.00 - 11.00)	5417.5	0.08	10.00 (8.00 - 11.00)	10.00 (8.00 - 11.00)	7777	0.96

IDA – iron deficiency anaemia, ID – iron deficiency, U – Mann Whitney U test statistic. Values are median (IQR).

Significant differences between those with and without anaemia, IDA or ID are shown as follows: *p < 0.05

Table 6.4 – A comparison between different fatigue scores (MFI-20) in those identified with HMB (n=112) between those with and without anaemia, IDA or ID. Anaemia ([Hb < 12.0 g·dL^{-1}), IDA ([Hb] < 12.0 g·dL^{-1} and serum ferritin < 15 µg·L^{-1}), and iron deficiency (ID; serum ferritin < 15 µg·L^{-1}).

	Anaemia n=17	**Not Anaemia n=43**	**U**	**p-value**	**IDA n=17**	**Not IDA n=43**	**U**	**p-value**	**ID n=29**	**Not ID n=31**	**U**	**p-value**
Total fatigue	47.00 (40.00 - 57.00)	48.00 (45.00- 57.00)	350.5	0.81	47.00 (40.00 - 57.00)	48.00 (45.00 - 57.00)	350.5	0.81	47.00 (40.00 - 57.00)	49.00 (45.50 - 57.00)	355.5	0.16
General fatigue	11.00 (9.00 - 14.00)	12.00 (9.50 - 13.00)	340	0.67	11.00 (9.00 - 14.00)	12.00 (9.50 – 13.00)	340	0.67	11.00 (9.00 – 13.00)	12.00 (10.00- 13.50)	418.5	0.64
Physical fatigue	11.00 (8.00 - 13.00)	9.00 (8.00- 11.00)	396	0.61	11.00 (8,00 - 13.00)	9.00 (8.00 - 11.00)	396	0.61	8.00 (8.00 - 12.00)	10.00 (8.00 - 11.50)	408.5	0.54
Reduced activity	10.00 (7.00 - 11.00)	9.00 (7.00 - 11.00)	394.5	0.63	10.00 (7.00 - 11.00)	9.00 (7.00 - 11.00)	394.5	0.63	9.00 (7.00 - 11.00)	10.00 (8.00 - 11.00)	398.5	0.45
Reduced motivation	9.00 (8.00 - 10.00)	9.00 (8.00 - 10.00)	332	0.58	9.00 (8.00 - 10.00)	9.00 (8.00 – 10.00)	332	0.58	8.00 (8.00 - 10.00)	10.00 (8.00 - 11.00)	378.5	0.29

Mental fatigue	10.00 (8.00 - 11.00)	10 (8-11)	398	0.59	10.00 (8.00 – 11.00)	10.00 (8.00 – 11.00)	398	0.59	10.00 (8.00 - 11.00)	10.00 (8.00 - 12.50)	371.5	0.24

IDA – iron deficiency anaemia, ID – iron deficiency, U – Mann Whitney U test statistic.

Values are median (IQR).

6.5 Reliability of the MFI-20

A summary of the reliability tests for the MFI-20 is given in Table 6.5. It was not necessary to remove any items based on the inter-item correlations and the corrected item-total correlations as these were all greater than 0.30. While Cronbach's ∝ showed acceptable reliability and good internal consistency, ∝ = 0.87, and when standardised ∝ = 0.92.

Table 6.5 - Mean and standard deviations for each aspect of fatigue, alongside inter-item correlations, Cronbach's alpha if the item is deleted, and the corrected item-total correlation.

	Mean	SD	GF	PF	RA	RM	MF	Cronbach's Alpha if item is deleted	Corrected Item-Total Correlation
General Fatigue	10.70	2.96	-	0.63	0.44	0.52	0.56	0.86	0.66
Physical fatigue	9.51	3.07	0.63	-	0.70	0.65	0.56	0.82	0.78
Reduced activity	9.28	2.65	0.44	0.70	-	0.64	0.49	0.85	0.68
Reduced motivation	8.96	2.55	0.57	0.65	0.64	-	0.56	0.84	0.74
Mental fatigue	9.49	2.82	0.56	0.56	0.49	0.56	-	0.86	0.64

SD – standard deviation, TF – total fatigue, GF – general fatigue, PF – physical fatigue, RA – reduced activity, RM – reduced motivation, MF – mental fatigue

6.5.1 Relationship between fatigue and markers of iron status

There was no strong evidence for a linear relationship between any of the MFI-20 subscales or total fatigue and either of [Hb] or serum ferritin independently (Table 6.6; all $p>0.05$).

Table 6.6 – The association between haemoglobin concentration ([Hb]) and serum ferritin and the five subscales of the MFI-20 and total fatigue.

	[Hb]		Serum ferritin	
	Correlation coefficient	p-value	Correlation coefficient	p-value
Total Fatigue	-0.01	0.83	0.05	0.44
General Fatigue	0.00	0.99	0.02	0.72
Physical fatigue	-0.05	0.38	0.10	0.12
Reduced activity	-0.01	0.92	0.04	0.49
Reduced motivation	0.04	0.53	0.02	0.75
Mental fatigue	-0.03	0.66	-0.01	0.84

[Hb] – haemoglobin concentration

6.5.2 Relationship between identified HMB, anaemia, IDA and fatigue

First, in an unadjusted logistic regression model, those identified with HMB were twice as likely to have anaemia (OR: 2.06, 95% CI: 1.05 – 4.03, *p=0.035*; Table 6.7) compared to those without HMB. Adjusting for mental fatigue slightly decreased the odds ratio between HMB and anaemia to 1.96 (95% CI: 1.00 – 3.65). In fact, as shown in Table 6.7, there was some evidence to suggest an increase in mental fatigue was associated with a 11% increase in likelihood of anaemia (OR 1.11, 95% CI: 1.00 - 1.24; *p=0.067*). There was no evidence for an interaction between identified HMB and mental fatigue (*p=0.20*) suggesting that the effect of HMB, when identified by this means, on anaemia is independent of mental fatigue.

After controlling for other covariates (BMI, age, weekly exercise volume, iron supplementation in the last 3 months), the relationship between identified HMB and anaemia was not strengthened and became insignificant (OR 1.79, 95% CI: 0.87 – 3.67; *p=0.11*), the relationship between mental fatigue and anaemia was slightly attenuated (OR decreased from 1.11 to 1.12, Table 6.7).

Table 6.7 – The unadjusted and adjusted results from the logistic regression of anaemia.

	Unadjusted model			Adjusted for mental fatigue only			Adjusted for mental fatigue and other covariates		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
HMB	2.06	1.05 – 4.03	0.035*	1.96	1.00 – 3.85	0.051	1.79	0.87 - 3.67	0.11
Mental fatigue				1.11	1.00 - 1.24	0.067	1.12	1.00 - 1.26	0.06
BMI							1.02	0.96 - 1.02	0.54
Age							1.02	0.99 - 1.06	0.18
Weekly exercise volume							1.00	1.00 - 1.00	0.96
Iron sup. (last 3 months)							2.54	0.71 – 9.05	0.58

OR – odds ratio, HMB – heavy menstrual bleeding, 95% CI – 95% confidence interval for the odds, BMI - body mass index

Significant interaction of the variable in the model are shown as follows: ***p < 0.005, **p < 0.01, *p < 0.05.

In an unadjusted logistic regression model, those identified with HMB were three times more likely to have IDA (OR: 3.23, 95% CI: 1.59 – 6.57, *p=0.001*; Table 6.8) compared to those without HMB. Adjusting for mental fatigue slightly decreased this relationship (OR: 3.10, 95% CI: 1.52 – 6.32, *p=0.002*; Table 6.8). The lack of evidence for an interaction between HMB and mental fatigue (*p=0.20*) suggests that the effect of HMB on IDA is independent of fatigue status.

After controlling for other covariates (BMI, age, weekly exercise volume, iron supplementation in the last 3 months), the relationship between HMB and IDA was only minimally attenuated (OR 2.65, 95% CI: 1.25 – 5.62; *p=0.01*; Table 6.8).

Table 6.8 – The unadjusted and adjusted results from the logistic regression of IDA.

	Unadjusted model			Adjusted for fatigue only			Adjusted for fatigue and other covariates		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
HMB	3.23	1.59 – 6.57	0.001*	3.10	1.52 – 6.32	0.002	2.65	1.25 – 5.62	0.01
Mental fatigue				1.10	0.97 - 1.24	0.138	1.09	0.95 - 1.24	0.21
BMI							1.04	0.97 - 1.12	0.26
Age							1.02	0.98 - 1.05	0.45
Weekly exercise volume							1.00	1.00 - 1.00	0.83
Iron sup. (last 3 months)							1.36	0.27 – 6.76	0.71

OR – odds ratio, HMB – heavy menstrual bleeding, 95% CI – 95% confidence interval for the odds, BMI - body mass index

Significant interaction of the variable in the model are shown as follows: ***p < 0.005, **p < 0.01, *p < 0.05.

6.5.3 Relationship between HMB and fatigue

In an unadjusted model, those identified with HMB scored 0.983 points higher for general fatigue than those without HMB (95% CI for difference: 0.14 - 1.83 points; *p=0.02*; Table 6.9) on average. When potential confounders were included, this relationship was lost, as the coefficient decreased from 0.983 to 0.846 (95% CI: -0.042 – 1.735; *p=0.06*; Table 6.9). BMI was related to fatigue in this mutually adjusted model. For every 1 $kg \cdot m^{-2}$ increase in BMI, mean fatigue score increased by 0.09 points (95% CI: 0.005 - 0.173 points; *p=0.04*; Table 6.9).

Using the Barron and Kenny method, there was no evidence to suggest that anaemia mediates the relationship between identified HMB and general fatigue. The pathway between anaemia and general fatigue was not significant (p=0.67), and a low proportion of the effect was mediated (0.0053). The Sobel test also provided no evidence of mediation (z-score 0.42; *p>0.05*).

Again using the Barron and Kenny method, there was also no evidence to show that IDA mediates the relationship between identified HMB and general fatigue. The pathway between IDA and general fatigue was not significant (p=0.87), and a low proportion of the effect was mediated (0.0030). The Sobel test also provided no evidence of mediation (z-score 0.17; *p>0.05*).

Table 6.9 - The unadjusted and adjusted relationship between identified heavy menstrual bleeding (HMB) and general fatigue.

	Unadjusted model			**Adjusted model**		
	B	**CI**	**p-value**	**B**	**CI**	**p-value**
Constant	10.483	10.085 – 10.882		9.662	6.744 – 12.580	
HMB	0.983	0.136 - 1.830	0.02*	0.846	-0.042 – 1.735	0.06
IDA				-0.357	-1.417 – 0.703	0.51
Age				-0.032	-0.070- - 0.007	0.11
BMI				0.089	0.005 – 0.173	0.04*
Periods in last 12 months				0.006	-0.119 – 0.132	0.92
Iron sup. (last 3 months)				-0.393	-1.174 - 0.387	0.32

B – coefficient, OR – odds ratio, CI – 95% confidence interval for the odds, HMB – heavy menstrual bleeding, IDA - iron deficiency anaemia, BMI - body mass index
Significant interaction of the variable in the model are shown as follows:, *p < 0.05.

6.6 Discussion

As outlined in Chapter 5, HMB, when identified using the criteria outlined in chapter 3 was common in this study population, affecting approximately a quarter of the participants. It was associated with an increased prevalence of IDA, anaemia and iron deficiency. Identified HMB was found to increase general fatigue, however this pathway was not mediated by anaemia or IDA. Anaemia was associated with an increase in mental fatigue, but neither IDA nor iron deficiency alone were related to any fatigue subscale, and there was no strong correlation between any aspect of fatigue and either [Hb] or serum ferritin. The association between IDA or anaemia and fatigue, and HMB and fatigue has previously been studied, but to our knowledge this is the first study to address the interrelation between all of these in an otherwise healthy, female exercising and menstruating population.

In the present study, those identified with HMB were twice as likely to have IDA when controlling for potential confounders, demonstrating a strong, if not unsurprising association given that HMB is associated with increased blood loss (37). The association between both IDA and anaemia with fatigue is well established, and iron therapy has been shown to be effective in reducing fatigue. However, research is inconclusive as to the association between iron deficiency alone and fatigue (56,226,247-249). Interestingly, in the present study while anaemia was associated with increased mental fatigue, neither the presence of IDA nor iron deficiency resulted in an increase in measured fatigue across any of the subscales. In light of previous uncertainties regarding serum ferritin cut-off values for iron deficiency, and the results in chapter 5, the WHO criteria indicating iron 'depletion' was specifically used here for the diagnosis of iron deficiency (serum ferritin < 15 $\mu g \cdot L^{-1}$) (40). Further, there was also no relationship between serum ferritin or [Hb] and any of the fatigue subscales. Interestingly, a lack of association between fatigue and serum ferritin has previously been found in an adolescent population (38). These findings question the applicability and power of serum ferritin as a sole means to establish iron status. Due to the acute response to inflammation that can ensue with serum ferritin, there is potential for an underlying iron deficiency being masked, thus distorting findings (36). This is inevitably particularly relevant amongst certain populations, including those with underlying inflammatory conditions, and in those who exercise, hence in this study population. This can lead to the misdiagnosis of both iron deficiency and IDA. However, providing no prior exercise had been done on the day of testing, one would expect inflammation in a healthy population, as in the present study, to be limited.

Both the results from the present study, and those in chapter 5 suggest that the use of ferritin as a means for diagnosing iron deficiency alone needs further evaluation.

Particularly given the apparent lack of increase in fatigue in either those with IDA or iron deficiency. It would therefore be advisable to measure other markers of iron status such as transferrin saturation, transferrin, serum iron and total iron binding capacity in addition to erythrocyte and reticulocyte indices prior to concluding any relationship between iron deficiency and fatigue, and future studies should address this. Significantly, in order to minimise risk of inaccuracies, testing should occur when participants are in a rested and fasted state, markers of inflammation should also be measured, and underlying medical conditions recorded and factored into the analysis.

In the present study, when identified using the outlined criteria, HMB was associated with increased general fatigue. Previous research has highlighted the impact of HMB on quality of life (372,408). Since HMB also increased risk of IDA, anaemia and iron deficiency it would seem plausible that the increase in general fatigue associated with HMB could be as a result of the increased loss of iron, especially given the association between IDA or anaemia and fatigue (405). However, neither IDA nor anaemia were found to mediate the relationship between HMB and general fatigue. The lack of association between iron deficiency alone and general fatigue also inevitably meant that this was not on the causal pathway between HMB and general fatigue. Further, when specifically looking at iron status in those with HMB, there was no difference in reported fatigue across any of the subscales between those with or without anaemia, IDA or iron deficiency, highlighting the independence of the HMB to fatigue pathway. In light of the relatively subjective nature of the HMB criteria, and in the absence of a universally validated means for diagnosis, further research is required, however it could be suggested that the sheer physical, social and mental distress alone caused by the increase in blood loss may be the primary cause of the increased fatigue seen here as opposed to the physical increased loss of blood. A previous study assessing causes for a reduction in quality of life amongst those with HMB supports this, finding no association between volume of blood lost and quality of life score, concluding that the physical impact of HMB alone was responsible for the reduction in quality of life (372,420). Alternatively, the impact of other underlying aetiologies associated with HMB also have the potential to be responsible for its association with increased fatigue, and could override any impact of iron status, so this clearly warrants further investigation, and this should be addressed in future work. For example, being an inflammatory condition, HMB is associated with an increased release of pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) (401). There is a strong relationship between TNF-α and fatigue (421), therefore it could be hypothesised that the increase in pro-inflammatory cytokines in HMB cause the ensuing fatigue. However, it is possible that TNF-α would only increase during menses, so if this is driving the fatigue, variations may be seen through the menstrual cycle.

It must also be considered that current phase of the menstrual cycle was not captured, and variation in iron status throughout the menstrual cycle is yet to be conclusively evaluated, therefore some individuals may not have been low in iron or feeling the effects of this at the time of measurement, skewing results. Further, it is significant to note that not all menstrual fluid is blood, the blood content, and therefore amount of iron which is lost can vary significantly. While the insidious nature of fatigue may have meant it was not appreciated and has become habitual to women with HMB or a compromised iron status, altering perception.

To our knowledge this is the first study to address the pathway between HMB and fatigue, and given that it appears to be independent of iron status it is important to gain a better understanding about the causes of the fatigue, in addition to identifying possible treatment options. Future studies should address the above outlined pointed alongside other accompanying factors that may be related to HMB.

The results from this study highlight that when an individual presents with either HMB and/or signs of mental fatigue it would be prudent to take a blood sample to check iron status, while this may not mediate the relationship between HMB and fatigue, and despite the impact of IDA and iron deficiency alone being unsure, there is still potential for these conditions to impact general health and well-being. Other markers of iron status should also be measured alongside these to increase reliability. Further, the independence of the association between identified HMB and fatigue in the present study, regardless of IDA or anaemia suggests that further research should address other factors associated with HMB such as an increased release of pro-inflammatory cytokines that may cause this. Additionally, research evaluating the impact of both IDA and iron deficiency is necessary, in addition to assessing the use of serum ferritin for diagnosis. This will be addressed in the next chapter.

6.6.1 Limitations

Firstly, the limitations with the 'Female Healthy Questionnaire' outlined in chapters 3-5 also apply in this chapter. Additionally, the language issues of the questionnaire highlighted in chapter 5 also require consideration here, and could have caused confusion or lack of understanding of some of the questions. The absence of a measurement for inflammation could have also impacted upon study data, considering that ferritin is an acute phase reactant. Iron deficiency can take a while to manifest, making the development of symptoms slow, so feelings of fatigue may have been adjusted to and missed.

Again, as previously highlighted, data collection only occurred on one day, and therefore other factors may have affected the results, for example specific day in the menstrual cycle or the presence of any illness. Since this population was assumed to be 'healthy', the potential for underlying medical conditions was not factored into the analysis, but this could have had an impact on the results. Finally, the potential bias around only using one healthcare body were outlined in chapter 5. In short, given the homogenous nature of Singaporean population in addition to the established position that SATA CommHealth have in Singapore, and the fact that the haematological data in this study are aligned to that outlined by the WHO and in the 2007 National Health Surveillance Survey the risk of bias is clearly minimised (116,394). Suggesting therefore that this sample is largely reflective of the general Singaporean population of reproductive women.

It is acknowledged that the current study is observational, therefore it is not possible to make strong causal inferences about the associations we have found. We have presented fully adjusted results, but this cannot account for unmeasured confounding. Finally, while the MFI-20 has been validated in multiple populations, it would increase reliability to conduct another questionnaire alongside this for purposes of validation.

6.7 Conclusions

This study showed that HMB and anaemia are both independently associated with an increase in fatigue in menstruating women. The general fatigue that ensued with HMB was not however mediated by anaemia or IDA. This suggests that the symptoms of HMB resulting in fatigue appear to be independent of iron status. HMB increased likelihood of IDA, anaemia and iron deficiency, while mental fatigue was independently associated with anaemia. Therefore, it would be prudent to measure iron status in women presenting with HMB and mental fatigue, however given the lack of association between IDA and iron deficiency and fatigue, the current definition of both of these also needs to be evaluated alongside any impact they may have.

To conclude, regarding the outlined hypotheses for this chapter, the first hypothesis that when identified using the outlined criteria, HMB, IDA, anaemia and iron deficiency increase fatigue cannot be accepted, while identified HMB, and anaemia were associated with an increase in fatigue, IDA and iron deficiency were not. The second hypothesis that a compromised iron status was the cause for the fatigue associated with HMB must be rejected since the associated between HMB and fatigue was independent of IDA or anaemia. Finally, the third hypothesis that HMB and fatigue together increased IDA or anaemia risk must also be rejected as there was no cumulative effect.

6.8 Future perspectives

Being an inflammatory condition, HMB is associated with increased release of pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) (401). This cytokine promotes hypoferremia, and is therefore associated with a systemic iron deficiency, resulting in a decrease in soluble transferrin receptor and serum iron. However, as an acute phase protein, serum ferritin will be transiently elevated in response to this inflammatory mediator (422). However, this would inevitably be dependent on the day of their cycle, since during menses, TNF-α would be higher, but this may return to normal during the rest of their cycle. The increased blood loss alone associated with HMB clearly increases iron deficiency risk, and here there is overt potential for this risk to be exacerbated. To my knowledge, research has only looked at these factors independently; the relationship between HMB and TNF-α, and the relationship between TNF-α and iron status, therefore further research is evidently necessary. However, this does again question the suitability and use of serum ferritin for iron deficiency diagnosis.

Further, given the association between TNF-α and fatigue, future studies should include measurement of pro-inflammatory cytokines alongside fatigue to see if these are in part responsible for the increased fatigue seen in those with HMB.

6.9 Acknowledgements

The author would like to thank SATA healthcare for their cooperation in facilitating access to the female participants.

6.10 Author Contributions

As with the Chapter 5, the 'Female Health Questionnaire' used in this study was written by the author, and the same collaboration between Professor Toby Richards, Mr Tim Cushway and the Singapore Anti-Tuberculosis Association (SATA) Healthcare group was utilised. Again, once the significant prevalence of HMB was established, there was a desire to establish the repercussions of this to emphasise need to change practice in this country, therefore a formalised analysis was deemed necessary.

The same total dataset was used as with chapter 5, but different inclusion criteria were applied by the author. The author extracted the relevant data and performed all the statistical analysis and interpreted all the data.

7 IRONWOMAN trial: the impact of intravenous iron on exercise and aerobic capacity, fatigue and mood disturbance in iron deficient, non-elite exercising women

7.1 Abstract

Introduction: Those who exercise are at an increased risk of iron deficiency, however diagnosis of iron deficiency, particularly amongst the exercising population has historically been problematic. With a lack of clarity around diagnosis and efficacy of existing markers, particularly in those who exercise, further research is required. Using the principle that a positive response to intravenous iron indicates deficiency, the primary aim of this study was to evaluate the impact of iron repletion on aerobic and exercise capacity in iron deficient, non-elite exercising women.

Methods: Thirty-two healthy exercising, iron deficient (serum ferritin ≤ 30 $\mu g \cdot L^{-1}$) women of a reproductive age were recruited to the study (mean age 35.0 ± 8.9 years, weight 61.9 ± 7.8 kg, height 1.65 ± 0.05 m, 22.7 ± 3.0 $kg \cdot m^{-2}$). Participants completed a maximal exercise test, a total haemoglobin mass test, had venous blood samples taken and answered questionnaires to assess fatigue and mood disturbance at baseline. They then received a single high dose of intravenous iron. Two weeks ± five days later baseline tests were repeated.

Results: Markers of exercise and aerobic capacity increased significantly from baseline to post-injection. Relative and absolute $\dot{V}O_{2max}$ increased by 3.30% (49.11 ± 6.41 $mL \cdot kg^{-1} \cdot min^{-1}$ to 50.73 ± 7.16 $mL \cdot kg^{-1} \cdot min^{-1}$; *p=0.003*) and 2.35% (2974 ± 357 $mL \cdot min^{-1}$ to 3044 ± 365 $mL \cdot min^{-1}$; *p=0.03*) respectively. Total haemoglobin mass also increased by 3.17% ($g \cdot kg^{-1}$; *p=0.005*) and 2.44% (g; *p=0.03*) after the iron injection. However, a significant degree of individual response was seen. When broken down into groups based on baseline serum ferritin, increase in exercise and aerobic capacity were only seen in those with more severe iron deficiency (serum ferritin < 15 $\mu g \cdot L^{-1}$). Despite this, there was no association between baseline serum ferritin and change in any markers of exercise or aerobic capacity. Fatigue and mood disturbance was reduced in all participants.

Conclusions: While there was an overall increase in markers of exercise and aerobic capacity, there was a significant degree of individual response. When results were broken down according to baseline serum ferritin, increases were only seen in those with more severe iron deficiency. This suggests that serum ferritin cut-offs often applied may be too high. However, the lack of association between baseline serum ferritin and exercise and aerobic response questions the appropriateness of serum ferritin as a diagnostic marker in isolation. Across the board improvements in fatigue may suggest that iron deficiency impacts upon cognition prior to aerobic and performance measures.

7.2 Introduction

Iron deficiency anaemia (IDA) and iron deficiency non-anaemia (IDNA) are more common in those who exercise than in sedentary controls (206). In agreement with previous research, approximately one third of athletes in Chapter 5 were found to be iron deficient when using serum ferritin for diagnosis (serum ferritin < 15 $g \cdot dL^{-1}$) (51,120,121). The evident impact of IDA on exercise performance and general health means that intervention is necessary. However, the equivocal research surrounding the impact of iron deficiency without anaemia, particularly in those who exercise has resulted in inconclusive advice as already highlighted in this thesis. This could in part be caused by the use of [Hb] and serum ferritin for diagnosis, both of which are susceptible to distinct variation, particularly in those who exercise (Section 2.5).

Previous studies evaluating the impact of iron deficiency have applied the principle that iron deficiency can be indicated if an individual responds positively to iron therapy (47). Since intravenous injections bypass the controlled absorption mechanism, these provide the most effective means for evaluation, however, recent research specifically in the exercising population using this method has only focused on elite and highly trained athletes, who, as a result of the increased physiological stress, may respond differently to a more general exercising and healthy population in whom research is sparse (7). Due to their substantial training status, potential for improvements in exercise and aerobic capacity in elite athletes may be very small, and statistically not significant. This may be a cause for the ambiguity in this area. Further, a degree of individual variation in response to iron therapy has been found (241), the reasons for this being unknown, but this could also be a reflection of the unsuitability of the markers used for diagnosis, or alternatively the inconsistencies in the protocol utilised, as previously explained. Utilising the principle that a positive response to iron therapy suggests iron deficiency (47), measures of iron deficiency could be improved.

No benefit has been demonstrated for supplementing with iron when iron sufficient (39), however, it is well known that coaches frequently advise supplementation without prior knowledge of iron status. In fact, there is potential for excess supplementation to impact iron absorption, causing a transient increase in hepcidin, countering iron absorption (51). In fact, recent research has highlighted alternate day oral iron supplementation is more effective than consecutive day supplementation (423). Further, unnecessary supplementation can have negative health implications, particularly in those who have genetic abnormalities that predispose them to iron overload (7). In Chapter 4, nearly half of the marathon runners (45.3%) and more than three quarters of the elite athletes (78.9%) were found to supplement with iron. While of those who indicated taking

supplementation, more than half (50.5%), reported no knowledge of their iron status. Since there is currently no evidence-based guidance for treating iron deficiency without anaemia in the exercising population, clarity is evidently required, and in accordance with this, awareness of best practices when supplementing is essential.

7.2.1 Study aims

1. The primary aim of this study was to assess the impact of iron repletion on exercise and aerobic capacity in iron deficient, but otherwise healthy exercising women when using existing criteria for the diagnosis of iron deficiency.
2. The secondary aims were then to assess the impact of intravenous iron on haematological markers and subjective markers of fatigue and mood disturbance in iron deficient, but otherwise healthy exercising women when using existing criteria for the diagnosis of iron deficiency.

7.2.2 Study hypotheses

1. Iron repletion improves exercise and aerobic capacity in iron deficient, non-elite exercising women.
2. Iron repletion improves haematological markers and subjective markers of fatigue and mood disturbance in iron deficient
3. Response to iron repletion will be greater in those identified with more severe iron deficiency.

7.3 Materials and methods

7.3.1 Ethics and participant consent

The IRONWOMAN trial is listed on the ISRCTN registry (ISRCTN14032359) and approved by both IRAS NHS Ethics Committee (15/LO/1570), and by the St Mary's University Ethics Committee. At the time of screening, all participants were required to complete and sign a consent form.

7.3.2 Inclusion criteria

- Female;
- Aged ≥ 18 years;
- Have a baseline serum ferritin level (≤ 30 $\mu g \cdot L^{-1}$);
- Undertake regular exercise (≥ 60 minutes/week); or
- Have not made any significant changes to training in the previous 6 months.

7.3.3 Exclusion criteria

- Pregnancy or lactation;
- A known reason for anaemia;
- A known history of iron overload or a family history of haemochromatosis, thalassemia or a transferrin saturation > 50%;
- Recent involvement in a study investigating a medical product (previous four weeks);
- Severe asthma or severe allergy requiring hospitalisation in the previous 12 months;
- Indication that they will be changing training over the time course of the study.

7.3.4 Participants

Recruitment of participants took the form of advertisement through various social media channels and word of mouth. Eighty-three regularly exercising women who indicated they either may be iron deficient or who had a history of iron deficiency had a full screening blood test in a fasted state and at rest (no exercise so far that day). A total of 32 women (as per the sample size calculation – Section 7.2.19.1) aged between 20 and 55 years met the inclusion criteria and were recruited to this study (Table 7.1). Since a lack of iron in bone marrow has been observed when serum ferritin is 30 $\mu g \cdot L^{-1}$, and using this cut-off value, sensitivity and specificity for iron deficiency diagnosis was found to be 92% and 96% respectively (42), this cut-off for serum ferritin for iron deficiency diagnosis was applied.

7.3.5 Study design

The IRONWOMAN trial was an interventional study conducted from March 2016 to October 2016. After initial screening, iron deficient, exercising women were recruited to this study. At baseline, all participants had a $\dot{V}O_{2max}$ test, the protocol for which is outlined in section 7.3.7.2, a total haemoglobin mass test, as outlined in section 7.3.8.2 and completed four questionnaires to profile fatigue and mood disturbance in addition to haematological analysis from a venous blood sample. They were then administered a single dose of intravenous iron (iron isomaltoside 1000, Pharmacosmos, UK). Each receiving 20 $mg \cdot kg^{-1}$ of bodyweight. Two weeks (±5 days) later, all baseline tests were repeated. An overview of the study is described in Figure 7.1. Prior to inclusion, the full screening blood test was performed on each participant when fasted and at rest.

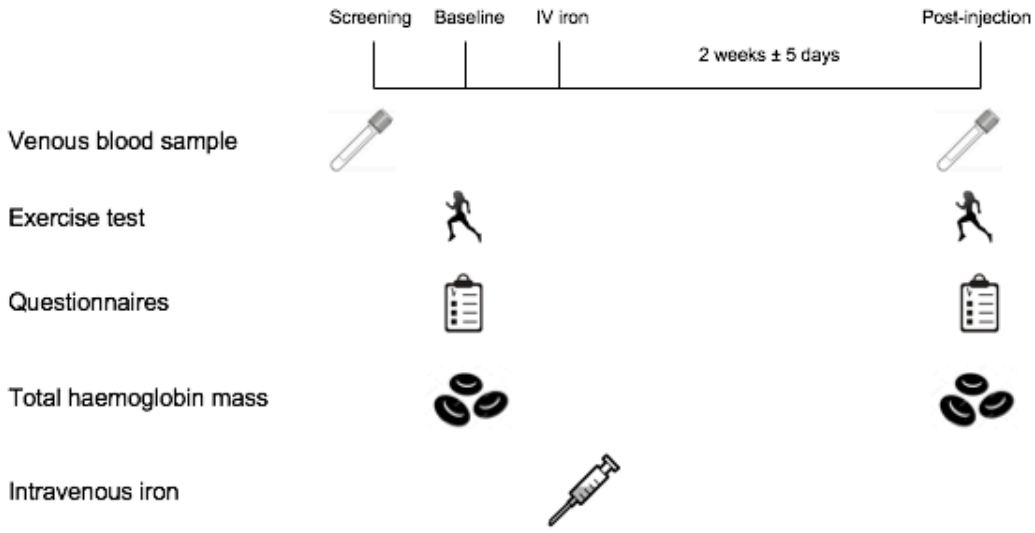

Figure 7.1 – Study design.

7.3.6 Measurements

7.3.7 Measurement of Exercise and aerobic capacity: Exercise test

Maximal aerobic capacity ($\dot{V}O_{2max}$), which is a component of physical fitness, was assessed on a treadmill both at baseline and post-injection. The same warm up protocol and set treadmill speed were used in both the baseline and post-injection tests.

Physiology of the measurement

In a healthy human, at rest, the amount of oxygen consumed ($\dot{V}O_2$) is equivalent to that required by cells, while the output of carbon dioxide ($\dot{V}CO_2$) reflects that produced. This is described by the Fick equation, showing that oxygen consumption is the product of cellular oxygen delivery and oxygen extraction (424):

$$\dot{V}O_2 = \dot{Q} \times (CaO_2 - CvO_2)$$

Where $\dot{Q}$ - cardiac output, CaO_2 - arterial oxygen content, CvO_2 - venous oxygen content.

Therefore, where maximal cardiac output is achieved alongside maximal oxygen extraction, a total value for maximal oxygen consumption ($\dot{V}O_{2max}$) can be obtained (424). This is a measure widely used in exercise physiology to indicate aerobic fitness.

$$\dot{V}O_{2max} = \dot{Q}_{max} \times (CaO_{2max} - CvO_{2max})$$

7.3.7.1 Equipment and calibration

All tests were performed on a Woodway treadmill in the sports performance laboratory at St Mary's University, Twickenham (Figure 7.2). Gas analysis was performed using an Jaegar Oxycon Pro (CareFusion, Hoechberg, Germany – running JLab software), which is an online breath-by-breath analyser. This measures $\dot{V}O_2$, $\dot{V}CO_2$, respiratory rate, minute ventilation, tidal volume and end-tidal gas tensions. Before each test, participant demographics were inputted into the software.

Prior to usage however, a number of calibration procedures took place:

1. The pressure analyser was calibrated using the in-laboratory barometer (Oregon Scientific).
2. The volume transducer was calibrated.
3. The oxygen and carbon dioxide analyser was calibrated.

Volume calibration

The flow sensor was calibrated using a 3-litre syringe according to the manufacturers guidelines (Viasys Healthcare, GmbH, Hoechberg, Germany). Smooth strokes were required for calibration. Any values outside ± 0.1 litres resulted in calibration failure.

Gas calibration

After switching on the Oxycon Pro, and allowing time for it to warm up in accordance with the instructions from the manufacturer, a two-point gas calibration was conducted. The first mixture was ambient air (20.93% oxygen and 0.03% carbon dioxide), the second mixture was calibration/reference gas (16% oxygen and 5% carbon dioxide). If gaseous values were not within 0.05% (0.02% for carbon dioxide in ambient air) of these values, the calibration process failed and would have to be repeated.

A facemask containing an ergospirometric device connecting it to the Oxycon Pro was fitted to participants to ensure all inspired and exhaled gas was collected. Potential for gaseous leaks was carefully assessed.

7.3.7.2 Test procedure

Participants performed a 10-minute warm-up at a self-selected speed which was monitored and recorded. After a 5-minute break, participants undertook a maximal exercise test. The treadmill was set at a continuous pre-determined speed. The gradient of the treadmill increased in an incremental manner by 0.5% every 30 seconds until volitional exhaustion

was reached. At this point a blood capillary sample was taken immediately from the earlobe for blood lactate analysis and measured using an automated analyser (Biosen C-Line, EFK Diagnostic, Barleben, Germany). Tests were stopped when participants either hit the 'stop' button on the treadmill or stepped off the treadmill. Participants were then required to undertake active recovery. The same warm up protocol and set treadmill speed were used in both the baseline and post-injection tests. Three different fixed treadmill test speeds were applied; fast (12 km·h^{-1}), medium (10 km·h^{-1}), and slow (8 km·h^{-1}). These were determined based on 5 km best times in the last six months. The groups were as follows:

Fast: those with times < 20 minutes: 15 seconds
Medium: 20 minutes: 15 seconds – 25 minutes: 15 seconds
Slow: > 25 minutes: 15 seconds.

Measurement of $\dot{V}O_{2max}$

Rolling 30-second average readings were provided for all gaseous measurements. Those sitting four standard deviations from the midpoint of a seven-breath average were removed. The highest average $\dot{V}O_2$ measure was deemed to be peak $\dot{V}O_2$, and therefore the $\dot{V}O_{2max}$. Similarly, peak $\dot{V}CO_2$ and the respiratory exchange ratio were also recorded.

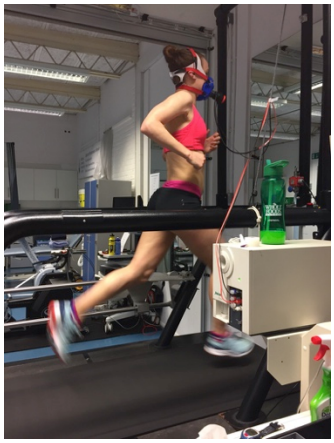

Figure 7.2 - $\dot{V}O_{2max}$ test in the St Mary's University performance lab.

7.3.8 Measurement of Exercise and aerobic capacity: Total haemoglobin mass test

The optimised carbon monoxide rebreathing test as described by Schmidt and Prommer (4), was used to measure total haemoglobin mass, blood volume and plasma volume at baseline and post-injection (Figure 7.3). A bolus of chemically pure carbon monoxide of 0.9 mL·kg^{-1} body mass was administered to participants through a closed glass spirometer

(Bloodtec, Bayreuth, Germany) and rebreathed for two minutes with 4 L of oxygen. A blood gas analyser (ABL80 Flex Radiometer, Denmark) was used to measure carboxyhaemoglobin, haematocrit and haemoglobin, from capillary blood samples that were taken from the ear lobe in triplicate at baseline and six minutes and eight minutes after carbon monoxide administration. A carbon monoxide analyser (Pac700, Drager, Lubeck, Germany) was used to measure residual carbon monoxide volume both before and four minutes after rebreathing. The remaining carbon monoxide in the spirometer was added to the end-tidal carbon monoxide concentration to calculate the total volume that had not been taken up by the body. These values were then used to determine total haemoglobin mass.

7.3.8.1 Test background

The optimised carbon monoxide rebreathing test as described by Schmidt and Prommer (4), was used to measure total haemoglobin mass, blood volume and plasma volume at baseline and post-injection. Unlike a venous blood sample providing a measure of [Hb], this test factors in plasma volume to provide an overall value for total haemoglobin mass (Section 2.6). Further, since the majority of oxygen travels in the blood bound to erythrocytes, total haemoglobin mass can be used to provide a measure of oxygen carrying-capacity. Each gram of haemoglobin can bind to 1.39 mL of oxygen. Particularly amongst athletes where plasma volume shifts are likely (28,392), haemoglobin mass has been suggested to provide a more accurate indication of oxygen transportation ability, in fact, Otto *et al.* (2013) showed total haemoglobin mass to correlate more effectively with $\dot{V}O_2$ peak than [Hb] (40), and Wachsmuth *et al.* (2015) showed a lower coefficient of variation in total haemoglobin mass when compared to [Hb] (415).

7.3.8.2 Equipment and test procedure

After a 15-minute period of seated rest to allow stabilisation of blood volume, residual carbon monoxide volume was measured using a carbon monoxide analyser (Pac700, Drager, Lubeck, Germany). Capillary blood samples were then taken from the earlobe in triplicate (80 µl – Hawksley & Sons), and were analysed immediately using blood gas analyser (ABL80 Flex Radiometer, Denmark) to measure for carboxyhaemoglobin, haematocrit and haemoglobin.

While still in a seated position, participants rebreathed a bolus of chemically pure carbon monoxide of a known volume (0.9 mL·kg^{-1} body mass) through a closed glass spirometer (Bloodtec, Bayreuth, Germany) for two minutes with 100% pure medical grade oxygen. A mouthpiece containing approximately 10 g of soda lime (to absorb the carbon dioxide) was

fitted to one end of the spirometer. A three litre bag containing the oxygen was fitted to the other end of the spirometer. Carbon monoxide was administered through a syringe (Figure 7.3).

Test procedure

After placing the nose clip on, participants took a full inhalation, followed by full exhalation to residual volume and were connected to the mouthpiece. Participants then inhaled deeply and held their breath for ten seconds as the carbon monoxide was administered in conjunction with opening the valve to release the oxygen. This was to enable diffusion of carbon monoxide into the bloodstream. Since haemoglobin has a much greater affinity for carbon monoxide than oxygen it is assumed that this will rapidly enter the bloodstream and bind to haemoglobin (425). After this, they then breathed normally within the closed system (426) for a further one minute and 50 seconds. The carbon monoxide analyser (Pac700, Drager, Lubeck, Germany) was used to check for any gas leaks from the mouth and nose.

Prior to disconnecting from the system, participants exhaled to residual volume, and this gas was collected in the three litre bag, allowing determination of the carbon monoxide that was not absorbed. Four-minutes after rebreathing the carbon monoxide, the participants repeated the assessment for the measurement of residual carbon monoxide volume, using the carbon monoxide analyser (Pac700, Drager, Lubeck, Germany). Blood capillary samples from the earlobe were then taken in triplicate both at 6-minutes and 8-minutes after rebreathing the carbon monoxide and were again measured for carboxyhaemoglobin, haematocrit and haemoglobin immediately, using blood gas analyser (ABL80 Flex Radiometer, Denmark).

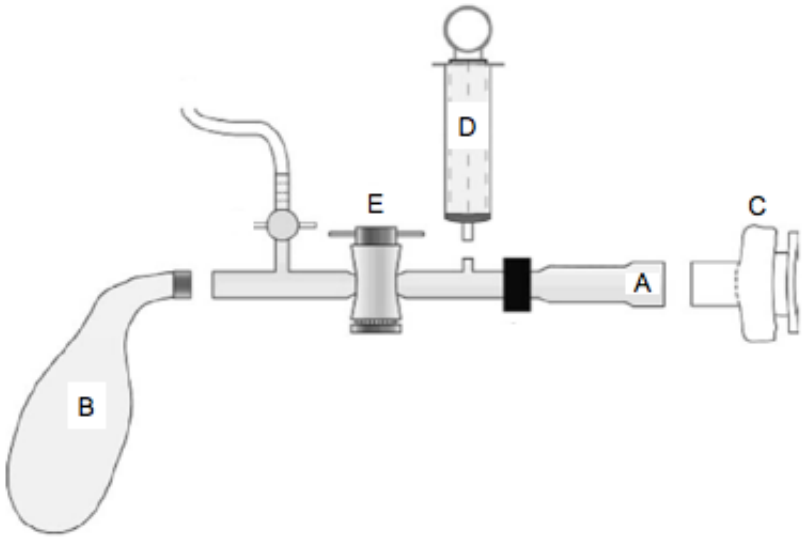

Figure 7.3 – Haemoglobin mass test equipment - glass spirometer (A), with bag (B) and mouthpiece attached (C), gas syringe (D), oxygen valve (E). Figure adapted from (4).

7.3.8.3 Calculation of total haemoglobin mass

Total haemoglobin mass was calculated using the following formula (as applied by Schmitt and Prommer, 2005) (4). This was inserted into an Excel Spreadsheet (Microsoft Excel for Apple Macintosh 2011) for automatic calculation.

$$tHbmass\ (g)\ = K\ \times\ MCO\ \times\ 100\ \times\ (\Delta HbCO\%\ \times\ 1.39)^{-1}$$

K = current barometric pressure $\times\ 760^{-1}\ \times\ [1 + (0.003661\ \times\ current\ temperature)]$

$MCO = CO_{\text{adm}} - (CO_{\text{system+lung (after disconnection)}} + CO_{\text{exhaled (after disconnection)}})$

Where:

CO_{adm} = the volume of CO administered into the system

$CO_{\text{system+lung (after disconnection}}$ = CO concentration in spirometer x (spirometer volume + lung residual volume)

$CO_{\text{exhaled (after disconnection)}}$ = end-tidal CO concentration x alveolar ventilation x time

$\Delta HbCO\%$ = difference between baseline %HbCO and %HbCO in the blood samples after rebreathing the carbon monoxide (the average between the samples collected 6-minutes and 8-minutes after rebreathing)

1.39 = Hüfners number (ml CO x g·Hb^{-1}) (427)

7.3.8.4 Calculation of blood volume (BV)

$$BV\ (ml)\ = \frac{tHbmass\ (g)}{[Hb](\mathrm{g}\cdot dL^{-1})} \times\ 100$$

7.3.8.5 Calculation of erythrocyte volume (EV)

$$EV\ (ml)\ = BV\ (ml)\ \times\ Hct\ (\%)$$

7.3.8.6 Calculation of plasma volume

$$PV\ (ml) =\ BV(ml) - EV(ml)$$

Example
If a participant's body mass is 54 kg, they will be given 48.6 mL carbon monoxide (given that 0.9 mL CO was given per kg of body weight). According to Hüfners number, 35 g of haemoglobin will be bound. Then if the change in baseline %HbCO and post rebreathing %HbCO is 5.2% (therefore 5.2% of the total Hb is bound to CO) of the total haemoglobin mass, their overall total haemoglobin mass will be 673 g (12.5 g·kg^{-1}).

7.3.9 Haematology and biochemistry

Venepuncture was used to collect venous blood from an antecubital vein in the forearm at baseline and post-injection. A 4 mL EDTA vacutainer was filled and inverted ten times, and a 10 mL SST gel vacutainer was filled and inverted five times prior to being chilled and either taken or sent for lab analysis at the Royal Surrey Hospital, Guildford, Surrey. Full

blood count data were provided using an ADVIA 2020i, and serum ferritin was measured using the ADVIA Centaur XP immunoassay system.

7.3.10 Fatigue

To ensure internal validation three fatigue scales were completed by participants at baseline and post-injection, including (Appendix 10.15):

Multidimensional Fatigue Inventory (MFI-20)

The Multidimensional Fatigue Inventory (MFI-20) is a self-reporting tool that includes 20 statements addressing five aspects of fatigue; general, physical, reduced motivation, reduced activity and mental fatigue (410). High scores indicate increased levels of fatigue. The MFI-20 has previously been used effectively in exercise and fatigue studies and content and construct validity has been shown in a general population and in exercise and iron repletion studies (414,428-430).

Piper Fatigue Scale

The Piper Fatigue Scale is a 22-part self-assessing fatigue scale designed to evaluate current fatigue levels (431). High scores indicate a higher degree of fatigue. This has previously been used effectively in iron repletion studies with iron deficient patients (226).

European Quality of Life – 5 Dimensions – 5 Levels (EQ-5D-5L)

The European Quality of Life – 5 Dimensions – 5 Levels (EQ-5D-5L) questionnaire is a brief, utility-based means for assessing health related quality of life (368,369). It consists of a health descriptive system and a visual analogue scale (EQ-VAS) for respondents to self-classify and rate their health on the day of administration of the instrument. It has been used widely in a range of different settings to assess quality of life.

7.3.11 Mood disturbance

Brunel Mood Scale (BRUMS) (Appendix 10.15)

The Brunel Mood Scale (BRUMS) is a 24-part questionnaire which is an abbreviated version of the Profile of Mood States (POMS) questionnaire (370). It addresses six factors including confusion, tension, depression, anger, fatigue and vigour (371). Participants select a value from 0 to 4 depending on how they feel at the time of asking. This has previously been used effectively in the sporting context (58,432).

7.3.11.1 Training and menstrual cycle questionnaire

Participants were asked a series of questions about their exercise participation, menstrual cycle and health. These were designed to obtain information about training volume and exercise participation level in addition to identifying current menstruation status, including the presence of heavy menstrual bleeding, diagnosed using a four-part criteria as used in Chapters 4 – 6 and in previous studies (28,392).

7.3.11.2 Physical state and menstrual characteristics

Participants were requested to complete a questionnaire (appendix 10.14) to establish training history and performance, in addition to and menstrual characteristics, in addition to enable identification of when in the menstrual cycle tests were conducted. Participants were also asked to maintain a similar training schedule and diet between baseline and post-injection tests. Therefore every effort was made to ensure the athletes did the post-injection tests in a similar physiological state and at a comparable time of day to their baseline tests. Participants were asked to report any deviations in training or nutrition. Two participants suffered ankle injuries between the baseline and post-injection tests, and as a result were unable to partake in the post-injection exercise test. No participants reported any recent changes in training in the 6 months prior to study involvement.

7.3.12 Iron administration

All participants were given a single dose of intravenous iron (iron isomaltoside 1000, Pharmacosmos, UK). Each receiving 20 $mg \cdot kg^{-1}$ of bodyweight administered as a single infusion over a period of 30 - 90 minutes.

7.3.13 Data analysis

7.3.13.1 Sample size calculation

Using external data, we estimated that the standard deviation of the change form baseline in $\dot{V}O_{2max}$ will be 2.5 $mL \cdot kg^{-1} \cdot min^{-1}$. Based on this, we calculated that 32 participants will provide 90% power at the 5% significance level to detect a mean change from baseline in $\dot{V}O_{2max}$ of 1.5 $mL \cdot kg^{-1} \cdot min^{-1}$. This calculation allows for a 5% loss to follow-up.

7.3.13.2 Statistical analysis

Statistical analysis was performed using a statistics computer package (IBM SPSS Statistics for Macintosh, Version 21.0, Armonk, NK: IBM Corp). Statistical significance was set at $p<0.05$. Graphpad Prism (GraphPad Prism version 7.00b for MacOS X, GraphPad Software, La Jolla, California, USA) was also used to visually display the data.

All data were tested for normality. Where data was normally distributed, paired t-tests were used to identify differences between baseline and post-injection data, and Wilcoxon signed-rank tests were used for paired non-normal data. The percentage change in all raw values from baseline to post-injection were calculated in an excel spreadsheet (Microsoft Excel for Macintosh, 2011).

Participants were divided into groups based on the World Health Organisation's serum ferritin cut-off value for iron deficiency of < 15 $\mu g \cdot L^{-1}$ (40). Those with a serum ferritin < 15 $\mu g \cdot L^{-1}$ were included in the 'severe' iron deficiency group, and those with a serum ferritin > 15 $\mu g \cdot L^{-1}$ but ≤ 30 $\mu g \cdot L^{-1}$ were placed into the 'moderate' iron deficiency group. Paired t-tests were then performed within each group to assess whether extent of iron deficiency affected response.

To assess the relationship between each of baseline [Hb] and baseline serum ferritin and both the change in $\dot{V}O_{2max}$ and change in total haemoglobin mass linear regressions were performed. To describe the relationship between the combination of baseline [Hb] and serum ferritin and change in $\dot{V}O_{2max}$ or change in total haemoglobin mass a multiple linear regression model was used. This was then adjusted for potential confounders including age, BMI, 5 km personal best time, total exercise volume each week and total running volume each week. Linear regressions were used to assess the relationship between change in [Hb] and change in serum ferritin and change in markers of exercise and aerobic capacity. Pearson's correlations were used to determine whether there was a relationship between baseline and post-injection $\dot{V}O_{2max}$ and total haemoglobin mass, in addition to assessing any association between the change in $\dot{V}O_{2max}$ and change in haemoglobin mass. While Pearson's or Spearman's correlations (depending on the type of data) were also used to determine whether there was a relationship between other baseline markers and change in the primary outcome variables.

The potential confounding effect of day of the menstrual cycle when looking at the association between baseline [Hb] or serum ferritin and functional outcome variables was assessed using multiple linear regression.

Wilcoxon signed rank tests were used to identify differences in fatigue (MFI-20 and Piper Fatigue Scale) and mood (BRUMS Mood Scale) between baseline and post-injection. To assess the validity and reliability and to ensure internal consistency of each of the five MFI-20 subscales both at baseline and post-injection, three reliability tests were performed; 1. inter-item correlation, 2. corrected-to-total correlation, and 3. calculation of the

standardised Cronbach's alpha coefficient. Inter-item correlations ranging from 0.30-0.70 were kept, while a value for the corrected item-total correlation of > 0.20 was deemed adequate (415). A relatively high reliability coefficient - Cronbach's ∝ ranging from 0.70-0.95 was considered to be sufficient (416). Cronbach's alpha if item was deleted were also calculated. Linear regressions were also run to assess the relationship between each of baseline [Hb] and baseline serum ferritin and change in the MFI subscales.

The EQ-5D-5L data was analysed by determining the percentage of individuals who reported no problems, in comparison to those who reported problems. A Wilcoxon signed-ranks test was then used to determine differences.

7.4 Results

7.4.1 Participant characteristics

Thirty-two participants completed the trial. Participant characteristics are detailed in Table 7.1.

Table 7.1 – Baseline participant characteristics.

Characteristic	Mean (± SD)	Range (min – max)
Age (years)	35.0 ± 8.9	20 - 55
Weight (kg)	61.9 ± 7.8	50.1 – 80.8
Height (m)	1.65 ± 0.05	1.55 – 1.78
BMI ($kg \cdot m^{-2}$)	22.7 ± 3.0	18.6 – 31.6
sFer ($\mu g \cdot L^{-1}$)	15.7 ± 7.0	7 – 30
[Hb] ($g \cdot dL^{-1}$)	12.4 ± 0.6	11.0 – 14.0
Average weekly exercise volume (h:min:s)	6:59.50 ± 3:34.16	1:00 – 15:30
5 km personal best (min:s)	24:02 ± 4:04	18:36 – 36:55
Heavy menstrual bleeding	64.5% (n=20)	-
Menstrual cycle affected training and performance	54.8% (n=17)	-

BMI – body mass index, sFer – serum ferritin, [Hb] – haemoglobin concentration
Values are mean ± SD.

7.4.2 Exercise and aerobic capacity

All markers of exercise and aerobic capacity increased significantly from baseline to post-injection (Table 7.2; Figure 7.4; *p<0.05*). Relative and absolute $\dot{V}O_{2max}$ increased by 3.30% (49.11 ± 6.41 $mL \cdot kg^{-1} \cdot min^{-1}$ to 50.73 ± 7.16 $mL \cdot kg^{-1} \cdot min^{-1}$; *p=0.003*) and 2.35% (2974 ±

357 mL·min^{-1} to 3044 ± 365 mL·min^{-1}; *p=0.03*) respectively. Overall, the $\dot{V}O_{2max}$ of 70% of the participants increased two weeks after the iron injection, with the mean increase being 1.62 mL·kg^{-1}·min^{-1}.

Total haemoglobin mass also increased by 3.17% (g·kg^{-1}; Table 7.2; Figure 7.4; *p=0.005*) and 2.44% (g; Table 7.2; Figure 7.4; *p=0.03*) after the iron injection, and on average participants were able to keep running on the treadmill for 56 seconds longer after the injection (*p<0.0005*).

Table 7.2 – A comparison between exercise and aerobic capacity, and haematology and biochemistry measures at baseline and post intravenous iron injection.

	Baseline	Post	Percentage change	p-value	
Exercise and aerobic capacity					
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})#	49.11 ± 6.41	50.73 ± 7.16	+3.30%	0.003**	
$\dot{V}O_{2max}$ (mL·min^{-1})#	2974 ± 357	3044 ± 365	+2.35%	0.026*	
tHbmass (g·kg^{-1})	9.93 ± 1.10	10.22 ± 1.29	+3.17%	0.007**	
tHbmass (g)	610 ± 65	618 ± 60	+2.44%	0.026*	
Blood Volume (g·kg^{-1})	87.2 ± 8.47	86.2 ± 11.19	-1.05%	0.53	
TTE (mins)#	10.13 ± 2.17	10.93 ± 2.29	+7.90%	<0.0005****	
Haematology and Biochemistry	**Baseline**	**Post**	**Percentage change**	**p-value**	**Clinical reference range**
sFer (µg·L^{-1})	15.7 ± 7.0	367.4 ± 144.3	+2242%	<0.0005****	15 – 250
[Hb] (g·dL^{-1})	12.4 ± 0.6	13.1 ± 0.6	+5.52%	<0.0005****	11.5 – 16.5
Hct (ratio)	0.37 ± 0.02	0.40 ± 0.02	+6.40%	<0.0005****	0.37 – 0.47
MCH (pg)	29.4 ± 1.8	29.8 ± 1.4	+1.49%	0.011*	26 – 35
MCV (fL)	88.5 ± 4.2	90.8 ± 3.9	+2.56%	<0.0005****	75 – 105
CH (pg)	29.03 ± 1.75	29.75 ± 1.63	+2.84%	<0.0005****	
CHCM (g·L^{-1})	329.23 ± 7.48	330.37 ± 10.58	+0.35%	0.50	
HDW (g·L^{-1})	24.96 ± 1.98	23.70 ± 1.73	-4.86%	<0.0005****	
Hyperchromia (%)	0.46 ± 0.24	0.51 ± 0.41	+22.52%	0.97	

Hypochromia (%)	2.35 ± 2.61	1.70 ± 1.94	-0.65%	0.022*	
Large platelets ($10^9 \cdot L^{-1}$)	7.39 ± 2.70	7.89 ± 3.64	+6.32%	0.29	
Lymphocytes ($10^9 \cdot L^{-1}$)	1.38 ± 0.36	1.68 ± 0.51	+24.21%	0.0003***	1.0 – 4.0
Macro (%)	0.54 ± 0.63	1.36 ± 1.25	+260.83%	<0.0005****	
MCHC ($g \cdot L^{-1}$)	33.20 ± 0.83	32.83 ± 0.83	-1.05%	0.073	290 – 350
Micro (%)	1.34 ± 1.69	0.93 ± 0.94	-15.02%	0.0042**	
Monocytes ($10^9 \cdot L^{-1}$)	0.31 ± 0.11	0.31 ± 0.10	+5.34%	0.99	0.2 – 0.8
MPC ($g \cdot dL^{-1}$)	23.73 ± 1.85	23.13 ± 2.01	-2.18%	0.16	
MPM (pg)	2.19 ± 0.20	2.16 ± 0.18	-1.38%	0.12	
MPV (fL)	9.75 ± 0.78	9.89 ± 1.11	+1.46%	0.36	
Neutrophils ($10^9 \cdot L^{-1}$)	2.97 ± 1.59	3.12 ± 1.16	+41.48%	0.46	2.0 – 7.5
PCT (%)	0.24 ± 0.06	0.24 ± 0.05	+5.00%	0.41	
PDW (%)	57.13 ± 24.56	52.56 ± 6.41	-2.51%	0.71	
Platelets ($10^9 \cdot L^{-1}$)	242.94 ± 61.19	248.94 ± 56.03	+4.49%	0.33	150 – 450
RBC ($10^{12} \cdot L^{-1}$)	4.23 ± 0.25	4.40 ± 0.27	+4.06%	0.0029**	3.5 – 5.5
RDW (%)	13.72 ± 1.60	14.37 ± 1.73	+4.92%	0.0016**	11.0 – 15.0
WBC ($10^9 \cdot L^{-1}$)	5.07 ± 1.83	5.37 ± 1.43	+12.67%	0.21	

tHbmass – total haemoglobin mass, TTE – time to exhaustion, sFer – serum ferritin, [Hb] – haemoglobin concentration, MCH – mean cell haemoglobin, MCV – mean cell volume,

CH – corpuscular haemoglobin content, CHCM – corpuscular haemoglobin concentration mean, HDW – haemoglobin distribution width, MCHC – mean cell haemoglobin concentration, MPC – mean platelet component, MPM - , MPV – mean platelet volume, PCT – platelet haematocrit, PDW – platelet distribution width, RBC – red blood cells, RDW – red cell distribution width, WBC – white blood cells

Values are mean ± SD.

Significant differences between baseline and post injection tests are shown as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0005.

[#] Note that due to sustained injuries n=30 for these data.

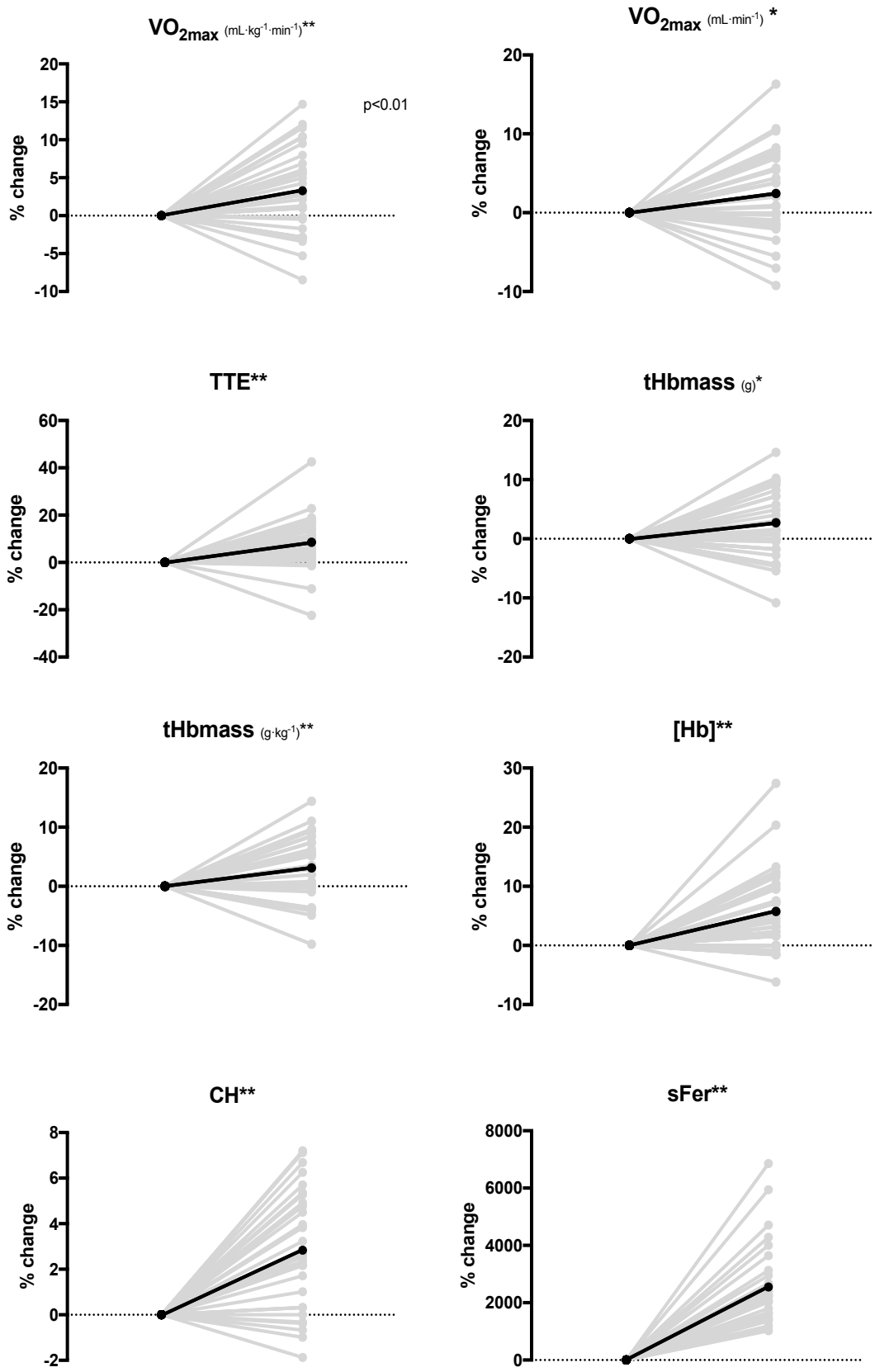

Figure 7.4 – Effect of intravenous iron on primary and secondary endpoints. Individual changes in $\dot{V}O_{2max}$, time to exhaustion (TTE), total haemoglobin mass (tHbmass), haemoglobin concentration ([Hb]), corpuscular haemoglobin content (CH) and ferritin

(sFer) from baseline to 2 weeks post intravenous iron. Data are presented as a percent change from baseline with individual changes shown in grey, and mean change in black. Significant differences between baseline and post injection tests are shown as follows: *p < 0.05, **p < 0.01.

7.4.3 Haematology and biochemistry

Full results are shown in Table 7.2. Both serum ferritin and [Hb] increased significantly from baseline to follow up (Table 7.2; Figure 7.4; *p<0.0005*). Other red cell measures including haematocrit (*p<0.0005*), corpuscular haemoglobin content (*p<0.0001*), mean cell haemoglobin (*p=0.01*), mean cell volume (*p<0.0005*) and red cell distribution width (*p=0.002*) also increased (Table 7.2).

7.4.4 The effect of baseline serum ferritin

Participants were grouped into 'severe' and 'moderate' iron deficiency using the World Health Organisation (WHO) cut-off value of a serum ferritin < 15μg·L^{-1} (40,41). Those with a serum ferritin < 15μg·L^{-1} were defined as having 'severe' iron deficiency, and those with a serum ferritin > 15 μg·L^{-1} but ≤ 30 μg·L^{-1} were defined as having 'moderate' iron deficiency. $\dot{V}O_{2max}$ and relative total haemoglobin mass only increased in those in the severely iron deficient group (Table 7.3; Figure 7.5; *p<0.05*).

Table 7.3 – Changes in exercise and aerobic capacity when comparing those with severe (sFer < 15 µg·L^{-1}) and moderate (sFer > 15 µg·L^{-1} but ≤ 30 µg·L^{-1}) iron deficiency.

	sFer < 15µg·L^{-1} (n=18)		**p-value**	**sFer > 15 µg·L^{-1} but ≤ 30 µg·L^{-1} (n=14)**		**p-value**
	Baseline	**Post**		**Baseline**	**Post**	
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})#	50.1 ± 5.11	52.5 ± 5.53	0.0009***	47.82 ± 7.84	48.43 ± 8.56	0.48
$\dot{V}O_{2max}$ (mL·min^{-1})#	2922.4 ± 295.53	3044.5 ± 310.08	0.0029**	3042.85 ± 426.88	3043.46 ± 440.46	0.99
TTE (min:sec)#	10.2 ± 2.29	10.83 ± 2:36	0.058	10.03 ± 2.09	11.05 ± 2.29	0.0052**
tHbmass (g·kg^{-1})	10.1 ± 0.80	10.4 ± 0.89	0.039*	9.61 ± 1.39	9.96 ± 1.69	0.066
tHbmass (g)	596.30 ± 57.33	609.8 ± 50.77	0.15	612.36 ± 65.01	629.46 ± 70.27	0.14
[Hb] (g·dL^{-1})	12.3 ± 0.7	13.1 ± 0.7	0.0014**	12.5 ± 0.6	13.0 ± 0.5	0.0083**

tHbmass – total haemoglobin mass, TTE – time to exhaustion, sFer – serum ferritin, [Hb] – haemoglobin concentration

Values are mean ± SD.

Significant differences between baseline and post injection tests within each group are shown as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0005.

Note that due to sustained injuries n=17 for sFer < 15µg·L-1 and n=13 for sFer > 15 µg·L-1 but ≤ 30 µg·L-1.

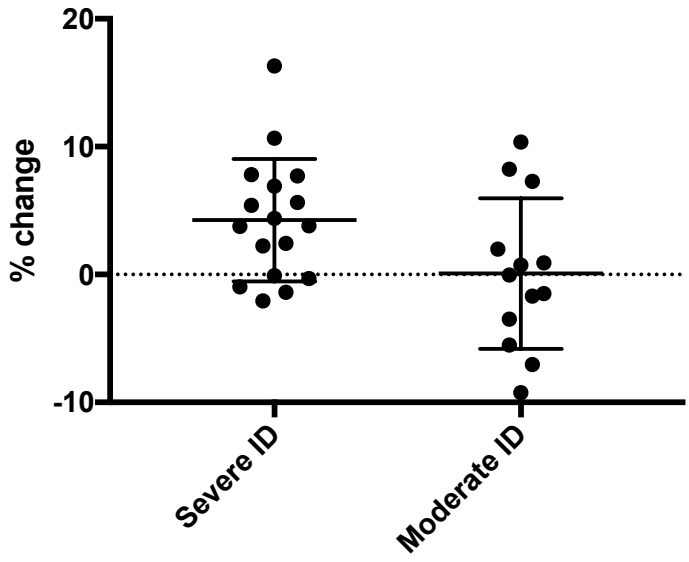

Figure 7.5 – Percentage change in $\dot{V}O_{2max}$ from baseline in response to intravenous iron when participants were grouped by baseline serum ferritin. Severe iron deficiency – sFer < 15 $\mu g \cdot L^{-1}$ and moderate iron deficiency - sFer > 15 $\mu g \cdot L^{-1}$ but ≤ 30 $\mu g \cdot L^{-1}$.

7.4.5 Relationship between baseline iron status and change in end points.

No relationship was found between either baseline [Hb] or serum ferritin and change in $\dot{V}O_{2max}$ independently (Table 7.4; [Hb] vs change in $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) – *p=0.66* and ($mL \cdot min^{-1}$) *p=0.71*; serum ferritin vs change in $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) – *p=0.20* and ($mL \cdot min^{-1}$) – *p=0.21*), or total haemoglobin mass (Table 7.7; [Hb] vs change in total haemoglobin mass – ($g \cdot kg^{-1}$) *p=0.42* and (g) *p=0.37*, serum ferritin vs change in total haemoglobin mass – ($g \cdot kg^{-1}$) *p=0.89* and (g) *p=0.96*).

Table 7.4 – The relationship between baseline haemoglobin ([Hb]) and serum ferritin (sFer) and the changes in $\dot{V}O_{2max}$ and total haemoglobin mass.

	[Hb]			sFer		
	B	**CI**	**p-value**	**B**	**CI**	**p-value**
Delta $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	0.36	-1.27 – 1.99	0.66	-0.091	-0.235 – 0.052	0.20
Delta $\dot{V}O_{2max}$ ($mL \cdot min^{-1}$)	-17.78	-115.59 – 80.03	0.71	-5.423	-13.999 – 3.154	0.21
Delta tHbmass ($g \cdot kg^{-1}$)	0.133	-0.199 – 0.465	0.42	-0.02	-0.032 – 0.028	0.89
Delta tHbmass (g)	9.22	-11.464 – 29.909	0.37	-0.047	-1.898 – 1.803	0.96

tHbmass – total haemoglobin mass, [Hb] – haemoglobin concentration, sFer – serum ferritin, B – co-efficient

In unadjusted models assessing the relationship between the combination of baseline [Hb] and serum ferritin and change in $\dot{V}O_{2max}$ or total haemoglobin mass, no association was found (Tables 7.5 – 7.8; all *p>0.05*). The inclusion of potential confounders did not affect any of these relationships (Tables 7.8 – 7.11; all *p>0.05*).

Table 7.5 - The unadjusted and adjusted relationship between the combination of haemoglobin ([Hb]) and serum ferritin and change in total haemoglobin mass (g).

	Unadjusted model			Adjusted model		
Variable	**B**	**CI**	**p-value**	**B**	**CI**	**p-value**
Constant	-99.73	-361.42 – 161.96	0.44	175.88	-143.58 – 495.33	0.27
Baseline [Hb]	9.44	-11.82 – 30.71	0.37	-7.15	-29.48 – 15.18	0.51
Baseline sFer	-0.15	-2.03 – 1.72	0.87	0.80	-1.16 – 2.77	0.41
Age				0.58	-0.84 – 2.01	0.41
BMI				-4.82	-10.23 – 0.59	0.08
5 km PB				-0.02	-0.10– 0.06	0.65
Total exercise/week				0.05	-0.02 – 0.12	0.16
Total running/week				0.03	-0.10 – 0.16	0.65

[Hb] – haemoglobin concentration, sFer – serum ferritin, BMI – body mass index, 5 km PB – 5 km personal best, B – co-efficient, CI – confidence interval

Table 7.6 - The unadjusted and adjusted relationship between haemoglobin ([Hb]) and serum ferritin and change in total haemoglobin mass ($g \cdot kg^{-1}$).

	Unadjusted model			Adjusted model		
Variable	**B**	**CI**	**p-value**	**B**	**CI**	**p-value**
Constant	-1.34	-5.53 – 2.86	0.52	3.118	-2.12 – 8.36	0.23
Baseline [Hb]	0.14	-0.20 – 0.48	0.41	-0.125	-0.49 – 0.24	0.49
Baseline sFer	-0.004	-0.03 – 0.03	0.81	0.011	-0.02 – 0.04	0.49
Age				0.008	-0.02 – 0.03	0.46
BMI				-0.084	-0.17 – 0.01	0.06
5 km PB				-0.000	-0.001 – 0.001	0.76
Total exercise/week				0.001	0.000 – 0.002	0.17
Total running/week				0.000	-0.002 – 0.003	0.68

[Hb] – haemoglobin concentration, sFer – serum ferritin, BMI – body mass index, 5 km PB – 5 km personal best, B – co-efficient, CI – confidence interval

Table 7.7 - The unadjusted and adjusted relationship between haemoglobin ([Hb]) and serum ferritin and change in $\dot{V}O_{2max}$ ($mL \cdot min^{-1}$)

	Unadjusted model			Adjusted model		
Variable	**B**	**CI**	**p-value**	**B**	**CI**	**p-value**
Constant	320.50	-889.41 – 1530.42	0.59	564.36	-1129.04– 2257.77	0.50
Baseline [Hb]	-13.40	-110.67 – 83.88	0.78	-14.72	-129.82 – 100.38	0.79
Baseline sFer	-5.33	-14.09 – 3.43	0.22	-7.53	-18.07 – 3.01	0.15
Age				-5.69	-13.35 – 1.98	0.14
BMI				-6.96	-41.66 – 27.74	0.68
5 km PB				0.10	-0.32 – 0.51	0.63
Total exercise/week				0.26	-0.10 – 0.61	0.15
Total running/week				-0.43	-1.13 – 0.27	0.21

[Hb] – haemoglobin concentration, sFer – serum ferritin, BMI – body mass index, 5 km PB – 5 km personal best, B – co-efficient, CI – confidence interval

Table 7.8 - The unadjusted and adjusted relationship between haemoglobin ([Hb]) and serum ferritin and change in $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$).

	Unadjusted model			Adjusted model		
Variable	**B**	**CI**	**p-value**	**B**	**CI**	**p-value**
Constant	-2.32	-22.43 – 17.79	0.82	2.56	-24.75 – 29.87	0.85
Baseline [Hb]	0.44	-1.18 – 2.05	0.58	0.50	-1.36 – 2.36	0.56
Baseline sFer	-0.09	-0.24 – 0.05	0.20	-0.13	-0.30 – 0.04	-1.57
Age				-0.10	-0.22 – 0.03	-1.65
BMI				0.20	-0.36 – 0.76	0.75
5 km PB				-0.003	-0.01 – 0.004	-0.91
Total exercise/week				0.000	-0.01 – 0.01	-0.10
Total running/week				-0.01	-0.02 – 0.002	-1.67

[Hb] – haemoglobin concentration, sFer – serum ferritin, BMI – body mass index, 5 km PB – 5 km personal best, B – co-efficient, CI – confidence interval

No relationship was found between change in [Hb] or change in serum ferritin and the changes in $\dot{V}O_{2max}$ and total haemoglobin mass. (Table 7.9; Figure 7.9f; all *p>0.05*).

Table 7.9 – The relationship between both change in haemoglobin ([Hb]) and change in serum ferritin (sFer) and the changes in $\dot{V}O_{2max}$ and total haemoglobin mass.

	Delta [Hb]			Delta sFer		
	B	**CI**	**p-value**	**B**	**CI**	**p-value**
Delta $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	0.41	-1.15 – 1.96	0.60	-0.004	-0.01 – 0.003	0.28
Delta $\dot{V}O_{2max}$ ($mL \cdot min^{-1}$)	14.70	-78.49 – 107.89	0.75	-0.16	-0.58 – 0.27	0.46
Delta tHbmass ($g \cdot kg^{-1}$)	-0.09	-0.35 – 0.18	0.51	0.00	-0.002 – 0.001	0.73
Delta tHbmass (g)	-7.90	-24.28 – 8.49	0.33	-0.02	-0.11 – 0.07	0.68

tHbmass – total haemoglobin mass, B – co-efficient, CI – confidence interval, [Hb] – haemoglobin concentration, sFer – serum ferritin

7.4.6 Relationship between $\dot{V}O_{2max}$ and total haemoglobin mass

There was a significant relationship between $\dot{V}O_{2max}$ and total haemoglobin mass both at baseline (*p<0.0005*; Figure 7.6) and post injection (*p<0.0005*; Figure 7.6). However, there was no relationship between the magnitude of the change in $\dot{V}O_{2max}$ and the magnitude of the change in total haemoglobin mass (r=-0.05; *p=0.81*; Figure 7.7).

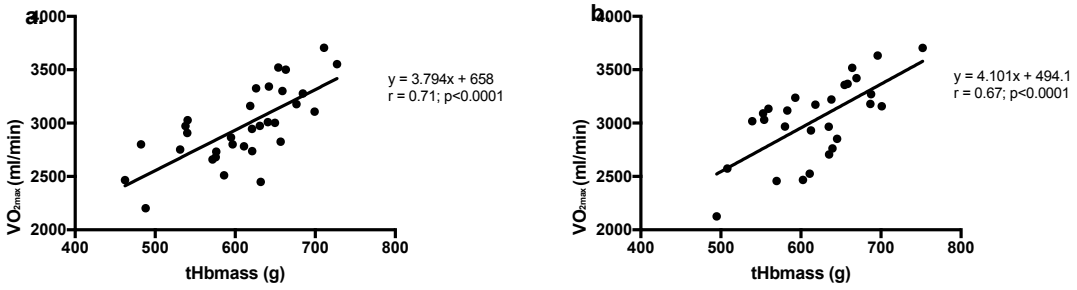

Figure 7.6 – The relationship between total haemoglobin mass and $\dot{V}O_{2max}$ at a. baseline and b. post injection.

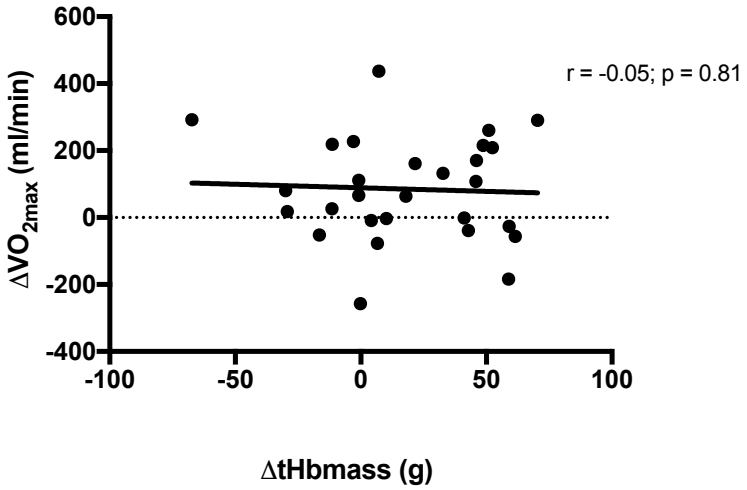

Figure 7.7 – The relationship between change in total haemoglobin mass and change in total $\dot{V}O_{2max}$ in response to the intravenous iron injection.

7.4.7 Other markers that are potentially related to change in endpoints.

There was a significant relationship between each of baseline $\dot{V}O_{2max}$ (*p=0.003*; Figure 7.8 c), reported 5 km personal best time (*p=0.02*; Figure 7.8 a), the total amount of exercise reported over a typical week (*p=0.009*; Figure 7.8 b), haematocrit (*p=0.02*; Figure 7.8e), and corpuscular haemoglobin concentration mean (*p=0.01*; Figure 7.8 d), and the change in haemoglobin mass after the iron treatment. Therefore, those who had a higher baseline $\dot{V}O_{2max}$, or who reported a faster 5 km personal best time were more likely to show a greater increase in total haemoglobin mass, and those who did more exercise each week were more likely to have a greater increase in total haemoglobin mass (Figure 7.8). A lower baseline CHCM, and a higher baseline haematocrit were associated with a larger change in haemoglobin mass (Figure 7.8 d and e). Change in haemoglobin mass was also related to both mean platelet component (*p=0.019;* Figure 7.9 a) and platelet distribution width (*p=0.032*; Figure 7.9 b).

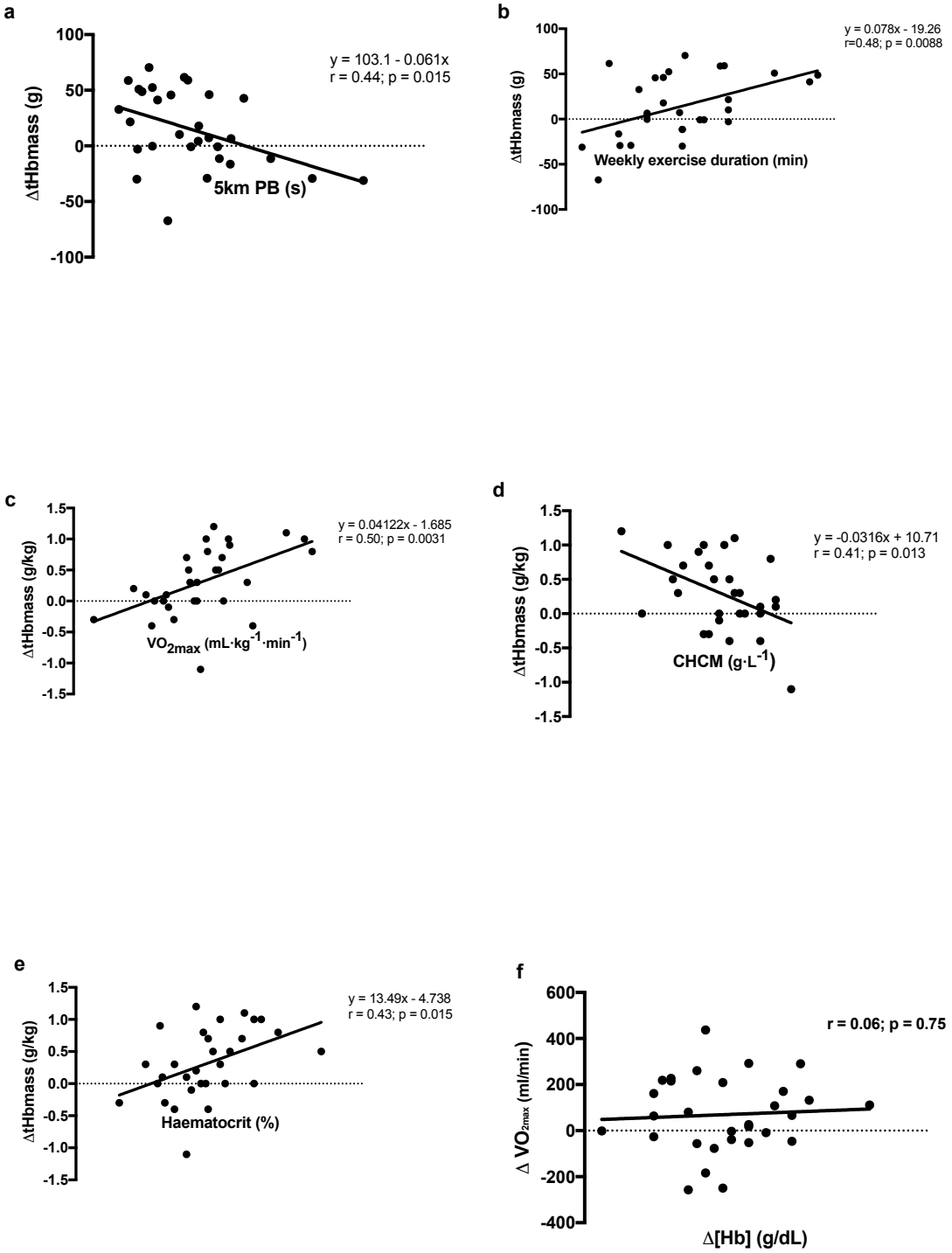

Figure 7.8 - Relationship between change in total haemoglobin mass and a. 5 km personal best time, b. weekly exercise volume, c. baseline $\dot{V}O_{2max}$, d. CHCM, and haematocrit in response to intravenous iron, f. Relationship between change in haemoglobin concentration ([Hb]) and change in $\dot{V}O_{2max}$ in response to intravenous iron. tHbmass – total haemoglobin mass, PB – personal best, CHCM - corpuscular haemoglobin concentration mean, [Hb] – haemoglobin concentration.

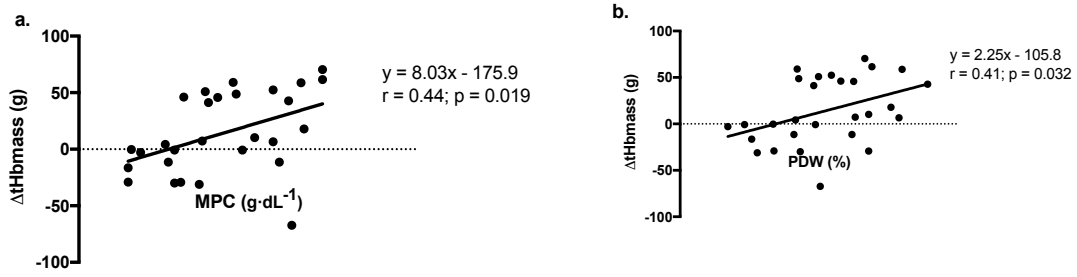

Figure 7.9 – Relationship between change in total haemoglobin mass (tHbmass) and baseline platelet biomarkers. a. association between change in haemoglobin mass and baseline mean platelet component, b. association between change in haemoglobin mass and platelet distribution width.

7.4.8 Training and menstrual cycle questionnaire

Controlling for day of the menstrual cycle had no impact on the association between baseline [Hb] or serum ferritin and any of the primary outcome measures (Table 7.10; all *p>0.05*). Nearly two thirds of the participants met the criteria for having a history of heavy menstrual bleeding (64.5%; Table 7.1).

Table 7.10 – The impact of day of the menstrual cycle on the relationship between baseline haemoglobin ([Hb]) and serum ferritin (sFer) and changes in $\dot{V}O_{2max}$ and total haemoglobin mass.

	Baseline				**Post-injection**			
	[Hb]		**sFer**		**[Hb]**		**sFer**	
	B	p-value	B	p-value	B	p-value	B	p-value
Delta $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	-0.89	0.40	-0.08	0.31	-0.69	0.50	-0.082	0.29
Delta $\dot{V}O_{2max}$ ($mL \cdot min^{-1}$)	-19.69	0.77	-4.64	0.36	-8.90	0.89	-4.26	0.38
Delta tHbmass ($g \cdot kg^{-1}$)	-0.077	0.70	-0.006	0.66	-0.04	0.86	-0.004	0.77
Delta tHbmass (g)	-2.26	0.86	-0.36	0.71	-0.13	0.99	-0.17	0.86

tHbmass – total haemoglobin mass, [Hb] – haemoglobin concentration, sFer – serum ferritin, B – co-efficient

7.4.9 Fatigue

7.4.9.1 Multidimensional Fatigue Inventory

A decrease in fatigue was seen after iron treatment when assessed using the multi-dimensional fatigue inventory (MFI), with a reduction observed across all subscales (all *p<0.01*; Figure 7.10).

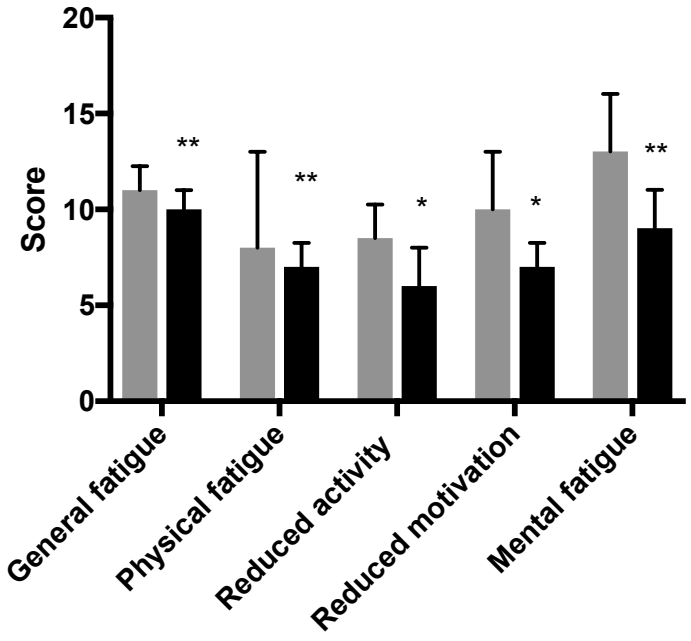

Figure 7.10 – Effects of intravenous iron on fatigue (MFI). A comparison between subscale Multidimensional Fatigue Inventory scores (MFI);
Values are median ± IQR at baseline and post-injection.
Significant differences between baseline and post injection tests are shown as follows: *p < 0.01, **p < 0.001.

A summary of the reliability tests for the MFI-20 both at baseline and post-injection is given in Tables 7.11 and 7.12. It was not necessary to remove any items in Table 7.11 based on the inter-item and corrected inter-item correlations as they were all greater than 0.30. While Cronbach's ∝ showed acceptable reliability and good internal consistency, ∝ = 0.81, and when standardised ∝ = 0.91. However as shown in Table 7.12, there were three correlations < 0.30 between general fatigue and each of reduced activity, reduced motivation and mental fatigue. Despite this, Cronbach's ∝ showed acceptable reliability and good internal consistency, ∝ = 0.79, and when standardised ∝ = 0.88.

Table 7.11 – Mean and standard deviations for each aspect of fatigue, alongside inter-item correlations, Cronbach's ∝ if the item is deleted and the corrected item-total correlation for tests at baseline.

	Mean (± SD)	TF	GF	PF	RA	RM	MF	Cronbach's ∝ if item is deleted	Corrected Item-Total Correlation
Total Fatigue	52.72 ± 13.88	-	0.69	0.85	0.81	0.87	0.77	0.86	1.00
General Fatigue	11.13 ± 2.54	0.69	-	0.78	0.35	0.41	0.36	0.80	0.64
Physical fatigue	9.86 ± 3.67	0.85	0.78	-	0.59	0.63	0.46	0.76	0.81
Reduced activity	9.25 ± 3.45	0.81	0.35	0.59	-	0.76	0.51	0.77	0.76
Reduced motivation	9.72 ± 3.66	0.87	0.41	0.63	0.76	-	0.64	0.76	0.84
Mental fatigue	12.77 ± 3.92	0.77	0.36	0.46	0.51	0.64	-	0.77	0.70

TF – total fatigue, GF – general fatigue, PF – physical fatigue, RA – reduced activity, RM – reduced motivation, MF – mental fatgue

Table 7.12 – Mean and standard deviations for each aspect of fatigue, alongside inter-item correlations, Cronbach's alpha if the item is deleted and the corrected item-total correlation for the post-injection tests.

	Mean ± SD	TF	GF	PF	RA	RM	MF	Cronbach's ∝ if item is deleted	Corrected Item-Total Correlation
Total Fatigue	41.91 ± 11.46	-	0.40	0.84	0.84	0.90	0.71	0.81	1.00
General Fatigue	9.75 ± 1.87	0.40	-	0.54	0.10	0.28	0.01	0.81	0.33
Physical fatigue	7.44 ± 2.61	0.84	0.54	-	0.55	0.67	0.50	0.76	0.80
Reduced activity	7.34 ± 3.53	0.84	0.10	0.55	-	0.83	0.47	0.74	0.79
Reduced motivation	7.47 ± 3.46	0.90	0.28	0.67	0.83	-	0.49	0.72	0.87
Mental fatigue	9.91 ± 3.44	0.71	0.01	0.50	0.47	0.49	-	0.76	0.62

TF – total fatigue, GF – general fatigue, PF – physical fatigue, RA – reduced activity, RM – reduced motivation, MF – mental fatgue

In an unadjusted model, neither baseline [Hb] nor baseline serum ferritin were associated with change in any of total, general, physical or mental fatigue or reduced activity (Table 7.13; all $p>0.05$). Baseline [Hb] was also not related to reduced motivation (Table 7.13; $p=0.99$), however this was related to baseline serum ferritin (Table 7.13; $p=0.02$).

Table 7.13 – The unadjusted relationship between MFI subscales at baseline and baseline haemoglobin concentration and level of serum ferritin. B – coefficient, CI – confidence interval, [Hb] – haemoglobin concentration

	Total fatigue			General fatigue			Physical fatigue			Reduce activity			Reduced motivation			Mental fatigue		
	B	**CI**	**p-value**	**B**	**CI**	**p-value**	**B**	**CI**	**p-value**	**B**	**CI**	**p-value**	**B**	**CI**	**p-value**	**B**	**CI**	**p-value**
Baseline [Hb]	1.32	-6.01 – 8.64	0.72	-0.28	-1.48 – 0.92	0.64	0.64	-1.23 – 2.51	0.49	-0.32	-2.37 – 1.72	0.75	0.01	-2.13 – 2.15	0.99	1.27	-0.58 – 3.11	0.17
Serum ferritin	0.60	-0.04 – 1.24	0.07	0.08	-0.02 - 0.19	0.12	0.08	-0.09 – 0.25	0.34	0.12	-0.07 – 0.30	0.21	0.22	0.04 – 0.40	0.02*	0.10	-0.07 – 0.27	0.25

Significant relationships are shown as follows: *p < 0.05.

7.4.9.2 Piper Fatigue Scale

When assessed using the Piper Fatigue scale, all dimensions of fatigue decreased (all *p<0.01*; Figure 7.11). All reported values reduced by more than one-point. Previous studies suggest a minimum of a one-point reduction for clinical significance (431).

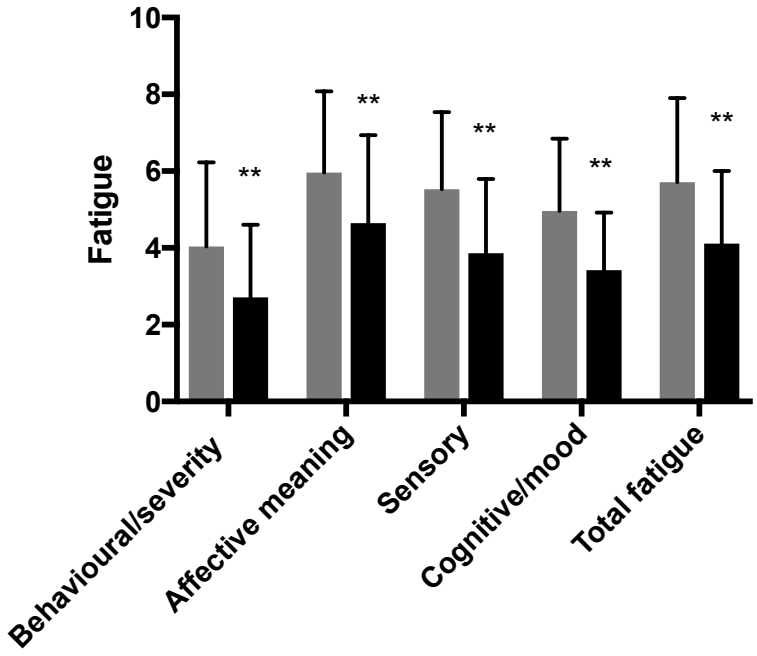

Figure 7.11 – Effects of intravenous iron on fatigue (Piper fatigue score). A comparison between subscale Piper fatigue scores (PFS; median ± IQR) at baseline and post-injection. Significant differences between baseline and post injection tests are shown as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0005.

7.4.9.3 EQ-5D-5L

When assessed using the EQ-5D-5L scale, impacts on usual activity (*p=0.008*), pain/discomfort (*p=0.*05) and anxiety/depression (*p=0.03*) all reduced (Table 7.14), while using the EQ-VAS, health overview increased from a median of 78.5 (70 – 85) to 85.0 (75 – 90) (z = 60.0; *p=0.001*). No problems in 'Self-care' were reported at baseline or post injection.

Table 7.14 – Effects of intravenous iron on feelings of health on the day of testing using the EQ-5D-5L questionnaire. The percentage of participants who reported no problem compared to those who reported any problem (from slight to extreme).

EQ-5D Dimension		Baseline	Post	p-value
Mobility	No problems	96.88%	100.00%	>0.9999
	Problems	3.13%	0.00%	
Self-care	No problems	100.00%	100.00%	-
	Problems	0.00%	0.00%	
Usual activity	No problems	75.00%	96.88%	0.0078**
	Problems	25.00%	3.13%	
Pain/discomfort	No problems	50.00%	50.00%	0.046*
	Problems	50.00%	50.00%	
Anxiety/depression	No problems	46.88%	59.38%	0.027*
	Problems	53.13%	40.63%	

Significant differences between baseline and post injection tests are shown as follows: ***p < 0.005, **p < 0.01, *p < 0.05

7.4.10 Mood disturbance

7.4.10.1 BRUMS

Iron supplementation had a significant impact on mood disturbance when using the BRUMS mood score. Perceived vigour increased (*p<0.0005*), while tension (*p<0.0005*), depression (*p=0.002*), fatigue (*p=0.001*), and confusion (*p=0.002*) all decreased in response to intravenous iron (Figure 7.12).

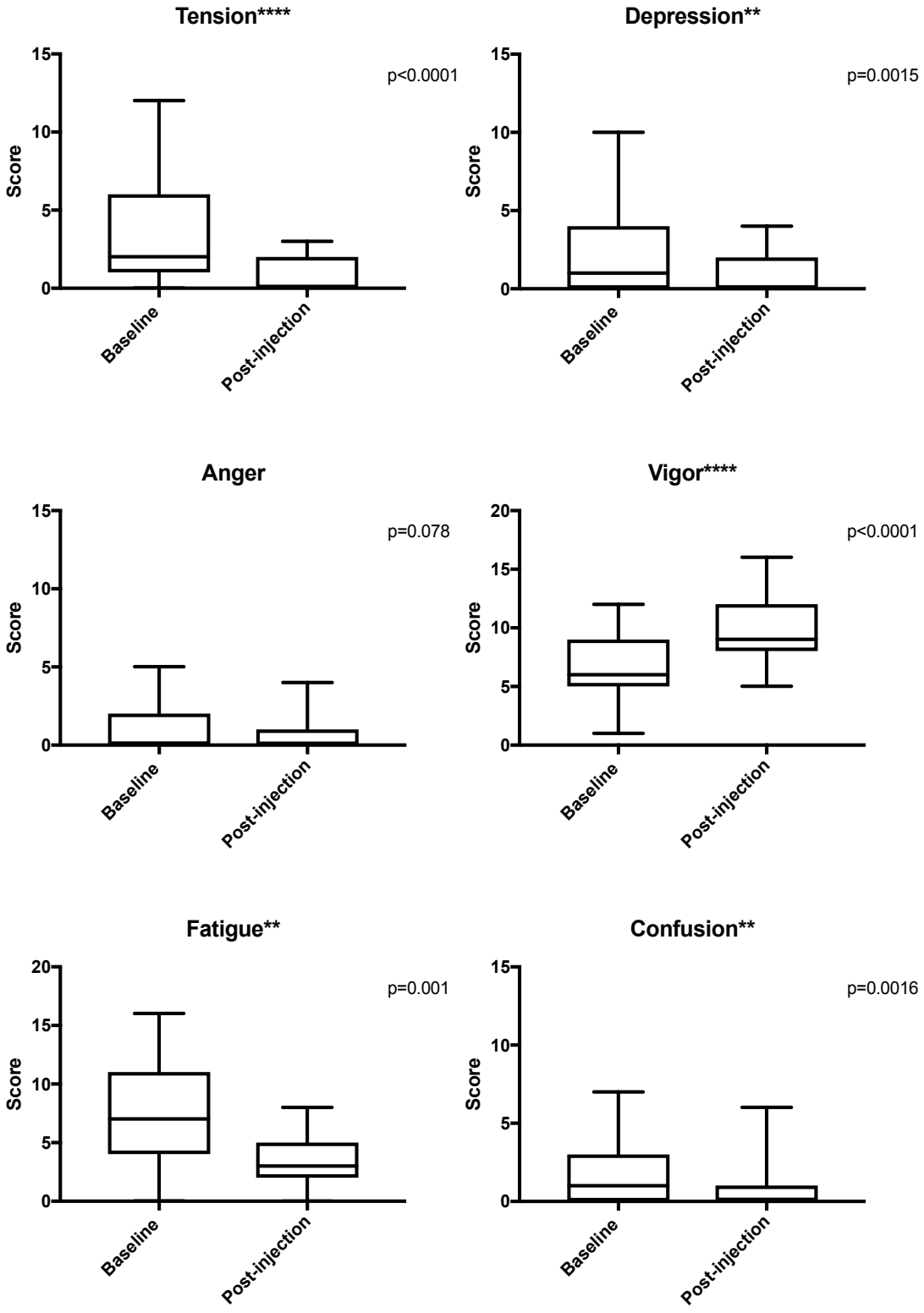

Figure 7.12 - Effect of intravenous iron on mood disturbance using the BRUMS mood scale. A comparison between mood disturbance at baseline and post-injection. Significant differences between baseline and post injection tests are shown as follows: ****p < 0.0001, ***p < 0.005, **p < 0.01, *p < 0.05.

7.5 Discussion

Previous research evaluating the effect of iron repletion on markers of exercise performance in iron deficient but not anaemic athletes has been inconclusive, resulting in a lack of clarity regarding both the impact and diagnosis of this condition. Studies are confounded by several inconsistencies which are likely to have exacerbated the variability in outcomes. The key finding in the IRONWOMAN trial was that a single high dose of intravenous iron was associated with an increased exercise and aerobic capacity in healthy iron-deficient, non-elite, regularly exercising women when measured two weeks after iron administration. Haematological markers of iron status improved, while fatigue and mood disturbance decreased. Since iron deficiency can be indicated by a positive response to iron therapy (47), it would seem prudent to suggest that the iron deficiency criteria (serum ferritin ≤ 30 $\mu g \cdot L^{-1}$) used in this study is appropriate for diagnosis. However, there was a distinct degree of individual variation in response, and when participants were divided into groups according to their baseline serum ferritin measurement, improvements in exercise and aerobic capacity were only seen in those with 'severe' iron deficiency (serum ferritin < 15$\mu g \cdot L^{-1}$). Yet, the magnitude of the change in exercise and aerobic capacity was not directly associated with baseline serum ferritin or baseline [Hb], questioning the suitability of these markers, which are typically the clinical markers used for the diagnosis of iron deficiency and IDA. To my knowledge this is the first study to measure all of these outcome variables at any one time in otherwise healthy, non-elite exercising women in response to an intravenous iron injection, and to evaluate the suitability of [Hb] and serum ferritin for diagnosis when assessing this using evaluation of key outcome variables.

As discussed throughout this thesis, and evaluated in Section 2.13.3, studies in sports science and sports medicine have typically defined iron deficiency using serum ferritin values ranging from 12 - 40 $\mu g \cdot L^{-1}$, and have used different protocols incorporating different supplementation routes (oral, intravenous or intramuscular) and dosing strategies, with equivocal results. These studies frequently have small sample sizes, and haven't conducted sample size calculations, and often differ through the involvement of athletes of varying degrees of training status (48-54). Injection studies where the controlled absorption process in enterocytes is bypassed are sparse, and have focused on elite athletes (51,52). The participants in this study were specifically non-elite, therefore representing a large proportion of the exercising population. Despite this, the overall magnitude of the improvements in $\dot{V}O_{2max}$ and total haemoglobin mass found here were similar to those seen in the intravenous iron group in the well trained runners in the study by Garvican *et al*. (2014) (52).

It has previously been suggested that the evident discrepancies in research may be due to some participants being slightly anaemic at baseline, therefore improvements in aerobic performance could be attributed to increases in [Hb] (52). Given the impact of IDA on aerobic performance, this seems plausible, and a number of studies support this hypothesis, showing increases in [Hb] alongside improvements in aerobic performance (236,243,433). The wide [Hb] reference range, typically 12.0 $g \cdot dL^{-1}$ – 16.0 $g \cdot dL^{-1}$ in women, means that there is also potential for significant decreases in [Hb] to occur before IDA is diagnosed as previously highlighted in Section 2.5, so a relative state of anaemia may be present, yet unknown and not diagnosed. However, the recent study by Garvican *et al.* (2014) found improvements in exercise measures without concomitant increases in [Hb] in response to intravenous iron in iron deficient endurance runners (52). In the present study, a mean increase in [Hb] was observed, however [Hb] did not increase in all participants. Further, increases in $\dot{V}O_{2max}$ and/or total haemoglobin mass were not always accompanied by increases in [Hb]. Where there was a change in both [Hb], and $\dot{V}O_{2max}$ or total haemoglobin mass, there was no association between the magnitude of these changes. In light of these results, this suggests that non-haematological effects of iron deficiency could be hypothesised.

According to the Fick equation, maximal oxygen consumption ($\dot{V}O_{2max}$) depends on cardiac output and oxygen extraction (section 7.3.7.1) (1). Since the transport of oxygen is dependent on [Hb], increases in [Hb] will clearly be a cause for increases in $\dot{V}O_{2max}$. However, there are a number of possible causes for increases in $\dot{V}O_{2max}$ without concomitant increases in [Hb], as seen here. Firstly, there could have been an increase in cardiac output, through a proportional increase in total haemoglobin mass and blood volume, or alternatively, oxygen extraction and utilisation may have improved through iron-dependent mechanisms (1). In instances where haemoglobin mass increased without an associated increase in [Hb], it is likely that blood volume increased proportionally with total erythrocyte mass.

As previously highlighted and shown in earlier studies, the suitability of [Hb] as a tool for the identification of red cell volume, and therefore the diagnosis of IDA is questionable (section 2.5). The use of total haemoglobin mass provides a more accurate means for identifying when oxygen transport capacity is limited, particularly amongst those who exercise, as this group are particularly susceptible to plasma volume shifts and changes in blood volume (108). Previous research has found total haemoglobin mass to correlate more effectively with $\dot{V}O_{2max}$ peak, with a lower coefficient of variation when compared to [Hb] (section 2.6) (241,434). Accordingly, total haemoglobin mass has been suggested to aid the accuracy of IDA diagnosis. A close correlation was also found in this study (Figure

7.6) in support of this, therefore providing further evidence for the use of haemoglobin mass, particularly amongst the exercising population. Future clinical practice could consider longitudinal monitoring of haemoglobin mass, where significant decrements may indicate a state of IDA.

Significantly, while there was a strong correlation between total haemoglobin mass and $\dot{V}O_{2max}$ both at baseline and post-injection, there was no relationship between the magnitude of the change in these markers in response to iron repletion. Again, this suggests that in some participants, the improvement in exercise capacity ($\dot{V}O_{2max}$) could have been caused by non-haematological factors, such as an improvement in iron dependent oxygen extraction and utilisation mechanisms, for example increased function of the iron-containing haem enzymes and iron-sulphur clusters. Therefore suggesting, in this non-elite, iron deficient population, that factors other than those associated with erythropoiesis may be limiting $\dot{V}O_{2max}$. While previously discussed in the literature, this has not been demonstrated before. To my knowledge, to date, one other study has evaluated the association between changes in $\dot{V}O_{2max}$ and total haemoglobin mass in response to iron therapy. Wachsmuth *et al.* (2015) assessed response to oral iron therapy over a 12-week supplementation period, and, unlike in this study, a direct relationship was found between the magnitude of changes in $\dot{V}O_{2max}$ and total haemoglobin mass (241). The reason for this discrepancy is unsure, however it is significant to note that in the present study. intravenous iron therapy was used as opposed to oral therapy, bypassing the controlled uptake mechanism in enterocytes. It is also possible that there was insufficient time for the full response to iron treatment to manifest here. The restoration of non-haematological impacts of iron deficiency could occur prior to those related to erythropoietic demands. Future studies should address this, considering other time points. However, in the study by Garvican *et al.* (2014), in those receiving four iron injections, the final tests were performed two weeks after the final injection (52), however Burden *et al.* (2015) had follow up tests after four weeks (51).

Evidently, the results in the present study beg the question of the impact of iron deficiency without anaemia. In addition to being a key component of haemoglobin, and being vital for oxygen delivery, iron is essential for numerous other functions as highlighted previously in this thesis. For example, its role in the electron transport chain, culminating in ATP production within mitochondria, utilising its ability to freely exchange electrons suggests that there are likely to be other non-haematological impacts of iron deficiency with the potential to affect exercise capacity. As previously highlighted, research in animals, in particular in rodent models, is far advanced of that in humans, where the non-haematological functions of iron have been studied, and the impact of iron deficiency in

the absence of anaemia is better understood (227). The sparsity of research in humans makes conclusions problematic, but it is possible that where increases in exercise capacity occurred in isolation of changes in red cell mass, and therefore oxygen transport capacity, these may be in part caused by improvements in mitochondrial or enzymatic functioning involving iron-dependent pathways for example, enhancing the production of ATP. This would be in accordance with the findings by Finch *et al.* in 1976, as iron deficiency without anaemia was found to impact mitochondrial activity in rats (227). Thereby iron repletion would cause functional improvements without an associated change in [Hb] or total haemoglobin mass. It is possible that this response may be more likely in non-elite exercisers where non-oxidative mechanisms may not be so well developed.

Significantly, in less well trained individuals, limitations in $\dot{V}O_{2max}$ have been attributed to oxygen consumption as opposed to oxygen supply (i.e. erythrocyte mass) (1). Therefore, in elite athletes with true IDNA, where oxygen supply is not limited, as [Hb] is not compromised, improvements in $\dot{V}O_{2max}$ may be less likely to be significant when compared to non-elite athletes since oxygen consumption mechanisms are already well-developed. This provides another potential cause for discrepancies in research.

It is also significant that an increase in total haemoglobin mass was seen in some participants without an increase in $\dot{V}O_{2max.}$ This is likely to be due to limitations to oxygen extraction and utilisation.

Despite the overall increase in exercise and aerobic capacity seen in this trial, when separating participants into groups according to baseline serum ferritin, increases were only seen in those with 'severe' iron deficiency (serum ferritin < 15 $\mu g \cdot L^{-1}$). This finding is similar to results from both Krayenbuehl *et al.* (2011) (56), when assessing fatigue in iron deficient non-athletes and Garvican *et al.* (2014) (52) evaluating exercise performance in iron deficient well-trained distance runners, both concluding that response to iron therapy was more significant in those who were more iron deficient. This suggests that, contrary to the belief of many, the criteria for iron deficiency diagnosis (serum ferritin ≤ 30 $\mu g \cdot L^{-1}$) in this study may be too high, and that lower serum ferritin cut-off values may be required. This could in part explain some of the ambiguity in previous studies, since many applied significantly higher serum ferritin cut-off values. Despite the WHO suggesting that serum ferritin < 15 $\mu g \cdot L^{-1}$ signifies iron depletion (100), a lack of iron in bone marrow has been observed when serum ferritin is 30 $\mu g \cdot L^{-1}$ (42). Accordingly, particularly amongst the athlete population, performance is considered to be affected much earlier than when serum ferritin < 15 $\mu g \cdot L^{-1}$, and as a result, clinically, treatment is typically initiated well before serum ferritin levels have dropped to this point (46,173). Appreciably though, despite the

improvements in aerobic and exercise performance seen in those with 'severe' iron deficiency, there was still a wide range of individual variation in response seen across all endpoints.

In addition to mean [Hb], mean serum ferritin and red cell indices (MCH, MCV, CH, RBC, RDW) all significantly increased in response to the intravenous iron in this study. An increase in serum ferritin in response to iron therapy has been widely shown and would be expected after administration of a high dose of intravenous iron directly into the circulation, bypassing the mechanism for controlled absorption in enterocytes (46,244). Despite a previous study showing a correlation between baseline serum ferritin and the magnitude of increase in total haemoglobin mass in response to oral iron therapy (241), when specifically looking at the individual results in this study, no relationship was observed between baseline serum ferritin and the magnitude of the change in either total haemoglobin mass or $\dot{V}O_{2max}$. Given the direct administration route, bypassing the gut, there was no confounding issue of absorption. Evidently, and in accordance with previous research, this study highlights that the suitability of serum ferritin for the diagnosis of iron deficiency in the exercising population needs to be questioned. A conclusion that was also drawn in Chapters 5 and 6, where a lack of association between serum ferritin and fatigue was observed. The use of other markers should be explored for diagnosis, particularly in those who exercise. However, in light of the individual responses seen here, particularly amongst those with a serum ferritin < 15 $\mu g \cdot L^{-1}$, one could also postulate that optimal levels of serum ferritin may be specific to an individual, and that for best practice, athletes should be monitored longitudinally over time, with intervention only when significant decreases from their normal occur. While it is significant that two weeks may not have been suffice to enable a full response to be established, some participants had not shown any indication of response by this point. Previous research has demonstrated that two weeks is suffice for a significant response to be evident (52,53,435).

If indeed optimal ferritin values are specific to an individual and longitudinal monitoring was possible, given the limitations associated with both serum ferritin and [Hb], as previously suggested, total haemoglobin mass measurements could be used alongside these to provide a better indication of the onset of iron deficiency. This has been used effectively in a previous study to monitor recovery from iron deficiency (241).

As [Hb] and serum ferritin are the primary indicators for the diagnosis of IDA, in the present study the combination of these was also considered, assessing whether, in conjunction, they were associated with changes in exercise and aerobic capacity, therefore establishing whether, despite no individual relationship between [Hb] and serum ferritin and response,

when combined they had a cumulative effect. However, no association was found, even after factoring in potential confounders, again supporting the questions surrounding their suitability. Given the possible implications associated with iron deficiency, in addition to the known negatives of iron treatment when not necessary, the lack of power that [Hb] and serum ferritin have in this study to determine response to intravenous iron, and therefore to indicate the presence of iron deficiency needs to be addressed. The power of other markers of iron status such as transferrin saturation, soluble transferrin receptor, hepcidin, and total iron binding capacity, at identifying response to iron therapy should be evaluated, possibly in conjunction with [Hb] and serum ferritin to provide a more robust analysis of iron status. In isolation, these markers (e.g. transferrin saturation) may not have the power to accurately identify iron deficiency, but power could be significantly increased when combined with other markers (436). Again, this need is likely to be particularly relevant in those who exercise who are subject to exercise related variation in haematological indices. The soluble transferrin receptor/log ferritin index has shown significant potential for example and should be investigated further (115).

Despite the lack of association between baseline serum ferritin and [Hb] and exercise and aerobic capacity, baseline $\dot{V}O_{2max}$, total weekly exercise volume and 5 km personal best time were all independently found to be associated with the change in circulating mass of haemoglobin. This suggests that these could in part be determinants of change in aerobic capacity. Hence, individuals who were fitter and who trained more responded better to iron treatment. While it should be highlighted that these relationships are only moderate to weak, it is interesting that three different indicators of fitness and exercise status point to a relationship. One could infer that since the follow-up testing was only conducted two weeks after the injection, the relationship could be as a result of an increased erythropoietic stimulus, triggered by the increased exercise volume and potentially increased intensity of exercise in those who train more and are fitter. Alternatively, these fitter individuals could have an accelerated and more efficient erythropoietic response. From a non-haematological perspective, it could be hypothesised that fitter individuals have a more efficient mechanism for iron utilisation and incorporation into enzymes, again meaning that they respond more quickly than those deemed less fit. Previous research has demonstrated changes in total haemoglobin mass (52,435), and $\dot{V}O_{2max}$ (53) in response to iron treatment across participants of all training levels within a comparable, and in some cases a shorter timescale, countering this. However, this is not to say that the time course for reaching the peak response is not dictated by fitness, so the full extent of response may not have been reached after two weeks. It is however significant that previous repletion studies using intravenous iron have only been conducted in elite and well-trained athletes, therefore this option needs further consideration in a more general exercising female

population. Again, as previously highlighted, there is a lack of studies in non-elite athletes, and future studies are required to evaluate difference in response time between elite and non-elite athletes.

A number of studies have also suggested that fitness may be a determinant in response. A recent meta-analysis found increases in $\dot{V}O_{2max}$ to occur only in trained women when compared to those untrained in response to iron treatment (232), and another study assessing response to oral iron therapy also demonstrated an increased response in those with a higher baseline $\dot{V}O_{2max}$ (241). Conversely, in a different meta-analysis, significant increases in $\dot{V}O_{2max}$ in response to iron repletion were only seen in those with a baseline $\dot{V}O_{2max}$ < 40 $mL \cdot kg^{-1} \cdot min^{-1}$, with no effect being concluded in those with a $\dot{V}O_{2max}$ > 45 $mL \cdot kg^{-1} \cdot min^{-1}$(46). This was thought due to a reduced potential for improvement (46). In the present study this was not the case, however owing to the specific lack of intravenous iron repletion studies in non-elite athletes, only studies involving oral and intramuscular administration routes were included in the meta-analysis (46). Further research is evidently required to evaluate the determinant of fitness on response. Markers of erythropoiesis such as reticulocytes, GDF-15, and erythropoietin, should also be included to provide a better mechanistic understanding of iron deficiency, while there is an evident need to assess the effect of low iron on mitochondrial function. It could be hypothesised that optimal serum ferritin levels are associated with fitness, suggesting that when using this for diagnosis, less fit individuals may not experience impairments in physical function as readily as those who are fitter, likely because there is more potential for underlying inflammation or a haemoconcentration to reduce accuracy of measurement in those who train more and at an increased intensity. However, when controlling for 5 km personal best time and total exercise and running time each week, there was still an absence of any relationship between serum ferritin and the magnitude of markers of exercise and aerobic capacity.

Baseline corpuscular haemoglobin concentration mean (CHCM) and haematocrit were also found to be associated with change in haemoglobin mass in response to iron repletion. These relationships are not surprising given they are associated with erythrocyte production, size and content. However, it could be suggested that future research could consider the use of these as part of a diagnostic panel for the diagnosis of iron deficiency, particularly given the ease of a blood test when compared to the measurement of total haemoglobin mass using the carbon monoxide rebreathing test.

Previous research has shown an association between markers of thrombocytosis and IDA (437). While specific markers of platelet function and therefore thrombocytosis were not measured in the present study, a relationship between both baseline mean platelet

component and baseline platelet distribution width and change in haemoglobin mass was observed. It is thought that there are some iron-containing enzymes in platelets that aid the haemostasis process, so when iron levels are insufficient, platelet function is compromised (438). Again, this is an area that should be addressed in future work.

As highlighted in Chapter 6, fatigue is a significant health issue which is particularly common in women (403,404). IDA is a leading cause of fatigue, but research evaluating the association between iron deficiency alone and fatigue is unsure. Most research in non-athletic iron deficient but not anaemic individuals suggests a reduction in fatigue and improvements in quality of life in response to iron therapy without a concomitant increase in [Hb] (56,226,247,248,439). However, some contradict this, finding no impact of iron repletion in the reduction of fatigue (249), again likely in part caused by the ambiguity surrounding the serum ferritin cut-off values used for the diagnosis of iron deficiency in addition to varying study protocols amongst the aforementioned other inconsistent factors (226,247). In Chapter 6, despite using the WHO's serum ferritin cut-off to indicate iron depletion, neither iron deficiency, nor IDA were associated with increased fatigue, however anaemia alone was ([Hb] < 12.0 $g \cdot dL^{-1}$). Due to the insidious nature of fatigue it must be appreciated that individuals may have adjusted to the fatigue and established a new sense of 'normal', which isn't realised until iron stores are repleted, hence repletion studies are required for a more accurate evaluation.

It has been suggested that for peak cognitive performance serum ferritin levels should in fact be higher than those viewed as optimal for physical performance, with some suggesting a serum ferritin cut-off of < 50 $\mu g \cdot L^{-1}$ (247). Accordingly, impacts of iron deficiency on exercise and aerobic capacity are hypothesised to be secondary to cognitive performance (58). There is however an absence of previous research assessing the impact of iron deficiency on fatigue and mood disturbance alongside exercise capacity and haematology in individuals who exercise. This has been highlighted in the literature, with only speculative prior hypotheses, thus making conclusions to this avail difficult (51,52). To my knowledge, the IRONWOMAN trial is the first study to capture all these factors in iron-deficient, but otherwise healthy regularly exercising women. In support of the hypothesis that cognitive performance is impaired prior to physical performance, there was a universal improvement in factors associated with cognition, i.e. a reduction in fatigue and mood disturbance, without a universal increase in exercise and aerobic capacity. It must however be appreciated that the reduction in fatigue and mood disturbance could be attributable to a placebo effect, particularly because this was an observational non-blinded study. In fact, a placebo effect has been found in previous studies regardless of blinding (56,226). In an attempt to ameliorate this impact in the present study, construct validity was

obtained by conducting three separate fatigue questionnaires, alongside a mood questionnaire. Reliability tests were also specifically performed on the MFI-20, and I believe that the sheer number of questions in addition to the time lag between questionnaire completion would also have helped to minimise the impact.

When specifically evaluating the responses from the MFI-20 questionnaire, the magnitude of the change in the fatigue subscales was not related to baseline [Hb], suggesting that fatigue reductions were not due to underlying IDA. Bar the association between serum ferritin and change in motivation, no other changes in fatigue subscales were related to baseline serum ferritin. Given that there was a decrease in fatigue and mood disturbance in some participants without a concomitant increase in total haemoglobin mass or [Hb], again, non-haematological effects of iron deficiency could be implicated, suggesting that an increase in oxygen transport was not responsible for the improvements seen. Iron is thought to have a number of roles in neurotransmitter biochemistry in the brain, including dopamine and serotonin signalling, the development of the neural network, energy metabolism and myelination (440). Therefore, it is possible that these could be compromised in states of iron deficiency, prior to functions associated with physical performance and oxygen carriage. This could potentially provide an explanation as to why cognitive performance could be impacted prior to physical function in iron deficient states.

With likelihood of iron deficiency being significantly greater in those identified with heavy menstrual bleeding (HMB) when utilising the criteria applied throughout this thesis as previously shown in Chapters 5 and 6 in this thesis, it was interesting to see that in the present study prevalence of this condition was nearly double that of the general exercising population discussed in Chapter 4 (35.5%) (28,392). This is in accordance with earlier findings that HMB increases risk of IDA and iron deficiency. A relatively recent study found an association between platelet dysfunction, IDA and HMB, suggesting that IDA causes the platelet dysfunction which is the reason for the increased menstrual blood loss (441). It could therefore be suggested, that the lack of iron could be responsible for HMB. Specific markers of platelet aggregation and secretion were not included in the present study, but should be considered in future work. Given that previous studies found iron therapy to reverse the presence of HMB (408), this could have significant future potential given the prevalence and impact of both HMB and IDA as shown in this thesis. There is a lack of previous studies addressing platelet aggregation and secretion in those with HMB and iron deficiency without anaemia, however, considering the results in the present study, further investigation is clearly warranted.

In this study, barring the two participants whose exercise results were excluded due to injury, no differences were reported in training pre- and post- supplementation, and no dietary alterations were highlighted. Further, controlling for day of the menstrual cycle had no significant impact on the results.

It is common for athletes to supplement with iron, often prior to knowledge of iron status. This was demonstrated in Chapter 4 where 78.9% reported supplementing with oral iron, despite more than half being unaware of iron status. Previous studies have demonstrated no benefit of iron supplementation in iron sufficient athletes, and given that not all participants showed an improvement in exercise and aerobic capacity despite evident increases in serum ferritin, this study is in agreement (51). In light of the placebo effect, it is difficult to gage the true source of the reduction in fatigue and mood disturbance. Further, as already highlighted in this thesis, given the side effects associated with oral supplementation, including gastrointestinal distress, nausea (257), increases in oxidative stress and inflammation (61), in addition to the upregulation of hepcidin expression, decreasing iron absorption and potentially worsening iron status (261), supplementation should only be used when necessary. Further, excess iron supplementation is a risk factor in those genetic abnormalities that predispose them to iron overload, for example in those with haemochromatosis (7). Elite endurance athletes may be at particular risk where mutations to the HFE haemochromatosis gene are more common than in the general population (259). Dependant on the circumstances, if iron deficiency is evident or thought possible, dietary modifications should be the initial treatment option.

While demonstrating that cut-off values for serum ferritin for the diagnosis of iron deficiency may be too lenient, the present study also highlights the potential unsuitability of both serum ferritin and [Hb] for the diagnosis of both iron deficiency and IDA. Other markers need to be assessed to determine whether they could provide a more accurate analysis, while longitudinal monitoring of iron status should be considered as it is possible that optimal values for markers of iron status are specific to an individual. Given the impacts that iron deficiency can have, as demonstrated here, in addition to the potential side effects of unnecessary supplementation, future research needs to focus on improving iron deficiency diagnosis. While the mechanism for iron deficiency should also be established.

7.5.1 Limitations

With no randomisation, this research is subject to bias caused by a placebo effect. However, while time to exhaustion, fatigue and mood are subject to variation to this avail, total haemoglobin mass and haematological indices cannot be altered, and providing $\dot{V}O_2$ peak is attained, this is also not subject to a placebo effect. The multiple different measures

collected here will reduce the impact of the placebo effect somewhat, however it cannot be ignored, and future studies should take the form of a randomised, double-blinded controlled trial.

The follow-up tests were only conducted two weeks after intravenous iron administration, therefore it is unlikely that the full response was captured. Additionally, the initial response (i.e. after 48 hours) as shown in previous research was not measured (51). However many studies have shown significant outcomes in a comparable time frame (52,53,435). Further, while participants were asked to maintain a normal diet through the duration of the study food diaries were not kept, so specific dietary intake was not monitored. Training diaries were kept; however, it was difficult to determine effort and physiological impact of different training sessions. In an attempt to reduce these effects, participants were requested to have post-injection tests in the same physiological state as those conducted at baseline, therefore they were asked to keep training and lifestyle as similar as possible in the couple of days prior to each test. This was very difficult to control as physiological state and wellness can be influenced by many external factors, and particularly since these athletes were not elite, other lifestyle impacts are likely to play a part such as diet and sleep. However, these factors should not impact total haemoglobin mass measurements, which have been found to be relatively stable in trained athletes (1), or haematological measures. The addition of reticulocyte measures, GDF-15 and erythropoietin would have enabled assessment of whether the improvements in aerobic and exercise capacity were due to a haematological response and future studies should include these. While measures of hepcidin and other iron status parameters would enable increased mechanistic understanding as to the factors driving the results seen, while also offering future possible diagnostic means.

While all participants had run on a treadmill before, they had not all previously undertaken a $\dot{V}O_{2max}$ test. Therefore, in the follow-up test they may have been more comfortable with the equipment. They were however blinded to total time on the treadmill.

7.6 Conclusions

A single high dose intravenous iron injection was associated with an increase in exercise and aerobic capacity in otherwise healthy non-elite, iron-deficient exercising women. However, there was a significant degree of individual variation despite increases in serum ferritin in all participants. When participants were divided into groups based on baseline serum ferritin, increases in $\dot{V}O_{2max}$ and total haemoglobin mass were only observed in those with severe iron deficiency (serum ferritin < 15 $\mu g \cdot L^{-1}$). Despite this, the magnitude

of improvements in exercise and aerobic capacity were not associated with either baseline serum ferritin or [Hb], suggesting response could be driven by a different mechanism, or questioning the suitability of existing markers used for iron deficiency diagnosis. Following iron repletion there was a universal decrease in fatigue and mood disturbance, while this may in part be due to a placebo effect, it could also be that iron deficiency affects cognitive performance prior to exercise and aerobic capacity.

To conclude, regarding the outlined hypotheses for this chapter, the first hypothesis that iron repletion improves exercise and aerobic capacity in iron deficient (serum ferritin < 30 $\mu g \cdot L^{-1}$), non-elite exercising women can be accepted. The second hypothesis that iron repletion reduces fatigue and mood disturbance in iron deficient (serum ferritin < 30 $\mu g \cdot L^{-1}$), non-elite exercising women can also be accepted. Despite, there being a significant degree of individual variation in response, and no direct correlation, the third hypothesis that those with a lower baseline serum ferritin saw greater increases in exercise and aerobic capacity and reductions in fatigue and mood disturbance can also be accepted.

7.7 Future perspectives

The IRONWOMAN trial clearly demonstrates the limitations of using serum ferritin as a sole marker of iron status in those who exercise. While serum ferritin may be able to establish an 'at risk' population, future studies should consider using other biomarkers in addition to, or as an alternative to this to improve diagnosis. Future studies should also include a blinded placebo group, to further ascertain whether iron deficiency first impacts cognitive performance prior to factors associated with physical performance. In an attempt to gain a better mechanistic understanding, muscle oxidative capacity should be measured.

The relationship between IDA and HMB should be further established, if indeed IDA can cause HMB, intravenous iron therapy could have fundamental use in treatment of this condition, which was shown in previous research to have a substantial impact.

7.7.1 Future studies

The present study clearly highlights the need for further research in this field. The following factors need consideration:

When using the same diagnostic criteria, the impact of iron repletion on markers of erythropoiesis and mitochondrial oxidative function. This will enable a better understanding as to whether the presence of non-haematological impacts of iron deficiency.

A randomised control trial needs to be conducted to minimise the impact of bias. This should involve both elite and non-elite athletes to enable determination as to whether baseline fitness dictates response. Based on previous research, more follow-up time points are warranted, for example at 48 hours post injection and four weeks post injection. Platelet measures should be investigated alongside HMB presence to identify any presence of a causal relationship.

7.8 Author Contributions

The author recruited all the potential participants, and conducted all screening blood tests and either organised for a courier to collect these for delivery to the laboratory or delivered them personally, following advice regarding the required environmental conditions. The author provided feedback to participants regarding whether they met the inclusion criteria and where this was met, scheduled the subsequent appointments (baseline test, iron injection and follow up tests). The author conducted all baseline and follow up tests (exercise and total haemoglobin tests and oversaw the completion of the questionnaires). A trained medical professional at the Iron Therapy Clinic administered the intravenous iron.

The author inputted all data onto a secured database and conducted the statistical analysis and the interpretation of the results.

8 General Discussion, overall conclusions and future research

In this section, the key findings in this thesis will be outlined, providing overall conclusions while also describing future perspectives, studies and recommendations.

Firstly, this thesis examined whether HMB, when identified using the outlined criteria for diagnosis, is a dysfunction that is common in exercising women. In the absence of a validated and universally accepted means for the diagnosis of HMB, a four-part series, similar to that which has been used previously was applied as explained in chapter 3. This relied on individuals to self-report symptoms experienced. After establishing, when using this diagnostic means that HMB was common, there was a need to evaluate unbiased prevalence. This was done using London Marathon runners. The presence of identified HMB and its association with individuals perceiving that their menstrual cycle disrupts their exercise training/performance was then evaluated. The prevalence of iron deficiency, iron deficiency anaemia (IDA) and anaemia in a different exercising cohort was then established. The association between these and HMB (when identified using the same means) both independently and together with the perceived menstrual cycle disruption to exercise training/performance or everyday lifestyle/exercise performance and fatigue was then evaluated. Finally, the impact of iron repletion in iron deficient non-elite exercising women was assessed when diagnosing iron deficiency using serum ferritin, which is the primary diagnostic marker used clinically. In so doing, the efficacy of serum ferritin for iron deficiency diagnosis was evaluated. Significantly, despite non-elite exercising women encompassing a very large and growing population, research as to the impact of iron deficiency both on physiological and psychological elements within this group is lacking.

8.1 Prevalence and impact of HMB in the exercising population

Despite the lack of a universal consensus on a means for HMB diagnosis, research has shown HMB to be common in the general population, previously found to affect 27.2% of a European population (28), and 10 - 35% of reproductive aged women in the United States (442). This condition has not however been researched in those who exercise, and this thesis provides new insights into the potential occurrence of HMB in this group. With the assumption that the primary menstrual cycle dysfunctions in those who exercise are amenorrhea and oligomenorrhea, little research has addressed other menstrual dysfunctions, yet both chapters 4 and 5 highlight that using a diagnostic series, incorporating aspects of other diagnostic means, (28) (218), HMB is a significant issue in this population. Appreciably, and contrary to many beliefs, HMB was also identified in elite athletes, affecting 36.7%. Despite prevalence in the Singaporean population in chapter 5

being lower (22.1%), evidently, HMB is still a significant issue amongst this exercising group. The differences in prevalence between the likely primarily British and Singaporean groups could be due to ethnicity, since, while not recorded, it can be assumed that the majority of participants in chapter 4 were Caucasian, and that the majority of participants in chapter 5 were Asian. While difficult to decisively conclude, since there is a lack of research comparing these two populations when using the same means for diagnosis.

The NICE define HMB as "excessive menstrual blood loss which interferes with a woman's physical, social, emotional and/or material quality of life" (26). However, this definition is purely subjective, relying on the perception of an individual, and suggests that it is the sheer physical impact of the blood loss that causes identification of HMB, not the actual volume of blood lost. The historical definition established by Hallberg et al. utilised an objective measure of 80 mL of blood loss (217), but one has to question the applicability of this discreet value. They used an alkaline haematin method to estimate haemoglobin loss. However, blood volume can vary significantly between women, therefore it would be more appropriate to identify HMB as a percentage of total blood volume, if indeed it is the blood loss that deems an individual to be a heavy bleeder. Further, the physical identification of blood loss is difficult, not only due to hygiene concerns, which are likely to lead to the lack of desire to measure, but blood is also lost when passing urine, defecating, or through flooding into clothing or bedding. Additionally, given that not all menstrual fluid is blood, this causes further problems in quantification.

The criteria utilised in this thesis, while not validated, provides a more objective, realistic and hygienic indication of blood loss, which could easily be used by practitioners. Despite still having a subjective, perceptual element, the questions have increased clarity when compared to the NICE definition, aiding individuals and practitioners with an indication of what may be abnormal. Significantly, the key consideration is the impact that HMB may have, if this is purely psychological then the need to intervene is different to if there is either an underlying aetiology, an associated impact on iron status or other resulting factors that could negatively impact health and well-being. A simple tool for quick evaluation of risk is warranted, and further evaluation can follow to better understand need for treatment. This thesis highlights that when applying the outlined criteria, there is potential for this tool to identify those who are at increased risk of a compromised iron status and those who may suffer disruption to lifestyle/exercise training/performance. For the purposes of this research it was important to establish whether HMB should be considered in those who exercise and whether there are negative repercussions associated with it, and these were clearly demonstrated. Further, as previously explained, iron deficiency can take a while to manifest, therefore it is very possible for the impact of HMB on iron status can take a while

to manifest. It is also significant to note that the aetiology of HMB is often unknown. A 2014 study in university students, which diagnosed HMB using the pictorial blood assessment chart, found only 20.7% of the participants with HMB (n=82) to have underlying bleeding disorders or menstrual cycle related pathologies (i.e. endometriosis or polycystic ovary syndrome) (Gursel et al. 2014). This is significant, suggesting the presence of HMB as a condition in its own entirety that needs to be appreciated.

Chapters 4 and 5 clearly demonstrate the potential for HMB, when using the outlined diagnostic series, to be a cause for disruption to exercise training/performance or to everyday lifestyle/exercise performance. The marathon runners with HMB in chapter 4 were more than three times more likely to report that their menstrual cycle disrupts their exercise training/performance than those without HMB, while those with HMB in chapter 5 were more than four times more likely to cite this when compared to those without HMB. Previous research in the general population has focused on the effects of HMB on quality of life, but as already outlined there is a dearth of research assessing prevalence and effects of HMB in those who exercise. In light of this, the mechanism for this association is unsure. Given the known association between HMB and iron deficiency, which was demonstrated in chapters 5 and 6, it would seem prudent to suggest that a lack of iron may be the cause for any detriment in performance. In fact, HMB is considered to be the primary cause of IDA and iron deficiency in the general population (443). Chapter 4 supported this hypothesis, finding a reported history of anaemia to be greater in those identified with HMB than in those without. However, despite confirming haematologically that identified HMB presence increased the risk of a compromised iron status in chapter 5, when factored into a model, the relationship between HMB and the perception that the menstrual cycle disrupts everyday lifestyle/exercise performance was found to be independent of either IDA or iron deficiency. This suggests that the mechanism by which HMB may cause this is independent of iron status. future research must address this, and there is a clear need for treatment options. Being an inflammatory condition, it is possible that the increased levels of inflammation may be a cause for performance detriment, however prior to establishing this further research is needed to objectively measure performance.

Interestingly, the identified prevalence of HMB in chapter 7 was significantly higher than that in the chapters 4 - 6 and in that found previously in the general population. It has been suggested that IDA can cause a platelet dysfunction which results in increased menstrual blood loss (441). As a result, it could be hypothesised that a lack of iron is causing HMB as opposed to the converse. In fact iron repletion has been found to be an effective treatment for HMB in these instances (408). Further research should address this

association, in particular in those without anaemia. Iron repletion could be considered as a potential treatment option.

The subjective nature of the question surrounding the perception that the menstrual cycle disrupts exercise training/performance makes the drawing of conclusions problematic, however, while not addressing physical performance and still including a subjective element, chapter 6 provided a different means for evaluating the potential impact of HMB. The presence of HMB was clearly associated with increased general fatigue.

While this thesis used one tool to establish prevalence of HMB, there is a clear need to create one universally agreed diagnosis means. As already highlighted in this thesis existing tools have elements which will affect accuracy. It would however be advisable to identify the significance and implication of identification of HMB when identifying the best tool to use. For example, this thesis has demonstrated the increased risk of a compromised iron status and an association with the perception that the menstrual cycle disrupts exercise training/performance in those identified with HMB. While this firstly suggests that this tool could be used by practitioners to identify when a blood test should be taken for iron status, the results from this thesis also highlight that associated effects of HMB need consideration, such as an increased production of inflammatory markers.

8.2 HMB, iron status and fatigue

The symptoms of HMB and IDA are similar, both impacting on quality of life and altering ability to execute everyday social, mental and physical activities (28). In light of this, it could be suggested that a poor iron status could be on the causal pathway between HMB and fatigue. However, to date, the interrelationship between HMB, a suboptimal iron status and fatigue has not been assessed. A recent study in adolescents evaluated the association between HMB and serum ferritin and the effect of HMB on fatigue independently, but did not evaluate whether iron status was responsible for the increased fatigue seen in those with HMB (38). This hypothesis was tested in chapter 6, where interestingly, the association between identified HMB and an increase in general fatigue was not found to be mediated by IDA or anaemia. This suggests that the fatigue associated with HMB is caused by a different mechanism, independent of iron status.

The results from chapters 5 and 6 demonstrate that HMB, when identified using the outlined criteria throughout this thesis, is a condition in its own entity, and that the repercussions that ensue with this are not simply those associated with IDA or iron deficiency. The increased release of pro-inflammatory cytokines such as TNF-α could be

the cause for this, and (401) the relationship between TNF-α and fatigue is well established (421). Impairments in exercise are also inevitable in response to increased levels of underlying inflammation, therefore future research should investigate this, as a better understanding of this could guide potential treatment mechanisms. This association has not previously been highlighted and given the demonstrated potential for exercise impairment and high prevalence in those who exercise, this is of particular significance. This could even provide another potential means for diagnosis.

8.3 Prevalence of iron deficiency

The prevalence of IDA in the exercising population in chapters 5 and 6 was 14.8%, this is comparable to previous studies reporting a prevalence of 8 – 14% (119-121), and likely slightly higher due to this being an exercising population. As highlighted already, the diagnosis of iron deficiency has historically been problematic, particularly in those who exercise, with prevalence ranging from 24 – 47% (59). In chapter 5, applying the WHO criteria for iron depletion (serum ferritin < 15 $\mu g \cdot L^{-1}$), 29.9% were iron deficient. When applying a more lenient criteria, in accordance with other studies in athletes, and in line with findings from Wish *et al.* (2006) (436), where bone marrow iron stores were found to be compromised when serum ferritin < 30 $\mu g \cdot L^{-1}$, prevalence was 48.0%. Unsurprisingly, and as previously shown, the identified presence of HMB, using the diagnostic series outlined, increased the likelihood of a compromised iron status.

With the potential for nearly one in two exercising women to be iron deficient, there is an evident need to evaluate the diagnosis and resulting need for treatment, optimal treatment options and the mechanism by which iron deficiency has an effect. As outlined in chapter 2, there are a number of reasons for the existing lack of clarity. Chapter 7 aimed to address this and to advance understanding, as will be discussed subsequently.

8.4 Impact of iron deficiency

8.4.1 Effect on exercise and aerobic capacity

Results from previous studies regarding the effect of iron deficiency on exercise and aerobic capacity are equivocal, in part due to inconsistencies in study protocol (e.g. study duration, supplementation route and outcome measurements), iron deficiency diagnosis, participant performance level and sample sizes (46). In chapter 7, while an overall increase in exercise and aerobic capacity was seen, when broken down into groups based on baseline serum ferritin, increases were only evident in those more severely iron deficient (serum ferritin < 15 $\mu g \cdot L^{-1}$). No association was found between serum ferritin and the

magnitude of change in outcome markers, and primary endpoints did not increase in all participants in the severely iron deficient group. This suggests that while a serum ferritin < 15 $\mu g \cdot L^{-1}$ could be used to identify increased risk, these results clearly question the suitability of the use of serum ferritin alone for iron deficiency diagnosis in those who exercise.

8.4.2 Effect on fatigue and mood disturbance

Iron deficiency is associated with fatigue, and previous studies have found a reduction in fatigue in response to iron repletion in iron deficient but not anaemic individuals (56,226,247,248,439), however this has not been found by all (249). While chapter 6 found anaemia to be associated with increased fatigue, these findings were not mirrored in those with IDA or iron deficiency. However, in response to iron therapy in chapter 7 there was a universal decrease in fatigue and mood disturbance. The potential for a placebo effect cannot be ignored here, however construct validity was obtained by conducting three separate fatigue questionnaires. It is important to consider that due to the insidious nature of fatigue, it can be missed, and may not be appreciated until iron stores are repleted. A lack of association was found between serum ferritin and fatigue in either chapter 6 or 7, and to date, only one other study has addressed this, also concluding no relationship, again highlighting the need to re-evaluate markers used for iron deficiency diagnosis (38), while a future double-blinded study is necessary.

8.4.3 Diagnosis

The majority of previous research has focused on assessing the efficacy of iron repletion, and has not specifically evaluated the suitability of markers to diagnose iron deficiency. Further, most recent studies in the sporting context have been conducted in elite or well-trained athletes, and there is a particular absence of studies using intravenous iron in non-elite exercisers. To my knowledge, the IRONWOMAN trial is the first study to assess iron repletion in non-elite exercising women when repleting iron stores using intravenous iron. This is significant because not only may elite athletes respond differently to non-elite exercisers (1), but this has the potential to influence a very large number of people.

Clinically being the primary marker used for the diagnosis of iron deficiency (40,100,444), serum ferritin was used for diagnosis throughout this thesis. However, the findings from chapters 5, 6 and 7 all clearly question its suitability in those who exercise. There is much ambiguity surrounding the appropriate serum ferritin cut-off in the literature and amongst medical professionals (46). In chapter 5, in an attempt to address this, when evaluating the association between identified HMB, iron status and reported menstrual cycle effects on performance, both a ‘severe’ and ‘moderate’ serum ferritin iron deficiency cut-off was

applied. The WHO's criteria for depleted iron stores (serum ferritin < 15 $\mu g \cdot L^{-1}$) was used to define 'severe' iron deficiency. Then a more lenient diagnosis (serum ferritin < 30 $\mu g \cdot L^{-1}$), reflecting that postulated by Wish *et al*. (2006) (436), and in line with that commonly used clinically and in sports science research, was used and termed 'moderate' iron deficiency. However, as neither 'severe' nor 'moderate' iron deficiency were associated with the perception that the menstrual cycle disrupts everyday lifestyle/exercise performance, this study did not help to address the ambiguity surrounding iron deficiency diagnosis. In light of this, chapter 6 only applied the serum ferritin cut-off for 'severe' iron deficiency. While HMB and anaemia were found to be associated with an increase in fatigue, there was no relationship between IDA or iron deficiency and fatigue, and significantly there was also no relationship between either [Hb] or serum ferritin and fatigue.

In chapter 7, increases in aerobic and endurance capacity were only seen in the group with severe iron deficiency (serum ferritin < 15 $\mu g \cdot L^{-1}$), yet response was not associated with or dictated by baseline serum ferritin levels. Factoring in [Hb] did not aid in the prediction of response, suggesting that an underlying state of IDA was not confounding results. It could be hypothesised that optimal levels of serum ferritin are specific to an individual, therefore for best practice, serum ferritin could be monitored regularly over time, ensuring that athletes are in a comparable state when tested (rested and fasted), with any significant declines in serum ferritin indicating need for intervention. Despite being subject to a placebo effect, the universal reduction in mood disturbance and fatigue is significant and needs further assessment, but again serum ferritin was not associated with this.

Evidently, the results from chapters 5, 6 and 7 highlight a need to reassess the use of serum ferritin alone as a primary means for indicating iron deficiency. There is a clear need to investigate the use of other markers. Given that nearly one in two were found to be iron deficient in chapters 5 and 6, and that the exercise industry is rapidly expanding, this has fundamental clinical significance. This need is exacerbated by the large number of individuals supplementing with iron. Clear guidelines are necessary.

8.4.4 Mechanism of iron deficiency

The findings in chapter 7 suggest that there are non-haematological effects of iron deficiency, which may occur prior to haematological effects. In some participants, $\dot{V}O_{2max}$ increased without concomitant increases in total haemoglobin mass or [Hb]. Non-haematological effects of iron deficiency on exercise capacity have not been shown before in humans. Research in iron deficient rodent models has shown mitochondrial function and composition to be affected in iron deficient states (227). However, as described in chapter 2, research in humans as to the impact and mechanism of iron deficiency is equivocal.

The increase in $\dot{V}O_{2max}$ without an increase in markers of erythropoiesis seen in chapter 7 suggests that the improvements could be due to enhanced cellular oxygen uptake or utilisation, both involving iron dependent pathways, for example enhanced iron containing haem enzyme activity in the electron transport chain in mitochondria. Indeed, in non-elite athletes there is increased potential for factors other than haemoglobin mass to impact upon $\dot{V}O_{2max}$, and research suggests that the $\dot{V}O_{2max}$ of non-elite athletes is more likely to be limited by cellular uptake kinetics than by oxygen supply when compared to elite athletes (1). The training status and performance level of athletes may therefore affect their response, and given the historical lack of research in non-elite athletes, particularly when using intravenous iron, this could be the reason for the novel non-haematological effects of iron deficiency found here. The use of intravenous iron in chapter 7 as opposed to oral iron meant that the controlled absorption route through enterocytes was bypassed. This provides a more reliable assessment of the effect of iron repletion and the resulting efficacy of markers of iron deficiency.

While inevitably subject to a placebo effect, the universal reduction in fatigue and mood disturbance seen in chapter 7 supports previous hypotheses that optimal serum ferritin levels for the prevention of impacts on cognitive performance may in fact be higher than often thought. It has been hypothesised that cognitive performance will be affected when serum ferritin < 50 $\mu g \cdot L^{-1}$ (247). This suggests that iron deficiency impairs cognitive performance prior to functional repercussions. However, to my knowledge no previous studies have evaluated both markers relating to fatigue and physical function in iron deficient women to further establish this, therefore to my knowledge this is the first study to assess this. Given the additional non-haematological roles of iron this is plausible, however as previously highlighted, studies looking at the mechanism for this in humans are lacking. The outcomes of this research could have significant implications, particularly given the sheer number of women with relatively low ferritin levels, in fact, a study in a general adolescent European population found mean serum ferritin in women to be 29.9 $\mu g \cdot L^{-1}$ (445). The addition of a blinded placebo group in a future study would be very insightful to aid further evaluation.

8.4.5 Awareness and supplementation

The findings of this thesis are in agreement with previous work highlighting a poor awareness of iron status, and the potential for a lack of awareness of HMB. Fraser *et al.* (2015) found that 46% of those with HMB have not sought medical help, suggesting no prior knowledge (28). In the marathon population in chapter 4, 55.6% had not sought advice/help for heavy periods, while 66.6% and 68.9% in chapters 5 and 6 had not sought

this. Worryingly however, in chapter 6, the prevalence of IDA, anaemia and iron deficiency did not differ in those with HMB who had or had not sought advice/help. While making an assumption, this could suggest that appropriate medical tests are not being conducted in response to HMB. A lack of awareness amongst medical professionals has previously been shown (384), and the ambiguity of the diagnosis of HMB is a likely confounder. In light of the significant impact of HMB shown here; being associated with perceived menstrual cycle driven disruptions to exercise training/performance , increasing fatigue and worsening iron status, there is an evident need to raise awareness of this condition. Further, when using this means for diagnosis, as it is clearly a prevalent condition amongst athletes and exercising women, sports medics should specifically be alerted to it. Given the ease of the applied diagnostic series, use of this or an abbreviation should be considered amongst medical professionals. However, the need for treatment options is imperative, and priority needs to be placed on research to this avail. For example, given the likely increase in inflammation, nutritional interventions with anti-inflammatory benefit could be prioritised during or prior to menstruation.

With less than half of those with IDA (41.2%) in chapter 5 reporting prior knowledge, and only 8.8% suggesting prior knowledge in the previous three months, awareness of iron status was poor. Given the known impact of IDA, this is concerning and more needs to be done to highlight the symptoms of this condition. Notably, many were found to supplement with iron despite a lack of prior knowledge of iron status, with nearly 80% of elite athletes in chapter 4 reporting supplementation. While of those in this study who indicated a lack of awareness of prior anaemia, more than half reported having taken iron supplementation. It is known that coaches encourage athletes to supplement with iron, however both chapter 7 and previous research (39) have demonstrated no benefit of unnecessary supplementation, and given the possible side effects associated with unnecessary supplementation, including an increase in oxidative stress and inflammation (61), and the consequences that this may have on iron absorption, it is important to improve education and clarify when supplementation should be used. Further, there are added risks associated with unnecessary supplementation, particularly in those with mutations to the haemochromatosis, HFE gene. Individuals with this genotype have an increased likelihood of iron overload. Significantly, this condition is particularly common in elite athletes (259), highlighting that supplementation must only be administered when iron status is known.

Throughout this thesis, it is evident that the use and suitability of serum ferritin for the determination of iron status particularly in those who exercise regularly is questionable. It is the most common and cost effective test used clinically, however its ability to diagnose iron deficiency appears limited, and has been found to exhibit significant disparity

throughout the day in athletes, with variation ranging from 13 – 75% (45). Chapters 5 - 7 show no evidence to specifically demonstrate that serum ferritin alone can be used to elucidate who will respond to iron therapy, and therefore to diagnose iron deficiency. While it is evident that those with serum ferritin < 15 $\mu g \cdot L^{-1}$ are at an increased risk of detriments to aerobic and exercise capacity, this was not universal. Further, while all participants in the IRONWOMAN study saw a reduction in fatigue and mood disturbance this was independent of baseline serum ferritin. With this in mind it would be prudent to investigate the use of other markers, possibly in conjunction with serum ferritin to determine iron status, while also considering whether optimal serum ferritin levels are specific to an individual. From the perspective of applying these findings clinically, it would be advisable to monitor athletes longitudinally, always testing them in a rested and fasted state, with intervention only when a significant decline is observed.

8.5 Limitations

Throughout this thesis a number of limitations have been outlined in each chapter. To summarise, it must be acknowledged that in the absence of any universally approved validated tool, the diagnostic series applied for the identification of HMB throughout this thesis was self-derived. This could obviously lean to significant inaccuracies, while also appreciating that the underlying goal of HMB identification still needs to be established. There were also a number of limitations associated with the female health questionnaires utilised, including inconsistency of time frames for reporting of factors such as performance times and training volumes, adding to risk of bias in the development of conclusions. It also must be appreciated that these questions were subjective.

The use of the convenience sample in chapters 5 and 6 also needs to be highlighted, this is a different population which may have encountered language barriers regarding questionnaire completion.

The lack of a control group or randomisation in the IRONWOMAN study meant that this is subject to a placebo effect, and this needs to be addressed in future studies. This study was also limited by only measuring one time point in follow-up and the lack of measurement of additional iron parameters and erythropoietic biomarkers.

8.6 Future studies and directions

There are a number of future directions that could be taken based on the findings throughout this thesis. Firstly, the limitations highlighted involving the female health questionnaires used in this thesis warrant the need for the development of a new version.

8.6.1 Future derivation of a Female Health Questionnaire

In order to develop this research further, and given the highlighted limitations with the female health questionnaires applied in this thesis, it would be advisable to develop a new questionnaire that can be used widely. In order to do this, it is vital for a universally recognised and approved diagnosis for HMB to be established. Essentially, as already highlighted consideration of the desired impact of HMB firstly needs to be identified; is it the physical or psychological impact that is the key factor. Once this is known, a realistic means for diagnosis needs to be used. There are a number of benefits to the application of the criteria used throughout this thesis as previously highlighted, including those related to hygiene, the increased element of objectivity and the ease of application, so a similar series could be applied. Even if this is used to highlight risk, it would really advance practice in this field, and evidently as identified here, the enhanced risk of a compromised iron status alone warrants its application.

Addressing the other issues with the Female Health Questionnaire, in order to gain association with awareness, individuals should specifically be asked whether they have sought medical advice about heavy menstrual bleeding and whether they deem themselves to have this condition. Timescales throughout the questionnaire also need to be consistent, however arguably, given that the repercussions of HMB can be slow to manifest the proposed time scale may be long in duration. Perhaps this could in part be overcome by simply asking whether heavy periods have disrupted exercise training/performance. Further, identification of performance level may, as opposed to performance times or training volume may be more appropriate.

Regarding use of hormonal contraception, a future questionnaire should establish whether any form of hormonal contraception is used, and could also consider asking whether this was initiated as a means to treat HMB. The presence of underlying pathologies should also be recorded, as it could help develop understanding as to the aetiology of the condition.

8.6.2 Other future directions

Despite the ambiguity surrounding HMB diagnosis, it is evident throughout this thesis that utilising the applied criteria, risk of a compromised iron status, fatigue and citation that the

menstrual cycle disrupts exercise training/performance. These areas all need to be considered in further details and future studies should be conducted to better understand these relationships. Primarily, due to the lack of involvement of iron status in either of the associations between identified HMB and fatigue or reported menstrual cycle-driven disruptions to exercise training/performance, other haematological markers should be measured. For example TNF-α, or oxidative stress, to determine whether these could indicate the primary biomarkers involved in these relationships. It would also be beneficial to more objectively measure performance and fatigue.

A randomised and blinded study needs to be conducted to minimise any element of bias in the IRONWOMAN trial – this is particularly significant from the perspective of impact on fatigue and mood disturbance. Future studies should also address other time points, and given the questionable ability of serum ferritin at identifying a compromised iron status throughout this thesis, future studies should include additional markers of iron status.

8.7 Conclusions

In conclusion, this thesis firstly addressed the prevalence of HMB when evaluated using a diagnostic series outlined in chapter 3, and then the association between this and the perception that the menstrual cycle disrupts exercise training/performance and fatigue. It then assessed the potential involvement of iron in these relationships, and finally evaluated the effect of iron repletion on markers of exercise and aerobic capacity and cognitive performance. In so doing, the efficacy of existing markers used for the diagnosis of iron deficiency was evaluated, while also highlighting the potential impact that both HMB and iron deficiency may have.

This thesis highlights the presence of some common issues experienced by women in sport that have the ability to significantly affect their exercise performance and general health. Given the increasing acknowledgement that the menstrual cycle can have an impact on exercise performance there is much scope for future research to evaluate further means in which the menstrual cycle can affect performance, while also identifying appropriate strategies to mitigate this. This is a particularly exciting time for research in female athletes.

Overall conclusions can be summarised as follows and are outlined in Figure 8.1:

i. When utilising the outlined criteria to identify presence, HMB is common in both exercising women and elite athletes. In light of the overt lack of investigation

surrounding HMB, this finding is of great significance, particularly given its association with: the perception that the menstrual cycle disrupts exercise training/performance; increases in fatigue; and an enhanced risk of iron deficiency, as evidenced in this thesis.

ii. The association between HMB (when identified using the outlined criteria) and the perception that the menstrual cycle disrupts exercise training/performance is independent of iron status. Other associated outcomes of HMB, e.g. release of pro-inflammatory cytokines, need to be assessed to determine whether this is overriding any impact of a compromised iron status. However, given the potential limitations associated with serum ferritin for iron deficiency diagnosis, this relationship should be further evaluated using additional iron status markers.

iii. The association between HMB (when identified using the outlined criteria) and fatigue is not mediated by IDA or iron deficiency when using serum ferritin and haemoglobin concentration for diagnosis. Again, factors associated with HMB need to be considered as these may be the primary in driving the increase in fatigue.

iv. An increase in endurance and aerobic capacity and a reduction in fatigue and mood disturbance was seen in non-elite, exercising, iron deficient women when repleted with intravenous iron. However, when individuals were separated into two groups based on severity of iron deficiency, endurance and aerobic capacity only increased in those with more severe iron deficiency as defined using serum ferritin. Despite this, there was a range of individual variation in response, and serum ferritin was not able to predict this. Those with an increased susceptibility to iron deficiency can be identified, but future work must address other markers of iron status alongside serum ferritin or as an alternative to this measure to enhance reliability of diagnosis. Considering the significant reported use of supplementation, this work is essential. Further, the impacts of iron deficiency on cognitive performance need further consideration, particularly since there is potential for these to be affected prior to impacts on exercise and aerobic capacity.

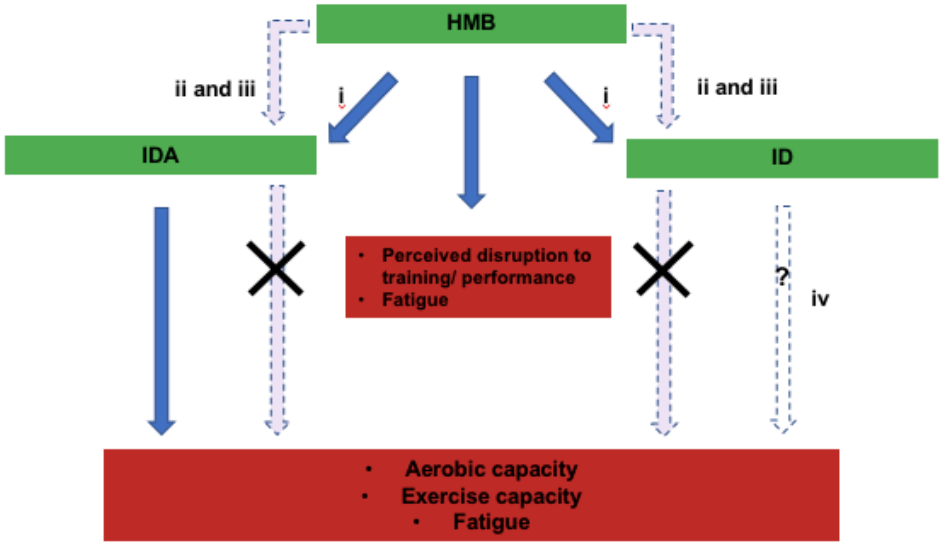

Figure 8.1 - An overview of the conclusions. i. There is an association between identified HMB and IDA and ID; ii. The association between HMB and the perception that the menstrual cycle disrupts exercise training/performance is independent of IDA and ID; iii. The relationship between identified HMB and fatigue is not mediated by IDA and ID; and iv. There was an overall increase in exercise and aerobic capacity in response to intravenous iron in iron deficient (serum ferritin < 15 $\mu g \cdot L^{-1}$), non-elite exercising women, but this response was not universal and independent of serum ferritin. However, a reduction in fatigue was observed in all. There is potential for IDA and possibly ID to actually be a causative factor for the development of HMB.

8.8 Key recommendations

- Diagnosis of HMB – a universal tool needs to be created for HMB diagnosis. This needs to factor in the subjective nature of this condition, but also the repercussions and implications that may ensue. Further agreement as to the desired outcome of HMB needs to be evaluated, be is psychological or physical.
- Awareness of HMB – with the majority of individuals who met the outlined HMB not reporting to have sought help/advice for heavy periods, it could be suggested that awareness that blood loss is potential abnormal and excessive is poor. In light of the negative implications that were found to be associated with HMB here an improved education is necessary. Firstly, appreciation amongst sports medical professionals that HMB is a common condition in athletes of all levels is necessary. Secondly, appropriate tests, e.g. measurements of iron status, need to be

performed where HMB is diagnosed. Finally, a greater understanding of HMB is needed amongst individuals, so that they know when to visit a medical professional. Excessive bleeding is evidently not a topic frequently discussed, however increased discussion and knowledge around this should reduce the potential reluctance of women to discuss this personal issue.

- Given the implications, priority should also be placed on finding appropriate treatment options for HMB alongside evaluating the aetiology of this condition – which could also aid in future physical diagnosis.
- The use of serum ferritin alone for iron deficiency diagnosis is evidently questionable. While it could be used to assess risk, measurement of other markers alongside serum ferritin is advisable, e.g. transferrin saturation. For best practice, iron status should be monitored longitudinally, alongside total haemoglobin mass, with intervention only when a significant decline is seen.
- Future research needs to address the potential for other more suitable markers for the diagnosis of iron deficiency.
- This thesis highlights that unnecessary iron supplementation is rife and does not benefit aerobic or exercise capacity. Many athletes, those who are elite in particular, supplement without knowledge of iron status. Given the potential adverse side effects of unnecessary supplementation it is important that individuals and their support personal appreciate that supplementation must only be taken when necessary. A food first approach at correcting iron status should be taken where possible.

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10 Appendices

10.1 Publication: The prevalence and impact of heavy menstrual bleeding among athletes and mass start runners of the 2015 London Marathon. (Letter to the Editor)

Bruinvels G, Burden R, Brown N, Richards T, Pedlar C. The prevalence and impact of heavy menstrual bleeding among athletes and mass start runners of the 2015 London Marathon. Br J Sports Med. 2016 May; 50(9):566.

LETTER

The prevalence and impact of heavy menstrual bleeding among athletes and mass start runners of the 2015 London Marathon

The single most common cause of iron deficiency anaemia in the developed world in premenopausal females is the menstrual cycle.[1] It is well recognised and reported that amenorrhoea and oligomenorrhoea are common in elite athletes typically as a result of relative energy deficiency;[2] however, little is known about the prevalence of other menstrual abnormalities. Heavy menstrual bleeding (HMB or *menorrhagia*) affects a quarter of the general population,[3] yet no data exist for athletes or exercising women. It is possible that HMB might impact significantly on women's participation in sport. HMB can lead to fatigue, anxiety, reduced mood and energy levels with a negative impact on quality of life and productivity.[4] Furthermore, iron turnover in exercising females is likely to be increased further due to factors such as haemolysis putting them at a high risk of iron deficiency anaemia.[5] Iron is an essential micronutrient required for numerous biological functions, and deficiency can result in adaptive changes limiting haemoglobin production and a state of iron deficiency anaemia. We sought to identify the prevalence and impact of HMB in exercising females where anaemia may have a significant effect on training and performance.

We recently conducted a 'Female Health Questionnaire', which incorporated a validated diagnostic HMB series, demographics, athlete ability data, training status, known anaemia, iron supplementation and questions concerning the effect of the menstrual cycle on training and performance. The survey was initially conducted online (n=789 women), advertised via social media. Subsequently, to obtain non-biased data, the same survey was then conducted via face-to-face interviews with runners during registration for the 2015 London Marathon Exhibition (n=1073 women). Among the group a total of 90 participants were classified as 'elite'. The key findings from this survey were that HMB was common in both groups; reported by over half of those online (54%), and by more than a third of the marathon runners (36%). In total, 55% (online) and 32% (marathon runners) stated that their menstrual cycle impacted on training and performance, this being more common in those with HMB (χ^2=183.4, p<0.01). Surprisingly, HMB was also prevalent among elite athletes (37%). Overall, 32% of all participants reported a history of anaemia, with this also being more common in those who have experienced HMB (41% vs 26%; χ^2=70.765, p<0.01), while 50% had previously supplemented with iron. Only a minority (22%) had sought medical advice. No significant association was found between average weekly exercise volume and HMB presence.

In summary, we found HMB to be highly prevalent in female athletes, associated with anaemia, an increased use of iron supplementation and reported negative impacts on performance. Somewhat unexpectedly, our results suggest that HMB is more common in the exercising population than in the general population. Although there are a number of limitations to this questionnaire-based study, we highlight that HMB may be underrecognised. Further research is needed to describe this issue and to understand its implications. Interventions to support female athletes such as iron therapy,[6] and an increased awareness of HMB among sports medicine professionals could have far reaching benefits for the female athlete.

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Contributors GB participated in protocol design, data collection, data analyses and manuscript preparation. CP, TR, RB and NB participated in the protocol design, data analysis and manuscript preparation.

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10.2 Publication: The Prevalence and Impact of Heavy Menstrual Bleeding (Menorrhagia) in Elite and Non-Elite Athletes. (Research Article)

Bruinvels G, Burden R, Brown N, Richards T, Pedlar C. The Prevalence and Impact of Heavy Menstrual Bleeding (Menorrhagia) in Elite and Non-Elite Athletes. PLoS One. 2016 Feb 22; 11(2):e0149881.

PLOS | ONE

RESEARCH ARTICLE

The Prevalence and Impact of Heavy Menstrual Bleeding (Menorrhagia) in Elite and Non-Elite Athletes

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Abstract

To identify the prevalence and impact of heavy menstrual bleeding (HMB) in exercising females where anemia may have a significant effect on training and performance a 'Female Health Questionnaire' was designed incorporating a validated diagnostic HMB series, demographics, exercise ability data, training status, anemia, iron supplementation and whether the menstrual cycle had affected training and performance. The survey was conducted in two stages; initially online, advertised via social media, and then repeated via face-to-face interviews with runners registered for the 2015 London Marathon. 789 participants responded to the online survey, and 1073 completed the survey at the marathon. HMB was reported by half of those online (54%), and by more than a third of the marathon runners (36%). Surprisingly, HMB was also prevalent amongst elite athletes (37%). Overall, 32% of exercising females reported a history of anemia, and 50% had previously supplemented with iron. Only a minority (22%) had sought medical advice. HMB is highly prevalent in exercising females, associated with self-reported anemia, increased use of iron supplementation and a perceived negative impact on performance. Further research is needed to investigate the impact of HMB, iron deficiency and anemia in exercising females.

Introduction

Heavy menstrual bleeding (HMB) is common, affecting a quarter of the female population.[1] HMB can negatively impact on physical, emotional and social quality of life and reduce work capacity.[2,3]

Diagnosing HMB can be subjective and definitions include; blood loss of more than 80ml per menstrual cycle or "excessive menstrual blood loss which interferes with a woman's physical, social, emotional and/or material quality of life".[3,4] In a recent Europe-wide study a diagnosis of HMB was given if two or more of the following criteria were met; 1. passing of large

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blood clots, 2. need for double sanitary protection (both towels and tampons), 3. need for frequent changes of tampons and towels (meaning changes every 2 hours or less, or 12 sanitary items per period) and 4. flooding through to clothes or bedding.[1]

The greater blood loss in HMB increases susceptibility to iron deficiency, which if left untreated may progress to iron deficiency anemia (IDA). Iron is an essential micronutrient required for numerous biological functions, including oxygen transport, cellular and mitochondrial respiration, electron transfer reactions, gene regulation, cell growth and differentiation.[5] Compromised iron stores cause adaptive changes, eventually resulting in limitations to the production of hemoglobin and a state of IDA. Menstruation is the most common single cause of IDA in females of a childbearing age,[6] with HMB specifically identified as the principal cause of iron deficiency and IDA in clinical practice in this population.[7] In a recent study of women with HMB, 63% of respondents reported being deficient in iron at some point.[1] However, despite the high prevalence of HMB, awareness is poor, with only a small minority (6%) of women seeking medical help annually.[8]

The impact of compromised iron stores on oxidative metabolism in endurance athletes can be significant, potentially reducing total hemoglobin mass, oxygen carrying capacity and performance.[9,10] Furthermore, those who exercise are at higher risk of iron deficiency as a result of increased iron loss through hematuria (blood in urine), gastrointestinal bleeding, sweating and hemolysis (particularly exacerbated in impact sports involving foot strike).[11–14] Research into the impact of iron deficiency without anemia is inconclusive, with an identified need for further research.[15]

While HMB has been shown to affect more than a quarter of women in the general population, the prevalence of HMB and the impact upon training and performance in exercising females has been unknown. We have recently published this headline data in a brief letter,[16] and in this paper we aim to 1. provide the full methods and results from this study, identifying the prevalence of HMB in exercising females; 2. determine any differential effect on exercisers of varying abilities; and 3. outline the perceived reported impact of HMB on training and performance which we were able to do through this research.

Materials and Methods

This research has been approved by the St Mary's University Ethics Committee. A 12-item 'Female Health Questionnaire' including free-text and yes-no polar questions was developed and designed to take 2–3 minutes to complete. The four-symptom definition of HMB [1] was used to identify HMB sufferers and information was collected on age, 'personal best' sports performance times, current training volume, previous history of anemia and iron supplementation (including as part of a multivitamin), the menstrual cycle and difficulties caused by it, and oral contraceptive pill (OCP) usage (S1 Appendix). The participants were informed that by indicating that they agree to the terms and completing the survey they have provided written informed consent for their information to be used in this study. The inclusion criteria were: female, aged $\geq$18 years, pre-menopausal and regularly exercising ($\geq$90 minutes/week).

Stage 1—online questionnaire

The questionnaire was administered online and advertised through social media including Twitter, Facebook, online blogs and forums, university newsletters, websites and by word of mouth between 22 January 2015 and 19 May 2015. A link was provided to the internet-based survey in addition to some brief information about the research.

Stage 2—marathon exhibition questionnaire

Females registering for the 2015 London Marathon at the pre-event exhibition were surveyed using the same questionnaire. No bias was applied when selecting females to question and to avoid a response bias a scripted standardized introduction was made providing no specific information about the context of the survey. To ensure maximum response yield, surveys were completed at the time of asking. The questions and format of the paper copies used at the Exhibition were identical to the online survey to maintain equivalency and reliability of this mixed mode strategy.[17]

Data Analysis

Data were analyzed descriptively to summarize the prevalence of HMB, known anemia, iron supplementation, the seeking of medical help and impact of HMB on training and performance in both stages 1 and 2. The statistical analysis was completed using a predictive analytic software statistics computer package (IBM SPSS Statistics for Macintosh, Version 21.0, Armonk, NY: IBM Corp.). Statistical significance was set at $P<0.05$. Chi-squared tests were used to determine whether there was an association between HMB and presence of anemia and HMB and self-reported impacts on training and performance. Mann-Whitney U and Kruskal Wallis H tests were used to determine whether age and average weekly training volume were related to HMB. A Kruskal-Wallis H test with *post hoc* analysis and correction was used to determine whether 5km personal best time was linked to the number of HMB symptoms experienced, and Mann-Whitney U tests and Chi-squared tests were used to determine whether participant performance level (based on 5km personal best) was related to HMB incidence.

After combining both groups, a sub-analysis was conducted to separate out elite athletes using the following criteria: 5km ≤18 minutes, 10km ≤36 minutes, half marathon ≤80 minutes, 2km row ≤7 minutes:45 seconds (elite running criteria defined using the 2015 'Great Run' series definitions of 'elite', rowing criteria defined by English Institute of Sport physiologist). Participants were split into the following groups based on typical total minutes exercised per week <90, 90–180, 180–360, 360–540, 540–720, and >720 minutes.

Results

Stage 1

A total of 789 surveys were completed online. More than half (54.1%) of the participants had experienced HMB at some point (Table 1).[16] 55.4% stated that their menstrual cycle impacts upon their training and performance (Table 1), with those meeting the HMB criteria (n = 427) being more likely to state this (69.3% vs. 39.0%; $\chi 2 = 867.593$, $p<0.01$) (Table 2).[16] Those with a history of HMB were found to be older (31 years ±9.32 vs. 29 years ±7.49; $H(2) = 10.392$, $p<0.01$).

Of the 427 participants who met the HMB criteria, 37.2% had sought medical help for heavy periods (Table 2).

Stage 2

1091 face-to-face surveys were collected and inputted into the Bristol Online Survey platform manually by the lead investigator and an assistant. Those with missing data or those completed by females who did not meet the inclusion criteria were excluded, resulting in a final sample size of 1073 for further analysis. Eight individuals declined to complete the survey once they had read the study information, and 61 declined answering the survey prior to being informed

Table 1. Self reported prevalence of heavy menstrual bleeding (HMB), the effects on training and performance, seeking of help, history of anemia and iron supplementation. [16]

	Stage 1 (n = 789)	Stage 2 (n = 1073)	Elite athletes (n = 90)
HMB	427 (54.1%)	381 (35.5%)	33 (36.7%)
Affects training and performance	437 (55.4%)	340 (31.7%)	46 (51.1%)
Sought help	190 (24.1%)	226 (21.1%)	21 (23.3%)
History of anemia	303 (38.4%)	300 (28.0%)	47 (52.2%)
History of iron supplementation	451 (57.2%)	486 (45.3%)	71 (78.9%)

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about the content typically citing a lack of time. Therefore, in stage 2, the survey was fully completed by 94% of randomly approached female marathon runners.

The prevalence of HMB in females undertaking the 2015 London Marathon was 35.5% (Table 1).[16] Overall nearly one third (31.7%) said that their menstrual cycle impacts upon their training and performance (Table 1).[16] This was more than twice as likely to be a problem in those with HMB (48.3% vs. 22.5%; $\chi 2 = 1151.481$, $p<0.01$; Table 2). Those who have experienced HMB were older than those who have not (35 years ±7.95 vs. 32 years ±7.83; $H(2) = 18.936$, $p<0.01$). Of the 381 participants who met the criteria for HMB, 44.6% had sought medical help (Table 2).

Across both groups, known anemia was reported by 603 (32.4%) participants, while 1049 (56.3%) specified that they were unsure whether they have had anemia.[16] Reported anemia was more common in those with HMB (40.7% vs. 26.0%; $\chi 2 = 70.765$, $p<0.01$).[16] Use of iron supplementation was also more common in those reporting HMB (58.4% vs. 50.3%; $\chi 2 = 39.199$, $p<0.01$).[16] Less than a quarter of all surveyed reported having sought help for heavy periods (22.3%), with this increasing in those who met the HMB criteria (40.7%).[16]

When a sub-analysis was conducted and elite athletes were separated out from both groups, 36.7% met the HMB criteria, with 51.1% indicating that their menstrual cycle has impacted upon their training and performance, with these being significantly related ($\chi 2 = 5.046$, $p<0.05$). A history of anemia was reported by 52.2%, with 78.9% having supplemented with iron (Table 1).

When participants who specified a 5km personal best time (n = 1166) were divided into groups based on the number of HMB symptoms they have experienced, a significant difference was found between groups ($H(4) = 11.464$, $p<0.05$), despite distributions looking similar. However, a *post hoc* analysis using pairwise statistics revealed no statistically significant pairwise comparisons. When simply comparing those with and without HMB, median 5km times were

Table 2. Self reported prevalence of heavy menstrual bleeding (HMB), its effects on training and performance, seeking of help, history of anemia and iron supplementation in those who have met the HMB criteria.

	Stage 1 (n = 427)	Stage 2 (n = 381)	Elite athletes (n = 33)
Affects training and performance	296 (69.3%)*	184 (48.3%)*	22 (66.7%)***
Sought help	159 (37.2%)*	170 (44.6%)*	14 (42.4%)**
History of anemia	184 (43.1%)*	145 (38.1%)*	19 (57.6%)
History of iron supplementation	262 (61.4%)***	210 (55.1%)*	27 (81.8%)

Significant differences between the values here from those meeting the HMB criteria and those who haven't are shown as follows:
*p < 0.001,
**p < 0.01,
***p < 0.05.

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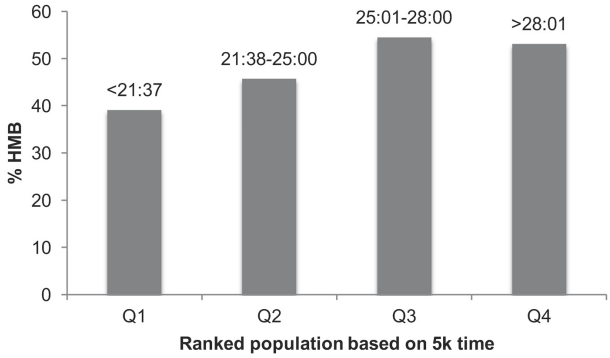

Fig 1. Prevalence of reported heavy menstrual bleeding (HMB) across participant performance level. 5km personal best times (minutes:seconds) are divided into quartiles, Q1 representing those with the fastest times, Q4 the slowest. A significant difference was found between groups *(p < 0.01)*.

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significantly different (25 minutes:0 seconds vs. 24 minutes:24 seconds; $z = 3.099$, $p<0.05$). When 5km personal best times were divided into quartiles (Q1 being the fastest athletes), a significant difference was seen in HMB prevalence between groups ($\chi 2 = 14.890$, $p<0.01$), the faster runners in Q1 being less likely to have HMB (39.1%) than the slower runners in Q4 (53.1%) (Fig 1).

No statistically significant association was found between average weekly exercise volume and HMB presence ($z = -0.811$, $p>0.05$). Those exercising for >720 minutes each week appeared as likely to suffer from HMB as those exercising for <90 minutes each week ($\chi^2 = 6.765$, $p>0.05$).

Discussion

This is the first study to identify that HMB is a common problem amongst exercising females. Stage 1 of this research was used to ascertain whether HMB was prevalent amongst exercising females. It is acknowledged that this online survey was likely to be biased because females with menstrual cycle issues were more likely to complete the questionnaire, however with 54.1% meeting the HMB criteria this demonstrated that this is a significant problem within this populace. To obtain unbiased prevalence data a large study (stage 2) incorporating a number of controls to prevent bias was conducted at the 2015 London Marathon Exhibition. This found that 35.5% marathon runners met the HMB criteria therefore confirming the outcome in stage 1 that this is a common problem amongst exercising females. HMB has previously been shown to affect more than a quarter of the general female population,[1] but this is the first study to investigate prevalence amongst exercising females.

Only 43.1% and 38.1% of those females from stages 1 and 2 with HMB had sought medical help. This highlights the need for increased HMB awareness. However, previous research has demonstrated that almost half of females meeting the criteria for HMB who sought help did not have HMB confirmed,[1] and the results of an audit conducted by the Royal College of Obstetricians showed that once diagnosed one third were not given treatment in primary care. [18] This suggests that from both a diagnostic and treatment perspective an increased

awareness of HMB prevalence and treatment options is required. However, a review of treatment methods has shown there to be considerable variation in the treatment procedures and medications used, highlighting the need for further research.[19]

Due to the increased blood loss, those with HMB are more likely to suffer from iron deficiency and anemia, and this is consistent with our finding that those meeting the HMB criteria were more likely to report previous diagnosis of anemia than those who do not (40.7% compared to 26.0%). This may be higher as more than half of all respondents said that they were unaware whether or not they have been anemic. In the general population IDA has been shown to affect two thirds of women with HMB.[3] Regardless of menstruation, those participating in endurance exercise are susceptible to iron deficiency due to increased iron losses as a result of foot strike hemolysis, sweating, and gastrointestinal bleeding.[11–14] Dietary intake of iron has also been found to be suboptimal in those who exercise, and particularly in females.[20] This iron deficiency and IDA risk is further exacerbated in those with HMB. Many elite athletes routinely supplement with iron—as shown here with 78.9% reporting supplementation. Coaches often encourage supplementation without knowledge of iron status due to the unfounded but common belief that iron deficiency is rife and supplementation may benefit performance. Less than half of those with HMB have sought medical help, therefore it is necessary to raise awareness as clinical iron deficiency and IDA can result in fatigue, weakness, impaired cognition and psychological morbidity, negatively impacting upon quality of life.[21]

Similar to the findings from Marret et al,[22] this study demonstrated that HMB was marginally more likely in those who were older. Unsurprisingly those with HMB were more likely to report that their menstrual cycle impacts upon their training and performance.

The sub-analysis from the elite athlete sample showed that more than one third met the HMB criteria. This is somewhat surprising because it is well documented that elite female athletes, particularly endurance athletes are susceptible to amenorrhea and oligomenorrhea often as a result of a relative energy deficiency associated with a high training volume.[23,24] This suggests that elite athletes may also be susceptible to other menstrual disturbances. Furthermore, it could be hypothesized that increases in training volume would equate to increased risk of amenorrhea or oligomenorrhea, potentially decreasing HMB incidence, but this was not the case here with no identified relationship between total number of minutes exercised per week and HMB presence. However, those with faster 5km personal best times were less likely to report HMB, with the median 5km time in the HMB group being slower. The slower times seen in Q3 and Q4 where HMB prevalence is higher when compared to Q1 could be caused by an increased incidence of IDA, which is impacting upon performance, alternatively increased rates of amenorrhea in Q1 could reflect the lower HMB incidence seen here. However these differences were only marginal, and further research is required before forming a definitive conclusion. Historically, much research has focused on the female athlete triad and the new term 'Relative Energy Deficiency in Sport'–RED-S, particularly in elite athletes.[25,26] These syndromes are characterized by amenorrhea or oligomenorrhea, however this study suggests that other menstrual cycle issues are also commonplace, highlighting the need for further more general research across other menstrual cycle irregularities in exercising women.

There are a number of limitations of this study. Firstly, the self-reported nature of this questionnaire could have resulted in inaccurate data, however the HMB diagnostic criteria does not lean itself to comparison bias. Secondly, stage 2 data was only collected in marathon runners, which may not be representative of other running events and sports. It has been shown that exercise increases susceptibility to iron deficiency,[27] however blood parameters including markers of iron status have not been measured therefore we are making an assumption. Additionally, the presence of illnesses (i.e. endometriosis) or the use of medication was not recorded, these could increase the likelihood of participants meeting the HMB criteria. To obtain

standardized performance comparisons a means for knowing finishing time in the marathon would strengthen ability to determine any relationships between participant performance level and HMB presence. Relationship between anthropometrical parameters and and HMB presence could also be explored. Further studies are required to address these limitations.

Conclusions

This study has demonstrated that HMB is common in the exercising population. HMB was associated with anemia, iron supplementation and slower performance times. Further research is however needed to explore the impact of HMB and iron deficiency on performance. The lack of medical help sought by the participants in this study suggests that either females don't feel or realize this is a problem, or have learnt to cope with it, highlighting that more research and awareness is needed. HMB is also surprisingly common amongst elite athletes, ostensibly impacting upon their training and performance, and potentially causing iron deficiency, although further research is needed to confirm this association.

Supporting Information

S1 Appendix. 'Female Health Questionnaire'. The 'Female Health Questionnaire' that was completed either online or at the 2015 London Marathon Exhibition by all surveyed (n = 1862).
(DOCX)

S2 Appendix. 'Letter to the Editor'. Letter to the Editor: The prevalence and impact of heavy menstrual bleeding amongst athletes and mass start runners of the 2015 London Marathon.
(DOCX)

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Author Contributions

Conceived and designed the experiments: GB RB NB TR CP. Performed the experiments: GB. Analyzed the data: GB RB NB TR CP. Contributed reagents/materials/analysis tools: GB RB NB TR CP. Wrote the paper: GB RB NB TR CP.

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10.3 Publication: Sport exercise and the menstrual cycle: where is the research? (Editorial)

Bruinvels G, Burden RJ, McGregor AJ, Ackerman KE, Dooley M, Richards T, Pedlar C. Sport exercise and the menstrual cycle: where is the research? Br J Sports Med. 2017 Mar;51(6):487-488

BJSM Online First, published on June 6, 2016 as 10.1136/bjsports-2016-096279

Sport, exercise and the menstrual cycle: where is the research?

G Bruinvels,[1,2,3] R J Burden,[2,3,4] A J McGregor,[5] K E Ackerman,[6] M Dooley,[7] T Richards,[1] C Pedlar[2,6]

Despite a decreasing gender gap in exercise participation, there still remains a significant under-representation of women included in sport and exercise medicine research studies.[1] A review of 1382 sport and exercise research studies involving over 6 million participants, from 2011 to 2013, found the representation of women to be 39%.[1] The complexities of the menstrual cycle are considered major barriers to the inclusion of women in clinical trials.

Historically, partially due to concerns of potentially damaging unborn fetuses, medical trials—including drug trials—were conducted solely in men. Further, women were perceived as more physiologically variable, therefore utilising only male participants would allow meaningful results with fewer participants and less funding. Since men were viewed as adequate proxies for women, the years of exclusion of female participants from research were considered inconsequential. However, it is now known that women can respond very differently to drug treatments than men. Evidence suggests that women are almost twice more likely to have an adverse reaction to a drug than their male counterparts, and 80% of drugs withdrawn from the market are due to unacceptable side effects in women.[2]

When research involving exercise metabolism includes women, participants are often tested in the early follicular phase of their menstrual cycle, when hormone levels are at their lowest, in order to minimise the possible impacts oestradiol and progesterone may have on the study outcomes.[3] This type of research practice leaves much ambiguity around how such hormones may influence the unique physiological processes in women, from blood pressure to substrate metabolism, thus perpetuating the significant gap in understanding how the menstrual cycle impacts exercise performance. Sheel[4] recently described a number of sex differences in the physiological response to exercise, likely caused in part by ovarian hormones, highlighting a lack of understanding and a need for further research.

We recently reported that 41.7% of exercising women believe their menstrual cycle has a negative impact on exercise training and performance.[5] However, largely due to the dearth of sports and exercise research in women, explanations for this are lacking. Heavy menstrual bleeding with unknown or undiagnosed iron deficiency could be a cause but this is speculative.

There is a clear need to gain better understanding of female physiology and to define the effects of the cyclical variations in hormones, both positive and negative, on athletic performance. Also, a greater understanding of the menstrual cycle is needed to address the reported negative impacts on exercise training in order to encourage participation and avoid further disparity in gender representation.

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Competing interests None declared.

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5 Bruinvels G, Burden R, Brown N, *et al*. The prevalence and impact of heavy menstrual bleeding (menorrhagia) in elite and non-elite athletes. *PLoS ONE* 2016;11:e0149881.

10.4 Other publications

Publications under review

Publication under second review:

Bruinvels G, Pedlar C, Burden R, Brown N, Simpkin A, Mansour D, Brown J, Richards T. The impact of heavy menstrual bleeding and iron status on fatigue in menstruating women.

Other publications during PhD

Ackerman KE, Holtzman B, Cooper KM, Flynn EF, **Bruinvels G**, Tenforde AS, Popp KL, Simpkin AJ, Parziale AL. Low Energy Availability Surrogates Correlate with Health and Performance Consequences of Relative Energy Deficiency in Sport (RED-S). Br J Sports Med. 2018 Mar; doi: 10.1136/bjsports-2017-098958

Lewis NA, Towey C, **Bruinvels G**, Howatson G, Pedlar CR. Effects of exercise on alterations in redox homeostasis in elite male and female endurance athletes using a clinical point-of-care test. Appl Physiol Nutr Metab. 2016 Oct; 41(10):1026-1032

Blagrove R, **Bruinvels G**, Read P. Early sport-specialization and intensive training in adolescent female athletes: risks and recommendations. Strength and Conditioning Journal. 2017:1 doi:10.1519/ssc.0000000000000315

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10.5 Oral presentations

Past

Bruinvels G, Durussel J, McClure JD, McBride MW, Wondimu DH, Wang G, Mooses M, Mooses K, Wang J, Murray A and Pitsiladis Y. Blood gene expression profiles of trained athletes in response to altitude exposure and differentiation from rHuEpo doping.

2014 In: Abstracts of the 93rd Annual Meeting of the German Physiological Society, Mainz, Germany. March 2014.

Bruinvels G. Iron Metabolism in Endurance Iron-Deficient, Non-Anaemic (IDNA) Athletes. Presented to the Robbins Group, Department of Physiology, Anatomy and Genetics at the University of Oxford. November 2014

Bruinvels G, Burden R, Brown N, Pedlar C, Richards T. The Prevalence and impact of heavy menstrual bleeding in exercising women. Female Athlete Conference, Boston. June 2015

Bruinvels G. Women's health in sport. The prevalence and impact of iron deficiency on exercise performance. Joint Women's Health and Patient Blood Management Initiative: Advisory Board, Singapore. January 2017

Ackerman KA, **Bruinvels G**. Periods, Performance, and the Pill – Effects of the Menstrual Cycle on Performance and Contraception Choices for the Modern Female Athlete. 2018 Annual Meeting, World Congress on Exercise is Medicine.

10.6 Abstracts and Posters

2016

Bruinvels G, Pedlar C, Burden R, Yong TT, Cushway T, Richards T. Heavy Menstrual Bleeding and iron status in exercising women in Singapore. Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis. 17th Annual Symposium, April 2016.

2017

Bruinvels G, Pedlar C, Burden R, Yong TT, Cushway T, Richards T. The impact of heavy menstrual bleeding (menorrhagia) and iron status in exercising females. British Jounral of Sports Medicine. Feb 2017, 51(4)304 IOC World Conference – Prevention of Injury and Illness in Sport

Bruinvels G, Pedlar C, Burden R, Brown N, Butcher A, Chau M, Richards T. IRONWOMAN Trial: The impact of intravenous iron on exercise performance in iron deficiency, exercising women. Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis. 18th Annual Symposium, April 2017

Bruinvels G, Pedlar C, Burden R, Butcher A, Chau M, Cushway T, Richards T. The impact of heavy menstrual bleeding and iron status on fatigue in menstruating women. Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis. 18th Annual Symposium, April 2017

Bruinvels G, Pedlar C, Burden R, Brown N, Butcher A, Chau M, Richards T. IRONWOMAN Trial: The impact of intravenous iron on exercise performance in iron deficiency, exercising women. Seventh Congress of the International Bioiron Society (2017)

Bruinvels G, Pedlar C, Burden R, Butcher A, Chau M, Cushway T, Richards T. The impact of heavy menstrual bleeding and iron status on fatigue in menstruating women. Seventh Congress of the International Bioiron Society (2017)

Yong TT, **Bruinvels G**, Pedlar C, Burden R, Cushway, T, Richards T. Heavy Menstrual Bleeding, iron status and fatigue in exercising women in Singapore. Royal College of Obstetrics & Gynaecologists World Congress 2017.

Parziale AL, **Bruinvels G**, Richards T, Pedlar CR, Ackerman KE. The Prevalence and Impact of Heavy Menstrual Bleeding Among Exercising Women. 2017 Annual Meeting, World Congress on Exercise is Medicine

10.7 Grants

SCA-Libresse-Bodyform – two-year grant for research
Pharmacosmos - £10,000 & intravenous iron for IRONWOMAN trial

10.8 Selected media exposure

Written Press

Live Science - http://www.livescience.com/54988-fewer-women-in-exercise-research.html

Motherboard - http://motherboard.vice.com/en_ca/read/why-are-sports-researchers-so-scared-of-menstruation

BuzzFeed News - https://www.buzzfeed.com/kellyoakes/we-need-more-women-in-sports-medicine-trials-say-scientists?utm_term=.oaJQNB196#.nn4G7RA8j

CBC News - http://www.cbc.ca/news/health/sport-exercise-menstrual-cycle-1.3618140

eCanada Now - http://www.ecanadanow.com/are-fewer-women-in-sports-studies-because-of-their-menstrual-cycle/99210/

The i Newspaper - https://inews.co.uk/essentials/sport/women-excluded-sport-studies-due-menstrual-cycle/

The Tech Times - http://www.techtimes.com/articles/163467/20160608/monthly-period-prevents-women-from-participating-in-sports-medicine-research.htm

Medical Daily - http://www.medicaldaily.com/gender-gap-sports-research-menstrual-cycle-389021

Parent Herald - http://www.parentherald.com/articles/48120/20160608/menstruation-keeping-women-sports-exercise-research.htm

Newsweek US - http://www.newsweek.com/having-periods-major-barrier-including-women-sports-medicine-research-467067

Think Progress - http://thinkprogress.org/health/2016/06/08/3785632/menstruation-clinical-research/

The Australian - http://thinkprogress.org/health/2016/06/08/3785632/menstruation-clinical-research/

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Athlete Muscle - http://www.athletemuscle.com/monthly-period-prevents-women-from-participating-in-sports-medicine-research/

Balita News Tabloid, the Philippines - http://balita.net.ph/2016/06/10/apektado-nga-ba-ng-menstrual-period-ang-pag-eehersisyo/

Axtop.net – Indonesian news site - http://axtop.net/apakah-wanita-yang-lebih-sedikit-dalam-latihan-studi-karena-periode-mereka/

Yahoo News - https://www.yahoo.com/news/fewer-women-exercise-studies-because-periods-145216028.html?ref=gs

Economia y Negocios, Chile - http://www.economiaynegocios.cl/noticias/noticias.asp?id=259858

Huffington Post - http://www.huffingtonpost.com/entry/ridiculous-reason-women-are-excluded-from-exercise-studies_us_57597f78e4b00f97fba7698f

http://www.huffingtonpost.co.uk/entry/women-excluded-from-sport-and-exercise-research_uk_575189ebe4b0b23a261a310f

Pacific Standard Mag - https://psmag.com/does-a-womans-period-affect-her-athletic-performance-2d7317f963c0#.478646ksn

Popular Science - http://www.popsci.com/surprise-researchers-think-women-are-being-excluded-from-clinical-trials?dom=rss-default&src=syn

On Medica - http://www.onmedica.com/newsArticle.aspx?id=17996adb-83f0-493e-8cfc-c758e8d2c261

Health, Diet, Fitness and Nutrition - http://livinghealthytips.xyz/women-still-excluded-from-meaningful-sport-and-exercise-research-argue-experts/310757/releases.html

True Viral News - http://trueviralnews.com/?p=198991

True Viral News - http://trueviralnews.com/?p=198458

Scimex - https://www.scimex.org/newsfeed/women-missing-from-sports-research

True Breaking News - http://science.truebreakingnews.com/?p=7108

Daily Magazine - http://dailymagazine.news/are-fewer-women-in-exercise-studies-because-of-their-periods-nid-184305.html

CanBan Bizz, Australia - http://canban.biz/menstruation-shuts-out-women-from-clinical-trials-editorial-54388.html

Sports Vice, USA - https://sports.vice.com/en_us/article/for-elite-athletes-periods-are-still-a-question-mark

Athletics Weekly - http://www.athleticsweekly.com/featured/special-report-menstrual-cycle-impact-on-athletics-performance-50765

Huffington Post - http://www.huffingtonpost.co.uk/entry/period-stigma-report-bodyform-most-girls-avoid-sport-while-on-period_uk_57b2f9eae4b0730aab6465c5

http://fusion.net/story/331665/olympics-periods-female-athletes/

Konbini - http://www.konbini.com/en/lifestyle/china-swimming-fun-yuanhui-tackles-period-taboo/

Straits Times - http://www.straitstimes.com/opinion/kudos-to-fu-yuanhui-for-being-open-about-her-period

Slate - http://www.slate.com/blogs/xx_factor/2016/08/16/fu_yuanhui_discussed_having_her_period_during_the_olympics.html

BBC World News - http://www.bbc.co.uk/news/world-asia-china-37081669

Women's SportsNet - 'Confronting the Taboo of Menstruation' - http://wsnet.co.uk/WSN-TV/confronting-taboo-menstruation-survey-yourstmarys-uni-london

BBC Sport - 'Periods in sport: New research on the menstrual cycle effect' – http://www.bbc.co.uk/sport/33858956

The Telegraph - 'Half of athletes don't perform well when menstruating' - http://www.telegraph.co.uk/women/womens-life/11794088/Periods-in-sport-Half-of-athletes-dont-perform-as-well-when-menstruating.html

Sport Executive - 'The last great sporting taboo' - http://sportexecutive.dk/last-great-sporting-taboo-2/; https://issuu.com/sportexecutive/docs/se_08_2015_sportex_uk_net/28

Women's SportsNet - 'The Prevalence and Impact of Heavy Menstrual Bleeding (Menorrhagia) in Elite and Non-Elite Athletes' - http://wsnet.co.uk/WSN-TV/prevalence-and-impact-heavy-menstrual-bleeding-menorrhagia-elite-and-non-elite-athletes

Radio and TV

BBC Sportsday – Women in Sport week

http://www.bbc.com/sport/get-inspired/37561658.app

BBC Radio 5 live

http://www.bbc.co.uk/programmes/b07xf34j

BBC Radio 4 Women's Hour

http://www.bbc.co.uk/programmes/b07mvxs6

CBC News

http://www.cbc.ca/news/health/sport-exercise-menstrual-cycle-1.3618140

Written article

Sport Health, Australia

https://asp-au.secure-zone.net/v2/index.jsp?id=684/750/8761&lng=en

10.9 Other work

Miles L, Bruinvels G, Otto JM, Chau M, and Richards T. Cochrane Protocol 0224: Iron therapy for iron deficient non-anaemic adults. Started June 2015, work still on going

10.10 IRONWOMAN Trial: Participant Information Sheet

PARTICIPANT INFORMATION SHEET
Version 3 – 8 October 2015

Study Title: IRON WOMAN – Iron therapy for female athletes

Chief Investigator: Professor Toby Richards
Student Researcher: Miss Georgie Bruinvels
Principal Investigators: Dr Charles Pedlar and Dr Richard Burden

You are being invited to take part in this pilot research study as a result of your recent expression of interest in our work. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Please do us if there is anything that is not clear or if you would like more information.

Study Purpose and Research Overview
This study is being conducted to fulfil the requirements of a Graduate Research Degree (PhD) being undertaken by Miss Georgie Bruinvels, Division of Surgery and Interventional Science at University College London (UCL).

Our research group aims to optimise the health and well being of athletes. The research group at St Mary's University, Twickenham in collaboration with UCL is specifically looking at the effects of low iron (i.e. iron deficiency anaemia and iron deficiency non-anaemia) and it's impact on performance.
Endurance exercise can cause small iron loss through haematuria (blood in urine), gastrointestinal bleeding, sweating and haemolysis (destruction of red blood cells, particularly exacerbated in impact sports involving foot strike). Females who participate regularly in endurance training therefore have an increased susceptibility to iron deficiency, and this is likely to be exacerbated in menstruating females. Iron is essential for the healthy functioning of the body and deficiency will eventually result in a reduction in energy levels, weakness, impaired cognition and motivation amongst other long-term detrimental affects to the human body.

The purpose of this study is therefore to see whether intravenous iron supplementation improves exercise performance and quality of life in iron deficient exercising women.

Why have I been invited to take part in this study?
We are contacting you because you have indicated that you may be low in iron and exercise on a regular basis.

Do I have to take part in this study?
You are under no obligation to take part in this research, participation is voluntary, but if you do decide to partake please read and keep this information sheet, and sign the consent form at the end. If you decide that you no longer want to be a part of this research then you will be able to withdraw safely, at any time with no notice or reasoning. Declining to take part or withdrawing from this study will have no impact on future care or involvement in other research.

What will happen to me if I take part in this study?
You are being contacted because you think you may meet our predefined criteria for being iron deficient. If you decide that you would like to continue involvement in this study you will undergo some tests and answer some questionnaires, then be given a single injection of iron. The initial tests and completion of questionnaires will then be repeated on one or two occasions. The aim of giving you iron is to return your iron levels to a 'clinically normal' level. You will need to visit St Mary's University,

Twickenham on four occasions for a number of tests, and 112 Harley Street, London as explained below;

1. Screening test – this will involve having a blood test to check your iron status to see if you are eligible for this research. During the visit to the lab you will be given an overview of the study and will be able to ask questions. This will take place at St Mary's University, Twickenham.

2. Baseline testing – you will be required to have an exercise test (the test is called a VO_{2max} test, and it measures the maximum amount of oxygen that you can take in and use while exercising. It is the main test used in exercise physiology to assess physical fitness, and a higher value indicates a higher level of fitness), a blood test, a total haemoglobin mass test (explained below), provide a urine sample and answer 5 questionnaires (approx. 2 hours for all tests). This will take place at St Mary's University, Twickenham.

3. Iron injection - after the baseline tests you will be required to go to the iron therapy clinic located at 112 Harley Street, London to receive an iron injection. This will be given by a trained medical professional. Iron will be infused over a minimum period of 15 minutes and you will be observed for 30 minutes after this. (approx. an hour in total)

4. Follow up testing – 2 weeks after you have had the iron injection you will be required to come back to St Mary's University, Twickenham and repeat the tests that were performed at baseline, including – an exercise test, a blood test, a total haemoglobin mass test, provision of a urine sample and to complete the 5 questionnaires. 3 months (12 weeks) after the iron injection you will be required to come back to St Mary's again for a blood test and to answer the 5 questionnaires.

How long am I likely to be in this study?
After you have come into the lab for your familiarisation trial you will complete your baseline tests, you will receive your iron injection within the next 2 weeks, follow up testing will take place 2 weeks and 3 months (12 weeks) after this. Therefore from the baseline testing you will be in the study for approximately 16 weeks.

What are the possible benefits of taking part in this study?
You will receive information about your current level of physical fitness, and will be given key target training and heart rate zones that you can use for your training. You will also gain information about your general health and well-being. If your iron deficiency is having an impact on your quality of life and exercise performance you will also benefit from the restoration of your iron levels.

What are the possible side effects, disadvantages and risks of taking part in this study?
The risks associated with a VO_{2max} test are minimal but include the following: fatigue, muscle soreness, irregular heartbeat and chest pain. We will conduct testing in the standardised procedure under the guidance of an experienced exercise physiologist; heart rate and rate of perceived exhaustion will be continuously monitored throughout the test in a controlled environment.

We will also require you to complete a total haemoglobin mass test. This test is routinely used in sport and exercise science and provides another way of measuring fitness, showing how much oxygen your body can transfer. The test involves breathing a very small amount of carbon monoxide, less than you would breathe in when sitting in a queue of traffic. This will be performed by a trained physiologist, and is very unlikely to have any effects on your health and wellbeing.

The most common reported side effects of intravenous iron are dizziness, high blood pressure and/or injection site reactions.

Other uncommon side effects (occur in less than 1 in 100 (1%) and more than 1 in 1,000 (0.1%) patients receiving iron) are allergic reaction (hypersensitivity), sensation of pain (paraesthesia), a change in your taste sensation (dysgeusia), high heart rate (tachycardia), low blood pressure (hypotension), redness in the face (flushing), difficulty breathing (dyspnoea), vomiting, upset stomach (dyspepsia), flatulence, abdominal pain, constipation, diarrhoea, itching (pruritus), hives (urticaria), redness of the skin (erythema), rash, muscle, joint and/or back pain (myalgia and arthralgia), muscle spasm, fever (pyrexia), tiredness (fatigue), chest pain, swelling of the hands and/or the feet (oedema peripheral), pain and/or chills.

In all the clinical trials reported to date (including over 6000 patients) there has been no report of increased side effects in patients receiving the intravenous iron compared to those patients who received the placebo.

If you encounter any problems following the IV iron treatment you should contact Professor Toby Richards immediately (Tel: 0207 679 6454).

Is it compulsory for me to take part?
No, it is not compulsory; participation is voluntary and specific to this study. If you do agree to be involved in this study it does not mean that you agree to being involved in other studies.

What are the alternatives for treatment of iron deficiency?
In the UK, typically iron is given in tablet form, however this is typically poorly absorbed and there are some side effects including abdominal pain, constipation and heartburn. Also, restoration of iron stores typically takes 3-6 months. Additionally, those who exercise may have increased levels of inflammation, which will hinder absorption. Conversely, intravenous iron which can be administered as a single treatment in a relatively short length of time (minimum of 15 minuets) enables rapid restoration of iron status. Intravenous iron is widely and effectively used in countries such as Switzerland and Australia.

Why will iron be delivered intravenously and not orally?
As restoration of iron stores is slow and poorly absorbed through oral iron treatment intravenous iron therapy is being used. This has been shown to be much more effective.

What will happen if I do not want to carry on with the study?
If you decide during the study that you no longer want to participate you can withdraw yourself at any time and do not need to give a reason. After study completion, any stored blood or tissue samples that can still be identified as yours will be destroyed if you would like.

If there is anything you do not understand or wish to ask questions about, please feel free to ask.

In the unlikely event of a loss of capacity to consent, the research team will retain tissue and personal data collected during this study and continue to use it confidentially for research purposes. This could include further research after the current project has ended.

What if something goes wrong?
In the unlikely event that you are harmed while taking part in this research project, there are no special compensation arrangements. But, if you are harmed due to someone's carelessness, then you may have grounds for legal action but you may have to pay for it.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. You will be referred to by a unique code, and any information about you will have your name and address removed so that you cannot be recognised.

Will my General Practitioner be notified?
On agreement, your general practitioner will be informed of your inclusion and will be provided with your results from this research.

What if new information becomes available?
Sometimes during the course of a research project, new information becomes available about the subject being studied. Should this happen a member of the research team will tell you about it and discuss with you whether you want to continue in the study.

What will happen to the results of the research study?
Results will be published in a peer-reviewed scientific journal once the study is completed. You will be given with a lay summary of the research results. You will not be identified in any publication.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the London-Westminster Research Ethics Committee.
The St Mary's University Ethics Committee has also reviewed and approved this research.

What will happen to any samples I give?
Any blood samples that are taken as part of the research study will be transferred to a central laboratory for analysis. The blood will only be identified by using your unique study number. The blood will be frozen at -80°C. The samples will be transported to a laboratory in central London called The Doctors Laboratory (TDL). The bloods will be analysed. Any serum excess (blood product) will be stored for future research. All results will be sent to the statistician who is based at the London School of Hygiene and Tropical Medicine (LSHTM). This is where the staff organising this study are based.
Unless you withdraw your consent, we will ask you to gift your blood to the people running the study and in so doing give up all future claims to its use that may include further research.

If you wish to find out more about this research study, you can contact:

Georgie Bruinvels – georgie.bruinvels@stmarys.ac.uk; georgie.bruinvels.14@ucl.ac.uk

Thank you for taking time to read this information.

10.11 IRONWOMAN Trial: NHS Ethics Approval letter

NHS
Health Research Authority
Research Ethics Service
London - Westminster Research Ethics Committee
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0207 104 8012

16 November 2015

Mr Toby Richards
University College London
Division of Surgery & Interventional Science
74 Huntley Street
London
WC1E 6AA

Dear Mr Richards

Study title:	**The role and effect of intravenous iron isomaltoside at improving functional performance outcome measures and quality of life in iron deficient exercising females**
REC reference:	**15/LO/1570**
IRAS project ID:	**180318**

Thank you for your submission of 03 November 2015, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair and Mr Robert Goldstein.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Ms Rachel Katzenellenbogen, nrescommittee.london-westminster@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the

study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet completed any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		20 July 2015
GP/consultant information sheets or letters [IRON WOMAN Trial - Letter to GP]	1	20 August 2015
IRAS Checklist XML [Checklist_24082015]		24 August 2015
Other [Response to validation query]		26 August 2015
Participant consent form [IRONWOMAN - Consent Form v2]	V2	03 November 2015
Participant information sheet (PIS) [IRON WOMAN Trial - Participant information sheet v3]	V3	03 November 2015
REC Application Form [REC_Form_24082015]		24 August 2015
Referee's report or other scientific critique report		31 October 2014
Research protocol or project proposal [IRON WOMAN Trial - Protocol]	1	20 August 2015
Summary CV for Chief Investigator (CI) [Toby Richards - 2 page CV]	1	18 August 2015

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

15/LO/1570	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

Dr Alan Ruben
Chair

Email:nrescommittee.london-westminster@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Mr Toby Richards, University College London

10.12 IRONWOMAN Trial: St Mary's Ethics Approval

Georgie Bruinvels (SHAS): 'Ironwoman – iron therapy for female athletes'.

29 February 2016

Dear Georgie

University Ethics Sub-Committee

Thank you for submitting your ethics application for the above research.

I can confirm that your application has been considered by the Ethics Sub-Committee and that ethical approval is granted.

Yours sincerely

Dr Conor Gissane
Chair of the Ethics Sub-Committee

Cc Charlie Pedlar
Richard Burden

10.13 IRONWOMAN Trial: Informed consent

St Mary's University Twickenham London

UCL

INFORMED CONSENT
Version 2 – 28 October 2015

Study Title: IRONWOMAN – Iron therapy for female athletes

Participant Identification Number for this trial: _____

Chief Investigator: Professor Toby Richards
Student Researcher: Miss Georgie Bruinvels
Principal Researchers: Dr Charles Pedlar and Dr Richard Burden

Please **initial boxes** to indicate agreement with each specific point below.

- I confirm that I have read and understand the information sheet dated _____ (version ____) for the above study. I have had the opportunity to ask questions and have had these satisfactorily answered. ☐
- I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason without my legal rights being affected. ☐
- I am satisfied that the researchers have explained the purpose, principles and procedures of the study, outlining any possible risks. ☐
- I understand how my data will be collected and used and that confidential information will only be seen by researchers. ☐
- I understand that I will have three venous blood samples and a urine sample collected, and I give permission for these to be stored in accordance with the Human Tissue Act for current and future research. ☐
- I understand that I will be required to perform two maximal exercise tests (and have a familiarisation trial). ☐
- I understand that I will have my haemoglobin mass measured on two occasions. ☐
- I understand that I am gifting my samples to the investigators and in so doing give up all future claims to its use that may include further research with the appropriate ethical approval. ☐
- I agree to my General Practitioner being informed of my participation in this study. ☐
- I agree to take part in the above study. ☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

10.14 IRONWOMAN Trial: Female athlete monitoring questionnaire

‹ Return PREVIEW Ironwoman Trial Female Health Questionnaire Skip: Next › 1 / 3

Ironwoman Trial Female Health Questionnaire

0% complete

Page 1

There is much ambiguity about the possible impacts of the menstrual cycle on exercise performance. Research in the female athlete at all menstrual cycle phases is lacking, however we are really keen to start addressing this and help understand why many females feel that their menstrual cycle holds them back.

In order to participate in this study you must be:

- Female
- 18 years or older
- Pre-menopausal

By completing this survey you are giving consent for the information you provide to be included in this study. Your participation is voluntary and is specific to this study, and shall not be taken to imply consent to participate in any subsequent experiment or deviation from that detailed. All information will remain confidential as to your identity, and you may withdraw from the study at anytime without reason. If there is anything you do not understand or wish to ask questions about, please feel free to ask.

Thank you for participating

Do you agree to these terms?

- ○ Yes
- ○ No

This part of the survey uses a table of questions, view as separate questions instead?

Please specify your personal best times for all/any of the below in the last year

	Time (minutes:seconds)
5km run (inc Parkrun)	
10km run	
Half marathon	
10M TT cycle	
25M TT cycle	
100km cycle	
2km row	

Do you use the oral contraceptive pill? ✱ *Required*

◯ Yes ◯ No

If you use any other contraceptive device that will stop regular bleeding please specify

Approximately how many periods have you had in the last year?

How long does your average menstrual cycle last (days)? - from the start of one bleed to the next

How long does each bleed normally last? (days)

Have you ever sought advice/help for heavy periods?

- ◯ Yes
- ◯ No

To your knowledge have you ever had anaemia?

- ◯ Yes
- ◯ No
- ◯ Don't know

Have you ever supplemented with iron?

- ◯ Yes
- ◯ No
- ◯ Don't know

Have you ever experienced any of the below during your period? (please select all that apply)

- ☐ Flooding through to clothes or bedding
- ☐ Need of frequent changes of sanitary towels or tampons (meaning changes every 2 hours or less or 12 sanitary items per period)
- ☐ Need of double sanitary protection (tampons and towels)
- ☐ Pass large blood clots

Do you feel that your menstrual cycle disrupts your training/performance?

- ◯ Yes
- ◯ No

This part of the survey uses a table of questions, view as separate questions instead?

How many days after you started your previous menstrual cycle were you at the following times:

	Day after started previous menstrual cycle (first day of bleeding)
Screening blood test	
Baseline tests	
Iron injection	
Follow up tests	

10.15 Questionnaires

10.15.1.1 The MFI-20

MFI® MULTIDIMENSIONAL FATIGUE INVENTORY

Instructions:

By means of the following statements we would like to get an idea of how you have been feeling **lately**.
There is, for example, the statement:

"I FEEL RELAXED"

If you think that this is **entirely true**, that indeed you have been feeling relaxed lately, please, place an **X** in the extreme left box; like this:

yes, that is true ☒1 ❑2 ❑3 ❑4 ❑5 **no, that is not true**

The more you **disagree** with the statement, the more you can place an **X** in the direction of "no, that is not true". Please do not miss out a statement and place only one **X** in a box for each statement.

1	I feel fit.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
2	Physically, I feel only able to do a little.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
3	I feel very active.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
4	I feel like doing all sorts of nice things.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
5	I feel tired.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
6	I think I do a lot in a day.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
7	When I am doing something, I can keep my thoughts on it.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
8	Physically I can take on a lot.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
9	I dread having to do things.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
10	I think I do very little in a day.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
11	I can concentrate well.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
12	I am rested.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
13	It takes a lot of effort to concentrate on things.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
14	Physically I feel I am in a bad condition.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
15	I have a lot of plans.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
16	I tire easily.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
17	I get little done.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
18	I don't feel like doing anything.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
19	My thoughts easily wander.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true
20	Physically I feel I am in an excellent condition.	yes, that is true	❑1	❑2	❑3	❑4	❑5	no, that is not true

Thank you very much for your cooperation

10.15.1.2 The EQ-5D-5L Questionnaire

EQ-5D-5L Questionnaire

Under each heading, please tick the ONE box that best describes your health TODAY

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

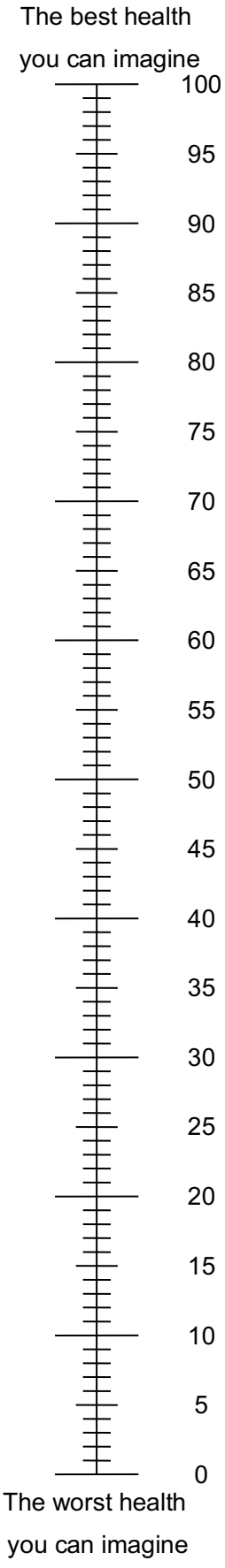
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the <u>best</u> health you can imagine.
  0 means the <u>worst</u> health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



10.15.1.3 Piper Fatigue Scale

1. To what degree is the fatigue you are feeling now causing you distress?

No Distress									A Great Deal
1	2	3	4	5	6	7	8	9	10

2. To what degree is the fatigue you are feeling now interfering with your ability to complete your work or school activities?

None									A Great Deal
1	2	3	4	5	6	7	8	9	10

3. To what degree is the fatigue you are feeling now interfering with your ability to socialize
with your friends?

None									A Great Deal
1	2	3	4	5	6	7	8	9	10

4. To what degree is the fatigue you are feeling now interfering with your ability to engage in sexual activity?

None									A Great Deal
1	2	3	4	5	6	7	8	9	10

5. Overall, how much is the fatigue which you are now experiencing interfering with your ability to engage in the kind of activities you enjoy doing?

None									A Great Deal
1	2	3	4	5	6	7	8	9	10

6. How would you describe the degree of intensity or severity of the fatigue which you are experiencing now?

None									Severe
1	2	3	4	5	6	7	8	9	10

7. To what degree would you describe the fatigue which you are experiencing now as being?

Pleasant									Unpleasant
1	2	3	4	5	6	7	8	9	10

8. To what degree would you describe the fatigue which you are experiencing now as being?

Agreeable									Disagreeable
1	2	3	4	5	6	7	8	9	10

9. To what degree would you describe the fatigue which you are experiencing now as being?

Protective									Destructive
1	2	3	4	5	6	7	8	9	10

10. To what degree would you describe the fatigue which you are experiencing now as being?

Positive									Negative
1	2	3	4	5	6	7	8	9	10

11. To what degree would you describe the fatigue which you are experiencing now as being:

Normal									Abnormal
1	2	3	4	5	6	7	8	9	10

12. To what degree are you now feeling:

Strong									Weak
1	2	3	4	5	6	7	8	9	10

13. To what degree are you now feeling:

Awake									Sleepy
1	2	3	4	5	6	7	8	9	10

14. To what degree are you now feeling:

Lively									Listless
1	2	3	4	5	6	7	8	9	10

15. To what degree are you now feeling:

Refreshed									Tired
1	2	3	4	5	6	7	8	9	10

16. To what degree are you now feeling:

Energetic									Unenergetic
1	2	3	4	5	6	7	8	9	10

17. To what degree are you now feeling:

Patient									Impatient
1	2	3	4	5	6	7	8	9	10

18. To what degree are you now feeling:

Relaxed									Tense
1	2	3	4	5	6	7	8	9	10

19. To what degree are you now feeling:

Exhilarated									Depressed
1	2	3	4	5	6	7	8	9	10

20. To what degree are you now feeling:

Able to concentrate									Unable to concentrate
1	2	3	4	5	6	7	8	9	10

21. To what degree are you now feeling:

Able to remember									Unable to remember
1	2	3	4	5	6	7	8	9	10

22. To what degree are you now feeling:

Able to think clearly									Unable to think clearly
1	2	3	4	5	6	7	8	9	10

10.15.1.4 BRUMS Mood Scale

	Not at all	A little	Moderately	Quite a bit	Extremely
Panicky	0	1	2	3	4
Lively	0	1	2	3	4
Confused	0	1	2	3	4
Worn out	0	1	2	3	4
Depressed	0	1	2	3	4
Downhearted	0	1	2	3	4
Annoyed	0	1	2	3	4
Exhausted	0	1	2	3	4
Mixed-up	0	1	2	3	4
Sleepy	0	1	2	3	4
Bitter	0	1	2	3	4
Unhappy	0	1	2	3	4
Anxious	0	1	2	3	4
Worried	0	1	2	3	4
Energetic	0	1	2	3	4
Miserable	0	1	2	3	4
Muddled	0	1	2	3	4
Nervous	0	1	2	3	4
Angry	0	1	2	3	4
Active	0	1	2	3	4
Tired	0	1	2	3	4
Bad Tempered	0	1	2	3	4
Alert	0	1	2	3	4
Uncertain	0	1	2	3	4