# Epidemiology of blood donor health in the context of increased frequency of donation: The INTERVAL trial


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This thesis is submitted for the degree of Doctor of Philosophy

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the prescribed word limit for the relevant Degree Committee

# Abstract - Epidemiology of blood donor health in the context of increased frequency of donation: The INTERVAL trial, Andrew Browne

Blood donation is central to maintaining adequate blood supply for routine hospital demands and ensuring preparedness for emergency situations. As blood donation is a voluntary activity, it is important that blood collection services ensure that blood donor health is not negatively impacted in the strive to maintain blood supply. However, health-related consequences of frequent blood donation are not fully understood, and the relevance of iron supplementation remains unclear. Thus, the aim of this thesis was to investigate different aspects of blood donor health (including quality of life, iron levels and symptoms related to iron deficiency) and to summarise key characteristics of donors who may be able to safely give blood donations more frequently.

The INTERVAL trial was a large pragmatic trial conducted to assess whether blood supply in the UK could be safely increased by reducing the interval between blood donations. A total of 45,263 UK blood donors (50% men and 50% women) were assigned to donate blood more frequently (men: 8 and 10 weeks; women: 12 and 14 weeks) than routine practice (men: 12 weeks, women 16 weeks). Interactions with randomised inter-donation interval were assessed to identify donor subgroups who may safely donate more frequently without affecting the trial's key safety outcome - low haemoglobin deferrals. Mediation analyses were performed to assess whether iron supplementation during the trial mediated the effect of randomised inter-donation frequency on donor reported symptoms potentially associated with iron deficiency, including tiredness, breathlessness, palpitations, and fainting. Modelling was performed to assess the effect of more frequent blood donation on donors' physical and mental wellbeing.

Among 252,528 donor attendances during the 2-year period of the INTERVAL trial, 222,370 (88%) resulted in successful donation and 13,099 (5%) resulted in low haemoglobin deferrals. Donors randomised to shorter inter-donation intervals were able to give more donations, but also had higher frequency of low haemoglobin deferrals. Further interaction analyses conducted in this thesis

suggested that increased frequency of donations with shorter inter-donation intervals was modified by age, donation history, iron multivitamin supplement use, physical and mental health scores and ferritin levels. The effect of randomised inter-donation interval on low haemoglobin deferrals was modified by baseline donation history, age, haemoglobin level and ferritin level.

Donors randomised to shorter inter-donation intervals reported higher frequencies of symptoms such as tiredness, breathlessness, fainting or feeling faint, and palpitations. Use of iron supplementation, a commonly studied therapy for relieving symptoms such as fatigue, also increased during the trial. However, the effect of shorter inter-donation intervals on the frequency of symptoms was the same irrespective of adjustment for iron supplementation use before or during the trial.

Donors in the INTERVAL trial had higher physical and mental health component scores (PCS, MCS) than the general population. While the PCS and MCS were associated with baseline characteristics such as age, weight, and previous diagnosis of anaemia, there was no significant effect of randomised inter-donation interval on any of PCS, MCS, or the eight sub-components that make up the two summary measures.

The findings from this thesis may help inform future directions for blood donation practice and management in the UK and elsewhere. Characteristics of donors able to safely donate blood more frequently and those who may be at greater risk of low haemoglobin deferrals were identified. Impact on donors' quality of life appears marginal. Some physical symptoms may be experienced by donors who donate more frequently, irrespective of iron supplementation use, suggesting need for further study to elucidate other mechanisms of mitigating post-donation symptoms.

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# Abstract

Blood donation is central to maintaining adequate blood supply for routine hospital demands and ensuring preparedness for emergency situations. As blood donation is a voluntary activity, it is important that blood collection services ensure that blood donor health is not negatively impacted in the strive to maintain blood supply. However, health-related consequences of frequent blood donation are not fully understood, and the relevance of iron supplementation remains unclear. Thus, the aim of this thesis was to investigate different aspects of blood donor health (including quality of life, iron levels and symptoms related to iron deficiency) and to summarise key characteristics of donors who may be able to safely give blood donations more frequently.

The INTERVAL trial was a large pragmatic trial conducted to assess whether blood supply in the UK could be safely increased by reducing the interval between blood donations. A total of 45,263 UK blood donors (50% men and 50% women) were assigned to donate blood more frequently (men: 8 and 10 weeks; women: 12 and 14 weeks) than routine practice (men: 12 weeks, women 16 weeks). Interactions with randomised inter-donation interval were assessed to identify donor subgroups who may safely donate more frequently without affecting the trial's key safety outcome - low haemoglobin deferrals. Mediation analyses were performed to assess whether iron supplementation during the trial mediated the effect of randomised inter-donation frequency on donor reported symptoms potentially associated with iron deficiency, including tiredness, breathlessness, palpitations, and fainting. Modelling was performed to assess the effect of more frequent blood donation on donors' physical and mental wellbeing.

Among 252,528 donor attendances during the 2-year period of the INTERVAL trial, 222,370 (88%) resulted in successful donation and 13,099 (5%) resulted in low haemoglobin deferrals. Donors randomised to shorter inter-donation intervals were able to give more donations, but also had higher frequency of low haemoglobin deferrals. Further interaction analyses conducted in this thesis suggested that increased frequency of donations with shorter inter-donation intervals was modified

by age, donation history, iron multivitamin supplement use, physical and mental health scores and ferritin levels. The effect of randomised inter-donation interval on low haemoglobin deferrals was modified by baseline donation history, age, haemoglobin level and ferritin level.

Donors randomised to shorter inter-donation intervals reported higher frequencies of symptoms such as tiredness, breathlessness, fainting or feeling faint, and palpitations. Use of iron supplementation, a commonly studied therapy for relieving symptoms such as fatigue, also increased during the trial. However, the effect of shorter inter-donation intervals on the frequency of symptoms was the same irrespective of adjustment for iron supplementation use before or during the trial.

Donors in the INTERVAL trial had higher physical and mental health component scores (PCS, MCS) than the general population. While the PCS and MCS were associated with baseline characteristics such as age, weight, and previous diagnosis of anaemia, there was no significant effect of randomised inter-donation interval on any of PCS, MCS, or the eight sub-components that make up the two summary measures.

The findings from this thesis may help inform future directions for blood donation practice and management in the UK and elsewhere. Characteristics of donors able to safely donate blood more frequently and those who may be at greater risk of low haemoglobin deferrals were identified. Impact on donors' quality of life appears marginal. Some physical symptoms may be experienced by donors who donate more frequently, irrespective of iron supplementation use, suggesting need for further study to elucidate other mechanisms of mitigating post-donation symptoms.

# Declaration

This dissertation does not exceed the word limit of the Degree Committee. It is my own work, and does not include work done in collaboration with others except specifically when mentioned in the acknowledgements below.

# Acknowledgements

This thesis would not have been possible without the help of many others. Particular thanks go to the Cardiovascular Epidemiology Unit, especially Sarah Fahle and the data management team who expediently processed the data I needed very quickly for me. I also generally thank PhD colleagues in the CEU for supportive environment in the PhD office, and Dr Adam Butterworth for his support during his time as Director of Doctoral Studies and of course my supervisors Dr Stephen Kaptoge and Dr Angela Wood, who provided advice and guidance on the thesis innumerable times. Thanks also to Wolfson College, especially my tutor Dr Giles Yeo who offered me invaluable advice and support on many occasions, which was critical to my completion. In addition, thank you to my funding body, the NIHR Blood and Transplant Research Unit in Donor Health and Genomics (NIHR BTRU-2014-10024).

Specifically in the context of this thesis:

**Chapter 1** was written by me with comments from my supervisors (Dr Stephen Kaptoge and Dr Angela Wood)

**Chapter 2** was lead by me in collaboration with Oxford University, who performed the original systematic review that this chapter updates. Carolyn Doree performed the literature search and de-duplication. I led screening of manuscripts for relevance on Cambridge's side, alongside Katya Masconi, Ryan Chung, Silvia Alonso Rodrigues, and Michael Sweeting. Sheila Fisher led screening on Oxford's side, alongside Akshay Shah and Mana Rahimzadeh. Stephen Kaptoge, Angela Wood, Sheila Fisher, Graham Smith, and Thomas Bolton provided comments on the text, with Sheila assisting greatly with the discussion.

**Chapter 3** was written by me with comments from Stephen Kaptoge and Angela Wood

**Chapter 4** was written by me with comments from Stephen Kaptoge and Angela Wood, who also both helped with example scripts to produce the marginal effect plots in Stata, and advice on statistical analysis more generally.

**Chapter 5** was written by me with comments from Stephen Kaptoge and Angela Wood. Stephen and Angela also provided assistance with essential Stata programming to produce the graphs.

**Chapter 6** was written by me with comments from Stephen Kaptoge and Angela Wood. Stephen and Angela also provided assistance with essential Stata programming to produce the graphs.

**Chapter 7** was written by me with comments from Stephen Kaptoge and Angela Wood

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# Chapter 1 – Introduction

## Summary

The UK blood services rely on donations from unpaid, voluntary donors. Blood donation in England is administered by NHS Blood and Transplant, which requires around 5,000 donations every day to meet the demand of hospital emergencies and planned procedures. The donation process lasts between 5-10 minutes, and each blood unit is separated into component parts which can be administered to different patients. The majority of blood is given by donors who are white, older than average, and male. The UK donor population is aging more quickly than the general population.

Despite concerns that the aging population would require more transfusions, transfusion demand has been declining globally. However, as it is possible that demand may outstrip supply in the future, and for rare blood groups the question of efficiently maximising blood collection from the blood donor population remains relevant. Currently in the UK, men can donate every 12 weeks, and women every 16 weeks.

The INTERVAL trial was a large multi-centre trial that recruited approximately 45,000 blood donors from the UK, who were randomised to give blood at different inter-donation intervals for two years. For men, the inter-donation intervals were 8, 10, and 12 weeks, and for women these were 12, 14, and 16 weeks. The aims of the trial included the feasibility of use of different donation intervals for subgroups of the population.

The objectives of this chapter are to provide a background to blood donation in the UK and an overview of the INTERVAL trial, the primary data source. It also examines recent trends in blood supply and demand and how these may change in the future, including how personalised inter-donation intervals that are more frequent than current NHS guidelines may become relevant.

The overarching aim of my PhD project was to identify donor characteristics associated with the ability to safely donate blood more frequently than current NHS guidelines, which could be used to develop stratification schemes to personalise the inter-donation intervals of blood donors.

## 1.1 Overview of Blood Donation in the UK

Blood donation in England is managed by the NHS Blood and Transplant (NHSBT). This body was formed in 2005 after the merging of the National Blood Service and UK Transplant. NHSBT currently needs approximately 5,000 donations a day to meet blood demand in England [1], which amounts to 400 new donors a day [2]. One donation removes 470ml of blood [3] and this is separated into components such as red blood cells, platelets, and plasma (**Figure 1.1**). These components can then be administered separately to patients who need them, allowing one blood donation to benefit multiple people [4].

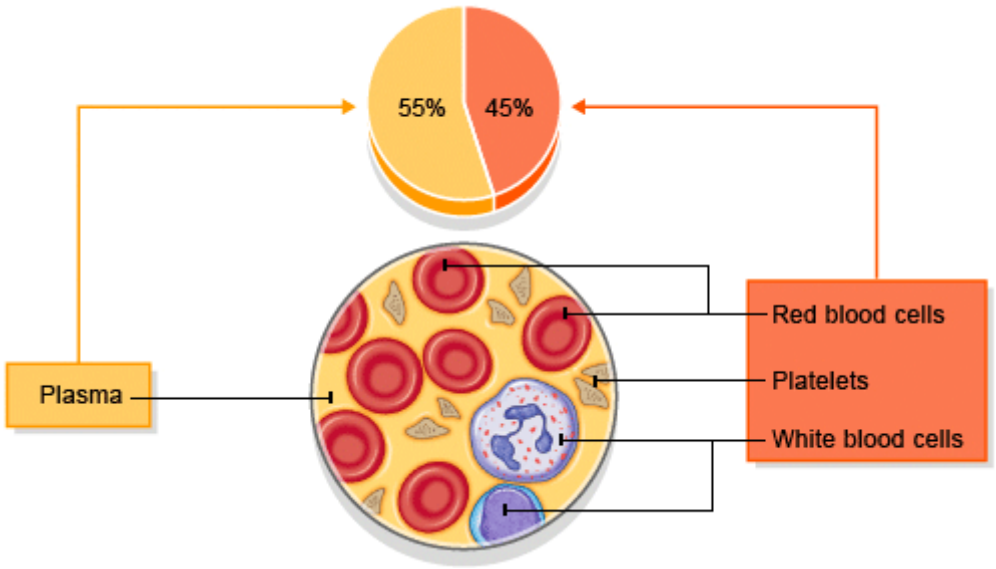



**Figure 1.1**: Diagram of key blood components [5]. Blood is made up of 55% plasma and 45% of cells, the most common being red blood cells, white blood cells, and platelets.

Blood donors in the UK must satisfy eligibility and exclusion criteria for blood donation (**Table 1.1**) [6]**.** In addition, they must satisfy a haemoglobin level screening test. This helps check that donors are not already iron deficient and minimises the risk of developing iron deficiency anaemia as a result of donation, with a loss of 250mg of iron per donation [7]. It also ensures that the blood collected by the

blood service is fit for purpose, and able to be safely used in a transfusion [8]. Currently, the haemoglobin cutoff levels for donation are 125g/l for women and 135g/l for men [9] in line with European guidelines [10].

Haemoglobin is a protein present in red blood cells with a primary physiological function for oxygen transport from lungs to cells and body tissues. Its molecular structure includes iron ions (Fe), which are essential for oxygen binding, and hence the regard of circulating haemoglobin concentration as one of key biomarkers of iron status in the blood [11]. However, as haemoglobin concentrations are also related to mechanisms that control the red cell mass, size and the plasma volume, haemoglobin screening alone may not detect iron deficient donors who should not be giving blood [12]. Ferritin is another key protein reflecting total body iron stores, primarily stored in body organs as the liver, spleen and muscle, but also present in circulation as a key carrier of iron in the blood [11]. Improvements in technology have recently allowed for quantification of haemoglobin concentrations in subpopulations of red blood cells, including reticulocyte haemoglobin concentration (i.e. in young red blood cells) suggested to be a better indicator of iron deficiency. Mean cell volume (MCV) is another iron-related circulating indicator that has been widely used to detect nutritional iron deficiency, however MCV values can be low due to other conditions such as thalassaemia and inflammation [12].

**Table 1.1**: Inclusion and exclusion criteria for blood donation in the UK

| Inclusion Criteria for Blood Donation | Common Exclusion Criteria for Blood Donation |
|---|---|
| Weight between 50 and 160kg | Receiving medical or hospital treatment |
| Aged between 17 and 66, or 70 if a previous donor | Taking medication |
| Over 70 and have given blood in the last two years | Travel outside of the UK |
| | Recent tattoo or piercing |
| | During and after pregnancy |
| | Feeling ill |
| | Cancer |
| | After receiving blood, blood products, or organs |

When a donor arrives at the blood donor centre to donate, they are asked to complete a questionnaire and undergo haemoglobin testing. The donor is also provided with 500ml of fluid which they are

advised to drink over 5 minutes. Then, a needle is inserted into the donor's arm to enable donation. Donors are advised to perform muscle tension exercises from this point of the donation process to maintain blood pressure and make the donation experience a more positive one. The amount of blood collected is measured by an agitator scale, and the process of drawing blood is stopped automatically when this reaches the required amount. The process of blood donation itself usually takes between 5 and 10 minutes.

Post-donation, donors can collect drinks and snacks from a refreshment table. They are advised to wait at least 15 minutes in the area and to take at least two drinks. Where possible, the donor is then encouraged to book an appointment for their next donation [3].

## 1.2 Low Haemoglobin Deferrals

One consequence of blood donation is an immediate decrease in iron stores followed by gradual recovery in the period thereafter. Low haemoglobin deferral occurs when a blood donor candidate fails to meet the haemoglobin threshold for donation. Low haemoglobin deferrals are very de-motivating for donors: studies have found that a low haemoglobin deferral can significantly reduce a donor's likelihood to return to donate [13, 14]. Even when considering donors who have donated regularly some may not return to donate after a low haemoglobin deferral [15].

While it is de-motivating for donors to be deferred, it is necessary to protect them from health risks associated with anaemia, which include fatigue, restless leg syndrome, and pica [16]. As blood donors are particularly at risk of iron deficiency [17], it is important to ensure that allocated inter-donation intervals minimise any potential harm to the donor.

Some donors may be more likely than others to be able to give more blood safely should inter-donation intervals be varied. There also exist certain donors who are more likely to be deferred with the current inter-donation intervals and some for which conclusive information is lacking, as will be reviewed in **Chapter 2**. It is possible that some of these subgroups could be more susceptible to low haemoglobin deferrals, as could others not yet apparent in UK populations. It is essential that these

aspects are rigorously assessed before implementation of a policy varying the inter-donation intervals for donors.

## 1.3 Blood Donor Motivations

With the United Kingdom's blood service relying on voluntary blood donors, it is important to understand donors' motivations as the demand for blood changes. Efforts to increase the donor base may be required as the current population of committed donors from the baby boomer generation become ineligible to donate [18].

Studies have been performed in the UK and elsewhere that have identified several motivating factors for donors to donate (**Table 1.2**) [13]. These include emotional and bond driven motivations, such as knowing others who donate, events such as natural disasters, and personal factors such as a feeling of accomplishment [19-21]. Some of these motivations vary by gender. More women respond to direct appeals for donation from blood services [22], while more men than women will come to donate in response to natural disasters, such as earthquakes, when more people are admitted to hospital hence more blood is needed [23, 24]. Men also have more external motivations to giving blood. These include personal benefits such as infectious disease testing [25] and general health screening [26]. In addition, more men than women report that they are influenced by friends and family, who encourage them to donate [25, 27]. This trend can also be seen in rural areas [19], where donors are proud to tell their village that they have donated, and blood donation is a part of the donor's identity. This would then encourage other donors to donate. However, donors who donate blood for reasons of reputation and recognition by peers, can be viewed as an example of "impure altruism" [28], which is described as a "warm glow" that donors experience – feeling good about themselves for donating because they believe they have done something good and altruistic. This is evident in both men and women and is in contrast to the traditional depiction of blood donation as a purely altruistic act i.e. donors are donating purely for the wellbeing of others. One study [29], reported that there has been a decline in altruism, which may partly explain the decline in new donor numbers.

Consideration of the impact of preceding motivations to donate blood need necessarily be taken into account when assessing the generalisability of findings from randomised trials in blood donors. While randomisation and analysis under the intention-to-treat principle provides unbiased estimates of the effects of the randomised intervention when there is no differential loss to follow up across randomised groups, the findings would still only generalise to the donor population similar in characteristics to the sample that participated in the trial. In the trial setting donors are expected to actively sign up to participate and comply with demanding trial protocols, such as donating blood at specific inter-donation intervals and completing questionnaires, which may skew recruitment to more altruistic and motivated donors than the general population. Consequently, it may be possible that the intervention effects estimated in a randomised trial may be of a higher magnitude than would be observed in practice, due to the variety of likely donor motivations that may impact recruitment into a randomised trial setting. Furthermore, substantial outcome-related differential losses to follow up across trial arms would bias the trial results.

**Table 1.2**: Motivators to donate blood by sex

| Motivates Men | Motivates Women | Motivates Both |
|---|---|---|
| Natural Disaster Response | Direct Appeals | Impure Altruism |
| Infectious Disease Testing | Social Motivations | Social Responsibility |
| Health Screening | Need for Blood | Altruism |
| Influence of Friends and Family | Religious Reasons | |
| Small Gifts | Concern for Others | |

## 1.4 The UK Blood Donor Population

The blood donor population in the UK is aging more quickly than the general population (**Figure 1.2**); the median (IQR) age of the UK general population and the UK donor population was 40 (31-51) and 45 (32-61) years in 2001 respectively, which rose to 44 (30-54) and 46 (31-62) years in 2011 [30]. A similar trend has been observed in other countries, for example, the over 65 donor population more than doubled between 1999 and 2009 in Japan [31]. In the USA, changes to the donor eligibility criteria increased the prevalence of donors aged 65 years and over [32]. The UK has an over-representation

of donors in the 40-60 year old age group [30]. The transfusion needs in the UK population are low, until ages 45-50 years, after which point red blood cell use increases exponentially [30]. Similar findings were observed in Switzerland [33], and Germany [18] with >60% of blood use occurring in those over 65 years old.

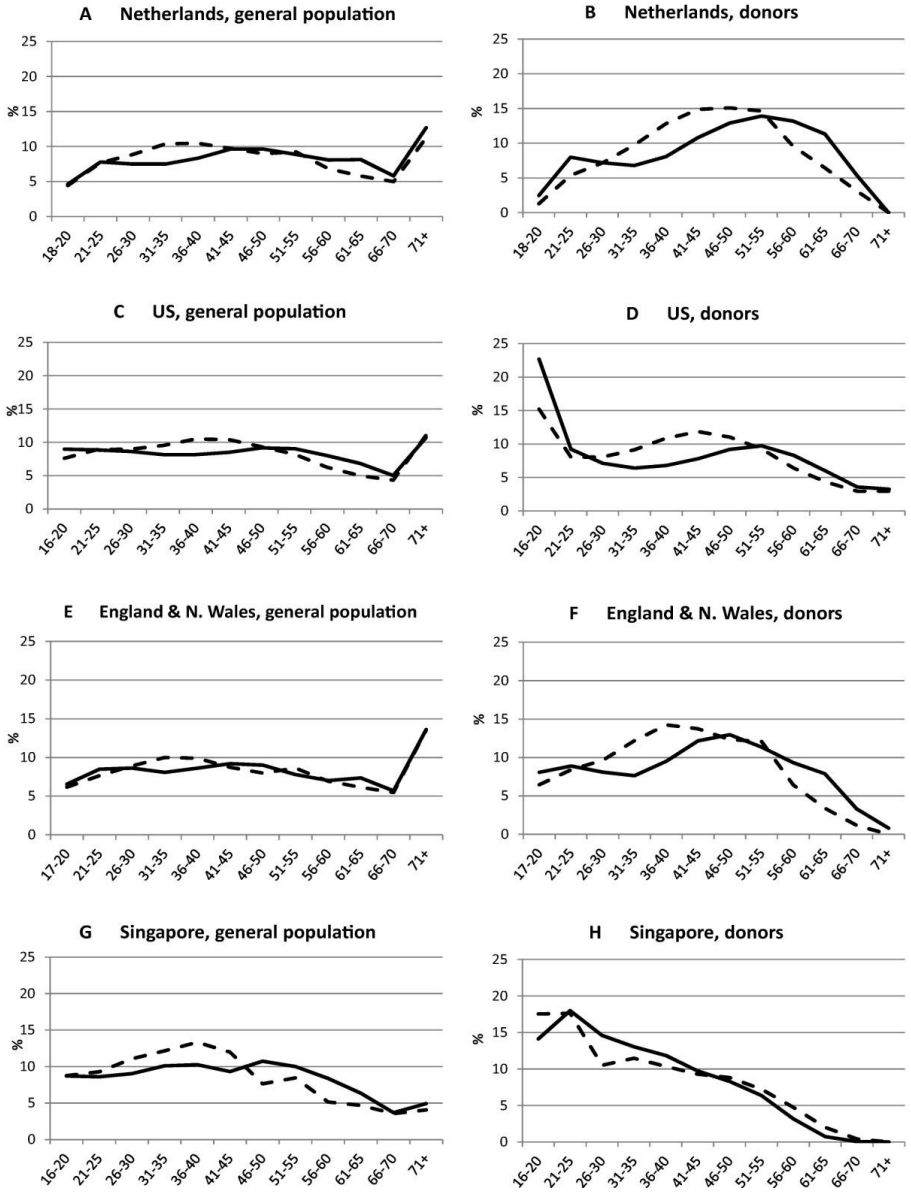



**Figure 1.2**: Age distribution of the general population and donor population in years in 2001 (– – –) and 2011 (—) in the Netherlands, USA, UK, and Singapore. Proportionally, the donor population is older in the UK and the Netherlands, and younger in the USA and Singapore. [34].

## 1.5 Donor Return Rates

Retention rates for new donors are low with only 55.2% of new donors in a UK study returning after 18 months [34]. There are many factors that affect whether a donor is likely to return to donate. A large Norwegian study found men significantly more likely to return to donate after 6 months than women (OR 1.09, 95% CI 1.08-1.11), although a more recent but smaller study found no significant difference (p=0.16) [35]. Other factors that affect blood donors returning to donate include adverse events and vasovagal reactions such as fainting, loss of breath, dizziness, and nausea [36], which can account for a drop of between 24% and 34% in return rates [8, 37-39]. Other individual level factors that reduce return rates are fear of needles, dizziness, and worries about the healthcare system and donation process [25, 40, 41]. Another key indicator of donor lack of return is missing an appointment [35]. Donors are more likely to continue to donate if they have a positive experience of or attitude to blood donation [33, 42, 43]. Another strategy that has proven effective in retaining donors is helping them to plan future donation, for example, by reminding them of donation appointments [43].

A key aim of donor retention should be to create blood donation as a part of a donor's routine, and the aforementioned systematic reminders can help donors to schedule donation into their lives as a routine activity. One study found that it takes four donations for blood donation to form a part of the donor's identity [44]. Another study established that donors are more likely than not to tell family and friends, and encourage them to donate [45].

## 1.6 Trends in Blood Donation

In the UK over 2 million donations per year are given by over 1 million donors [46], although donation rates per donor are falling [34]. The UK's donor population is reliant more on donors who are white, older, and male [34]. Donor numbers in London are significantly lower than elsewhere in the UK. This is in concordance with international studies that found that donors in urban areas are more likely to lapse from donating blood (cease donation) than those who live in rural and suburban areas [39, 43]. Ethnic diversity is increasing in the UK, with a rise from 11% of the UK population identifying as an

ethnic minority in 2001, to 20% in 2011 [47]. Despite this, ethnic minorities are under-represented in the donor population [34] and ethnic minority donors donate less frequently than white donors [34, 48, 49], and retention rates for ethnic minority donors, as well as first time donors, are low [34].

## 1.7 Changes in Transfusion Demand

Despite the aging population, transfusion demand is falling globally. All Western countries have found a reduction in the demand for blood [33, 50-53]. In the UK, there was a 7.3% decrease of RBC transfusions between 2011 and 2013, alongside a 12.1% decrease in collections [32]. Transfusion rates per 1000 of the population have fallen from 45.5 in 1999 to 36 in 2009 [51, 52]. In Germany, the number of transfusions fell by 13% between 2010 and 2015 [54], and in the wider time frame between 2005 and 2015, the largest reduction in transfusion demand came from the 20-45 and 65-80 year old age groups [18]. Like in the UK, donor numbers are falling, with a 12.4% decrease in donation rates for women. A Dutch study found decreased transfusions, with a 20% reduction in RBCs issued between 1996 and 2005, and a continued decrease of 9%, between 2012 and 2013 [53]. In addition, the number of hospital inpatients who did not require a transfusion increased from around 30% in 2002 to over 40% in 2011 [55]. There are several possible explanations for the decline in transfusion demand. The first is a change in the demographics of hospital procedures, particularly a large decrease in surgical patients requiring transfusion. Studies have found that surgical transfusion RBC use decreased by 40% or more [18, 51], and those without quantifiable results also stated that the surgical transfusion rate is falling [51-53, 56]. Noteworthy is that this is the type of procedure that affects the oldest populations most, and so counteracts fears of the aging population requiring more transfusions [18, 51, 53].

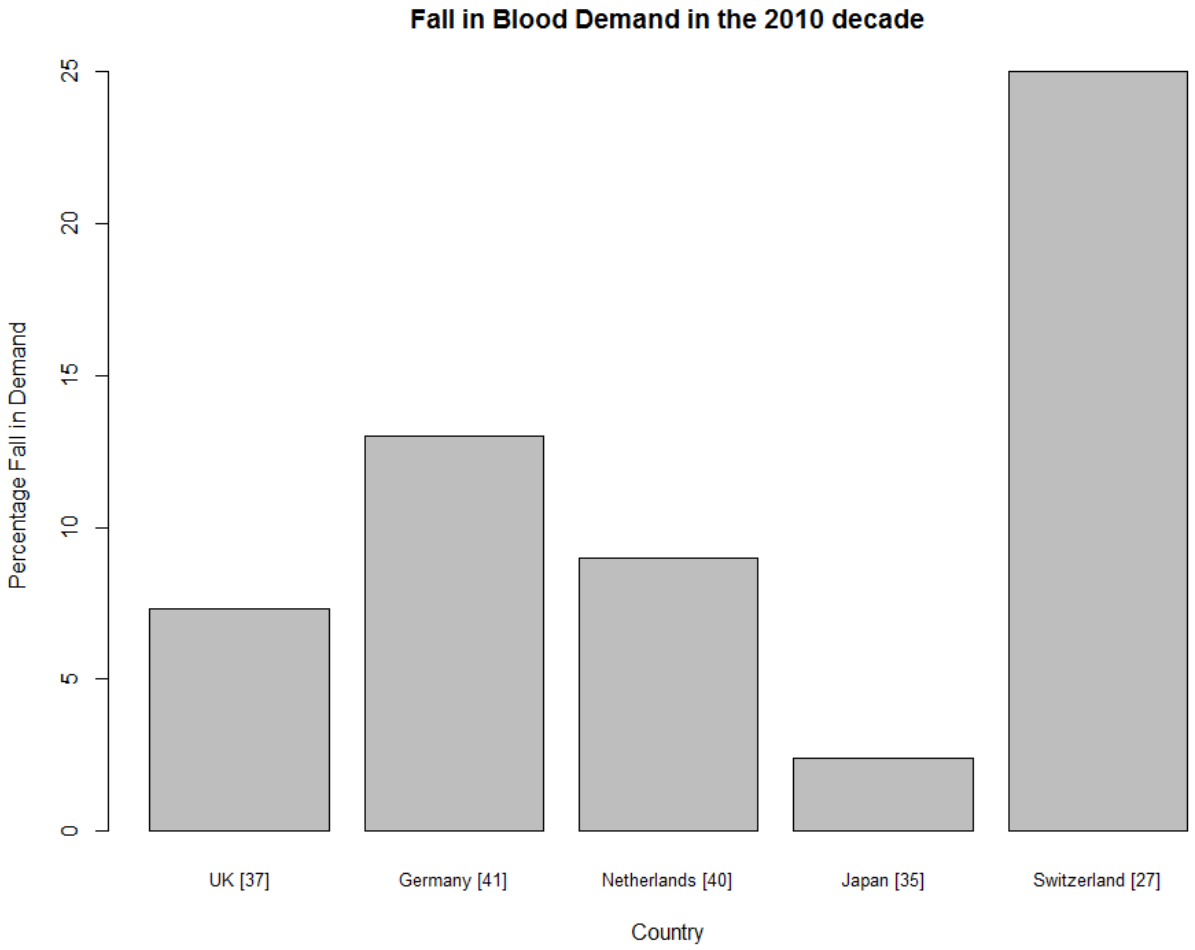



**Figure 1.3**: Observed changes in blood demand in the 2010 decade in the UK, Germany, the Netherlands, Japan, and Switzerland. All countries have seen a fall in demand in the 2010 decade.

### 1.7.1 Patient Blood Management

Another explanation for falling transfusion rates is the growing focus on patient blood management (PBM) techniques. PBM aims to optimise the haemoglobin concentration of patients, and to minimise blood loss [57]. Successful PBM reduces unnecessary transfusion and side effects, ensures blood products are available when needed, improves patient outcomes, and reduces costs [58]. A wide variety of PBM techniques can be used, including parenteral iron supplementation, oral iron supplementation, topical/systemic haemostatic agents, restrictive use of phlebotomy and restrictive transfusion practices [32], all of which have seen a significant increase in usage recently in the UK, despite inconsistent implementation amongst UK hospitals [58]. One hospital studied in Manchester, that implemented a wider range of PBM initiatives when compared with other hospitals in Europe,

had a lower numbers of transfusions per bed (14.9) and low proportion of transfused surgical patients (2.5) [56].

PBM has also been credited with declining blood use in the USA [59-62], with one study finding that giving less blood through more restrictive haemoglobin triggers (reducing the threshold at which a transfusion is performed) gave the same or better outcomes for most patients. This, used alongside other PBM techniques, contributed to a fall in RBC use per 1000 patients from 338 pre PBM to 228 post PBM. The effect was further pronounced in the over 65s, with these numbers being 547 vs 313 [63]. A change in haemoglobin thresholds advised before transfusion has also been implemented in the UK [52]. In the Netherlands, a reduction of 10-15% in the use of the overall blood supply has been achieved in the past decade due to increasing awareness of blood conservation techniques and reduced preoperative allogenic transfusion [64-66]. Another study found that the number of procedures which required just one unit per transfusion increased from 4 to 8%, and for medical procedures the use of three or more decreased [53]. A single unit transfusion policy was named as one of the PBM priorities for the UK hospital in a European PBM study [56] and as such, it is possible that future transfusion demand will decrease further if this practice is adopted more generally.

An additional explanation for more efficient transfusion practices is a large decrease in wasted blood products. A study from the USA found that outdated blood fell by 17.3% between 2011 and 2013 [67]. In another study, this figure was 19.8%. In particular, when examining platelet use in the same study, platelet collections and total distribution were not significantly different, but hospital use increased. Combining this with fewer outdated platelet donations suggests hospitals are using products more efficiently, and that this improved product management may have a greater effect than PBM initiatives [32].

## 1.8 Predictions for the Future of Blood Donation

Due to increased life expectancy, the aging of the baby boomer generation, and a decrease in birth rates coupled with increasing affluence, the demographics of the United Kingdom's blood donor

population may be expected to change in the coming decades [34]. This shift in demographics could lead to an increased demand for blood [30, 68-70]. The aforementioned faster aging of the donor population compared to the general population [30] could expediate such a problem. While there is a current surplus of blood for transfusions, this may change in the future. The primary cause for concern in the next 10-15 years is that the current set of donors from the baby boomer generation, who are currently most committed and give the most donations, become ineligible to donate because of age or other health problems [18], and new donor levels will not match the shortfall caused by these donors becoming ineligible to donate. It has been suggested that a way to mitigate this is to encourage repeat donationas soon as possible, as well as to encourage repeat donors to persuade peers to donate. As such, maximising the blood supply from the current donor base may become a relevant question for in the future.

The future of blood supply and demand remains uncertain throughout the world. Due to the aging population worldwide, studies predict that there will soon be large shortfalls in blood supply in many countries [33], some stating these could occur as soon as 2020 [70]. However, a study from Japan incorporated advances in medical technology into their models, and found that, after a slight increase in demand in 2022 to outstrip supply, demand would fall due to technological progress by 2027 and would be met by predicted supply [31]. However, Japan permits 200ml blood donations in addition to 400ml donations, and this could increase supply as donors unable to endure a full 400ml donation can still donate blood. Despite this, it is possible that these technological advances will be seen in other countries, thus similarly reducing demand for blood.

## 1.9 Blood Donation Intervals in the UK and Europe

The minimum length of time between blood donations (i.e. inter-donation interval) in England and Wales is twelve weeks for men and sixteen weeks for women [71]. These are some of the longest inter-donation intervals in Europe (**Table 1.3**). In other countries, the donation interval can be as little as eight weeks for both sexes, e.g. in the USA [72], and some countries in Europe. In Austria, Germany,

France and Finland, men can donate every eight weeks, and in Austria and Ireland women can donate every ten weeks [7]. Decreasing the donation interval for blood donors in the UK could be an answer to the declining number of new donors.

**Table 1.3**: Inter-donation intervals (in weeks) in England and selected countries from the rest of the world

| Country | Men | Women |
|---|---|---|
| England | 12 | 16 |
| USA | 8 | 8 |
| Austria | 8 | 10 |
| France | 8 | 12 |
| Ireland | 10 | 10 |
| Denmark | 12 | 12 |
| Slovenia | 12 | 16 |
| Scotland | 16 | 16 |

## 1.10 The INTERVAL Trial

The INTERVAL trial was a large randomised trial that assigned men and women to donate more frequently than current NHS guidelines, with a control arm donating at the current inter-donation intervals. The shorter inter-donation intervals trialled were 8 and 10 weeks for men, and 12 and 14 weeks for women [7]. The trial aimed to identify those who were able to safely donate blood more frequently than current guidelines, maximising the number of units of blood collected from the UK's decreasing donor population.

The trial ran for two years between 2014 and 2016, and randomised 45,263 donors, 22,466 men and 22,797 women, to donate blood at one of the donation intervals, in a 1:1:1 split between donation groups [73]. Donors had to donate in one of the 25 fixed donation centres in the UK during the trial period.

The primary outcome was the number of units of blood collected during the trial period. Quality of life, as measured by the Physical Component Score (PCS) and Mental Component Score (MCS) of the SF-36 questionnaire, was a key secondary outcome, and was assessed at baseline and end of trial using the SF-36 questionnaire, as well as every 6 months using the SF-12 questionnaire.

[7].

The INTERVAL cohort was around 4% of the UK's total donor population at the time. The sample of donors included in the trial was largely similar to the general donor population aside from having a higher mean age, lower proportion of men, and a longer and more active donation history [73].

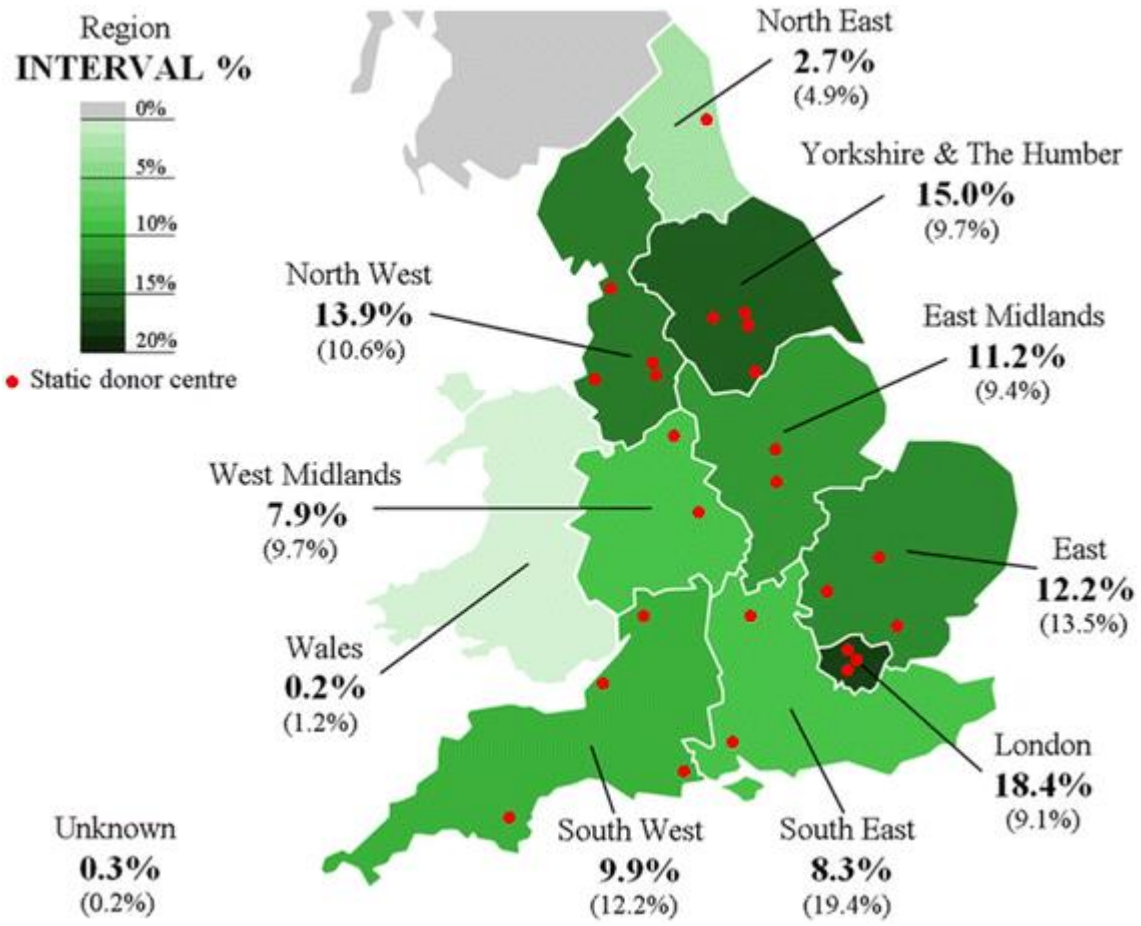



**Figure 1.4**: Distribution of INTERVAL participants (NHSBT general population in brackets), red dots are the donor centres. Darker shades of green indicate where more INTERVAL participants were located [73].

Previously published results from the trial have shown that for both sexes, donors on the shorter inter-donation intervals gave significantly more units of blood but also had a significantly higher frequency of low haemoglobin deferrals, as well as lower haemoglobin and ferritin concentrations at 2 years than those on the longer intervals. Men on the 8 week interval had a 33% higher mean difference in units of blood donated during the study. For women on the 10 week interval, this was a 24% increase. In addition, there was no impact on the overall quality of life scores due to increased donation, but

those on shorter donation intervals did experience more post-donation symptoms such as tiredness, restless leg syndrome, and feeling faint, especially men [74]. An extension study of the INTERVAL trial involved participants agreeing to continue donating blood at allocated intervals for up to four years. While donors in the extension study were older and more committed donors, with fewer deferrals and symptoms observed during the main trial, they had decreased haemoglobin concentrations and more self-reported symptoms compared with the initial two years of the trial. Furthermore, there was an increase in reported frequency of iron supplements prescription or use, particularly in the shorter inter-donation intervals [75].

## 1.11 PhD Project Rationale

While some studies have retrospectively assessed how prolonging the inter-donation interval would affect the blood supply in the USA [76], before INTERVAL there was yet to be a randomised trial to find the optimal inter-donation intervals at which individuals or groups should donate blood to maximise the amount of blood collected while minimising deferrals and effects on quality of life to donors.

The objectives of this PhD project are to identify donor characteristics associated with ability to safely donate blood more frequently than current NHS guidelines, which can then be used to develop stratification schemes to personalise the inter-donation intervals of blood donors.

The thesis is structured as follows:

- In **Chapter 2**, I present a systematic review of factors that influence a donor's inability to meet pre-donation haemoglobin thresholds.
- **Chapter 3** describes the INTERVAL trial and data used for analysis in the remaining chapters.
- In **Chapter 4**, I assess interactions with randomised inter-donation intervals to identify characteristics of donors that may potentially inform personalised donation in relation to the amount of blood collected, the number of low haemoglobin deferrals and haemoglobin and ferritin levels.

- **Chapter 5** presents the association between donation frequency and the risk of symptoms related to iron deficiency, including tiredness, breathlessness, palpitations and fainting. Mediation analyses are performed to assess whether iron supplementation mediated the effect of donation frequency on health-related outcomes.
- In **Chapter 6**, I perform analyses to quantify the effect of more frequent inter-donation interval on wellbeing, measured by the physical and mental component scores of the Sf-36 and Sf-12 questionnaires and the sub-components that comprise these.
- **Chapter 7** provides a general discussion, conclusions, and future directions of research arising from this thesis.

# Chapter 2 – Donor Deferral due to Low Haemoglobin – a Systematic Review Update

## Summary

Blood donors attending a donation session may be deferred from donating blood due to a failure to meet low haemoglobin (Hb) thresholds. This costs the blood donor service, and donors, valuable time and resources. In addition, return rates of donors deferred for low haemoglobin are lower, even amongst repeat donors. Moreover, even when donors do return, they take a long time to do so. It is therefore vital that low haemoglobin deferral is minimised to maintain the donor population. A systematic review of these factors was conducted in 2012. The current update further synthesised the growing evidence base, including quality assessment of relevant studies.

Studies were identified by searching MEDLINE, EMBASE, *The Cochrane Library* and the WHO International Clinical Trials Registry from 2012 onwards, and the results were added to the previous review. Demographic characteristics, donor history, haematological/biological factors and the primary outcome of deferral due to low Hb were extracted. Descriptive and quantitative analyses were conducted; pooled odds ratios (ORs) were obtained by meta-analysis.

Following re-assessment of the studies in the original review, 116 studies met the inclusion criteria between this and the previous search. A consistently higher rate of low Hb deferral was reported in females compared with males. Meta-analysis showed a significantly higher odds of deferral due to low Hb in females compared with males in studies with universal Hb thresholds for males and females (OR 14·42, 95% confidence interval (CI) 12·24–16.97) and those with sex-specific thresholds (OR 5.98, 95% CI 4·46–8.02). Other characteristics associated with increased rates of deferral due to low Hb included increasing age in men, low body weight, shorter inter-donation interval, donors of Hispanic or African descent, higher ambient temperature, donors with low ferritin levels, donation in a fixed donor centre,

and geographical location. There was conflicting evidence on the association of new and repeat donor status, and blood group with low Hb deferral.

This work has strengthened the evidence of the previous systematic review by further identifying key characteristics that may be considered in studies of donor deferral. The current review has also highlighted areas in need of further study, including blood group, previous platelet donation, diet, smoking, time of day, genetic data, and rhesus status.

## 2.1 Introduction

Faced with dwindling numbers of new donors, blood services globally need to be able to better understand how to retain donors. One of the main reasons why donors may stop donating blood is because they received a temporary deferral for low haemoglobin (Hb) [15]. While it is widely understood that women are more likely to be deferred for low Hb than men, there is limited evidence on the contribution of other donor characteristics to low Hb deferral including demographics, or physical and environmental factors.

A previous systematic review from 2013, which included 55 studies, identified a variety of characteristics associated with a higher risk for low Hb deferral (LHD) in blood donors [77]. These included female gender, the season of donation (spring and summer), older male donors, non-white ethnicity, and new donors [77]. Other potential factors identified from individual studies were difficult to evaluate due to the small number of studies reporting each factor.

The objective of this review was to identify new research findings after the publication of the previous review and re-assess the previous review. This review also included a formal quality assessment of all studies. The information obtained in this review expands the evidence base of factors that influence low haemoglobin deferral in blood donors, and helps to inform criteria that should be considered in trials of donor management.

## 2.2 Materials and Methods

The protocol for this review was prospectively registered on PROSPERO (CRD42017071105). The review was carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [78]. Full details of the search strategy, eligibility, data extraction and quality assessment are provided in the **Appendix A**. Briefly, literature searches were carried out up to March 2019 with eligible studies identified and summarised in **Figure 2.1**. Screening for eligibility and data extraction was performed independently in duplicate by two reviewers.

Extracted data included characteristics of study participants (sex, age, ethnicity, weight, number of donations during study, season of donation, type of donor etc.), Hb thresholds for deferral from donation, outcome data (number of donors deferred and/or number of donation attempts resulting in LHD) and any other reported factors which may affect donation. Quality assessment methods were adapted from the RTI Item Bank for assessing risk of bias and confounding in observational studies [79].

The primary outcome was deferral due to low Hb. Both qualitative syntheses and quantitative analyses were performed. Random effects meta-analyses were used to account for the expected clinical and statistical heterogeneity between studies and provide pooled association estimates based on unadjusted count data, which should be interpreted with caution. Summary measures were presented as unadjusted odds ratios (ORs) with 95% confidence intervals (CIs). Heterogeneity was assessed using the $I^2$ statistic which measures the percentage of variance in a meta-analysis which can be attributed to heterogeneity [80]. Statistical analysis was carried out in R v3.4.2 (http://www.r-project.org/) and Review Manager 5.3 [81].

## 2.3 Results of Literature Search

### 2.3.1 Study Selection

The PRISMA study flow diagram is shown in **Figure 2.1**. After de-duplication, 2,518 records were initially screened. Of these, 102 eligible records contributed to 76 independent studies. Thus, together with 40 studies from the previous review, 116 studies were included in this update.

### 2.3.2 Description of Included Studies

A summary of the characteristics of the studies can be found in **Appendix B**. Of the 116 included studies, the majority (n=80) included single unit red blood cell (RBC)/whole blood donations only. Other donation types included platelets, double red cell and multicomponent donations (Table 2.1). The studies were carried out in 35 countries across six continents.

Hb deferral thresholds were reported in 85 studies, and four studies used haematocrit (HCT) levels. A universal threshold of 125 g/L was used in 31 studies, while sex-specific thresholds were used in the remaining studies, where reported. The threshold for men was between 120 and 135 g/L, and for women between 110 and 125 g/L. A variety of screening methods for Hb levels were used, including gravimetric estimation, involving a drop of blood into a copper sulphate solution, Hb measurement in venous or capillary blood samples, spectrophotometric estimation of haemoglobin (Hemocue), and automated analyser (for example Sysmex or Coulter analysers).

### 2.3.3 Quality Assessment

The risk of selection bias was low in the majority of included studies. A moderate risk of attrition bias was observed due to some studies failing to report the number of successful donations or deferrals due to other reasons. Few studies adequately reported baseline characteristics of donors. The risk of attrition bias was lower in new studies, as was the risk of confounding, however few studies performed multivariable analyses and as such the residual confounding may be relatively high (**Figure 2.2**).

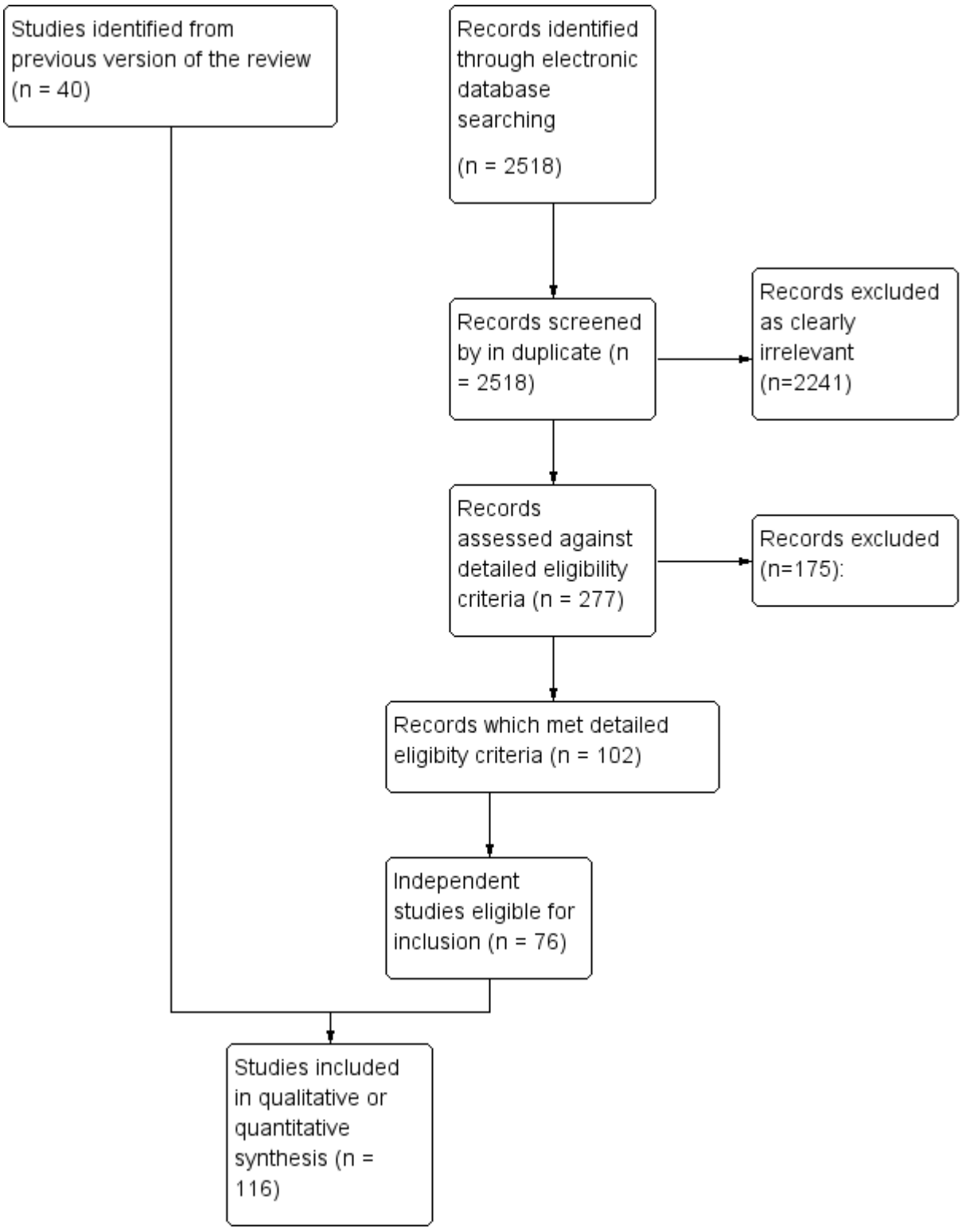
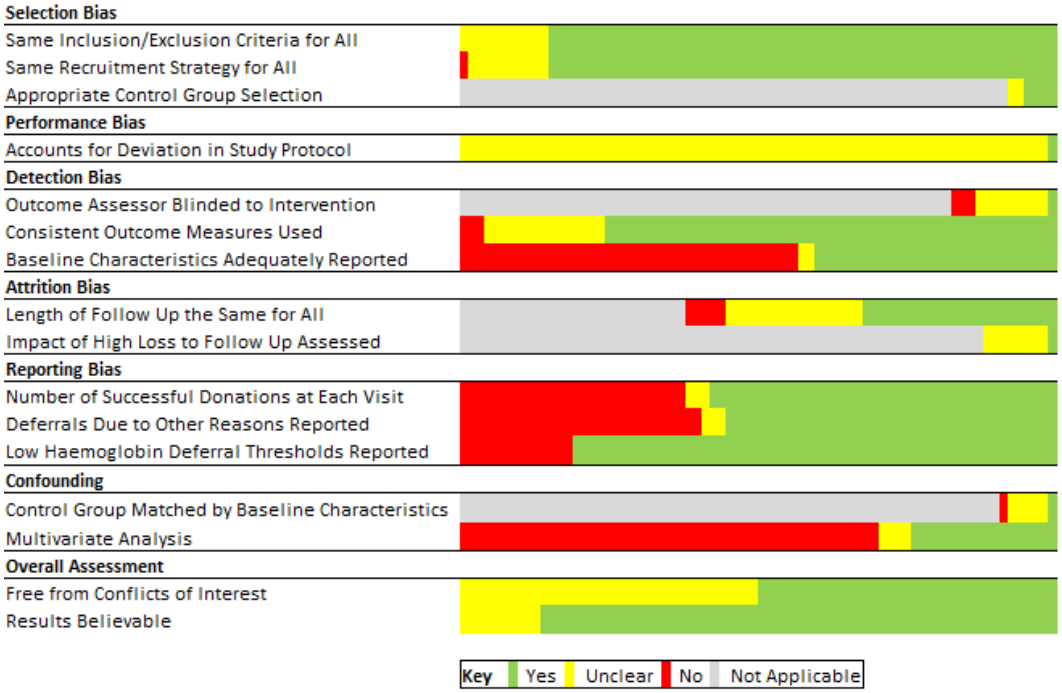



**Figure 2.1**: PRISMA flow diagram of study selection. 76 new studies were identified and added to the 40 studies from the previous review that remained after screening.

A




B

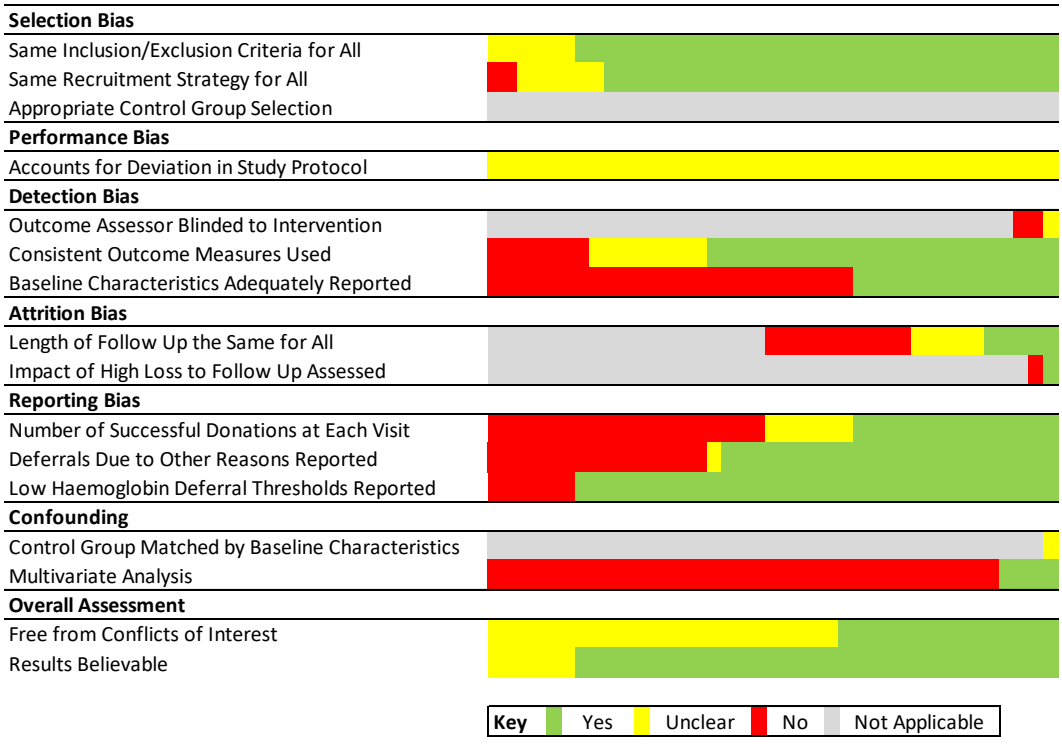



**Figure 2.2**: Comparison of Risk of Bias observed in original and new studies. A: studies new to this review update; B: studies included in the previous version of this review. Bias was similar in both the original review studies and those in the updated review.

## 2.4 Factors Associated with Low Haemoglobin Deferral (LHD)

### 2.4.1 Sex

Sex specific LHD rates were reported by 64 studies. The male to female ratio varied widely across studies, with the percentage of female participants ranging from 1.7% [82] to 100% [83]. Studies from India had significantly fewer female participants (3.96 - 20.2%), whereas studies performed in Europe and the USA generally had more equal proportions of male and female participants. LHD rates were higher in females in all studies. Studies which used universal Hb thresholds for both male and female donors showed a 14-fold higher odds of LHD for females (OR 14.62, 95%CI 12.43-17.19). An increased risk of LHD in females remained for those studies which applied lower Hb thresholds for female donors (OR 5.73, 95%CI 4.36-7.53) (**Figure 2.3**). High heterogeneity between studies was observed in both analyses ($I^2$>95%).

Studies that reported male and female deferral numbers separately were stratified by geographical location of study, and results of these meta-analyses are presented in **Figure 2.4**. Notably, differences between studies performed in different geographical settings were observed even when similar Hb thresholds were applied. For example, the increased risk of LHD in females compared with males was significantly higher in studies from Africa (OR 5.24, 95%CI 4.07-6.74) than in studies from Europe (OR 2.85, 95%CI 2.21-3.68) despite both sets of studies using predominantly sex-specific Hb thresholds (where reported). The greatest difference in the risk of LHD between men and women was observed in India (OR 19.11, 95%CI 13.95-26.16).

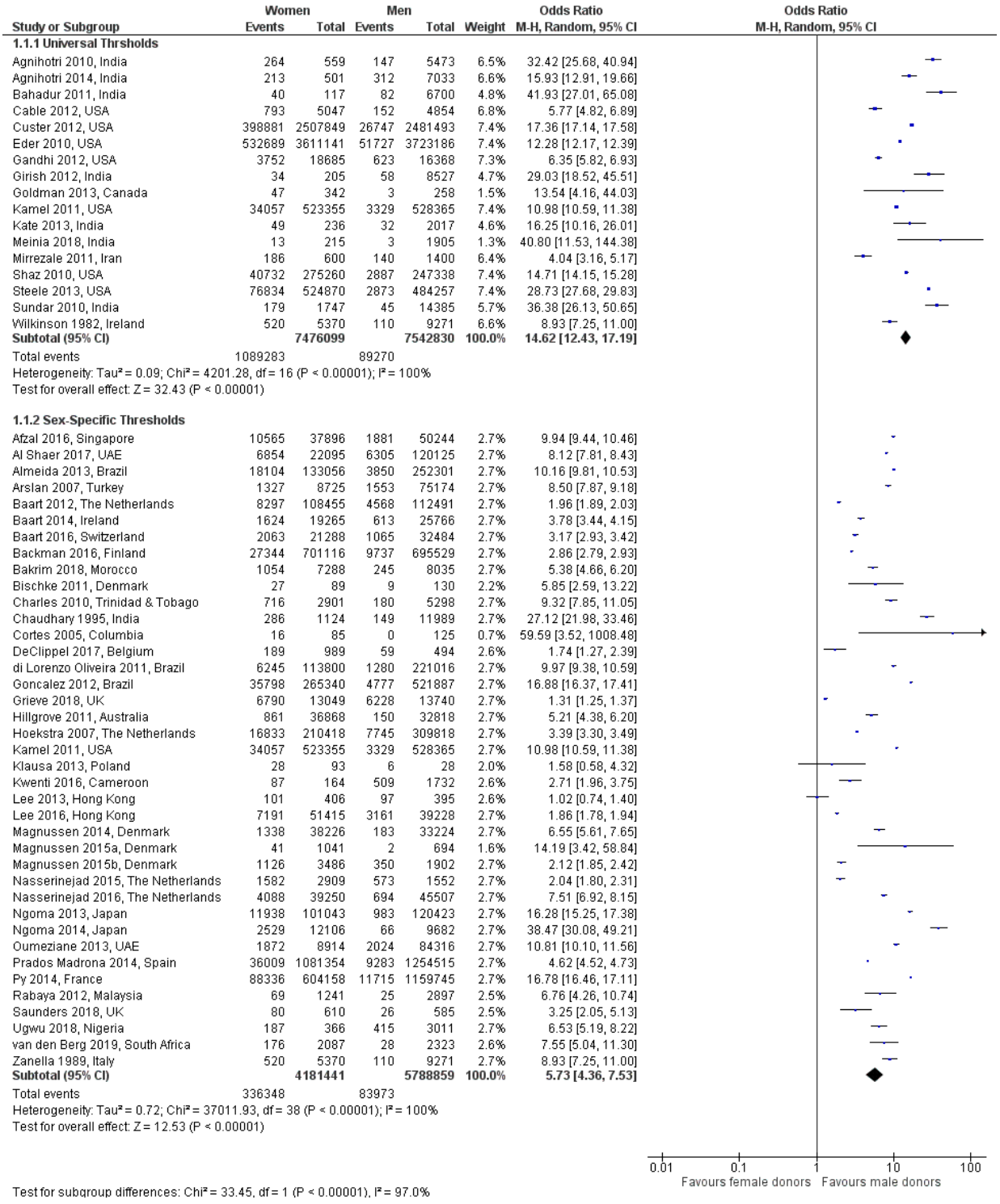



**Figure 2.3**: Meta-analyses of low Hb deferral for females compared with males stratified by whether the studies used universal or sex-specific deferral thresholds. There was a higher rate of low Hb deferral for women regardless of whether universal or sex-specific thresholds were used.

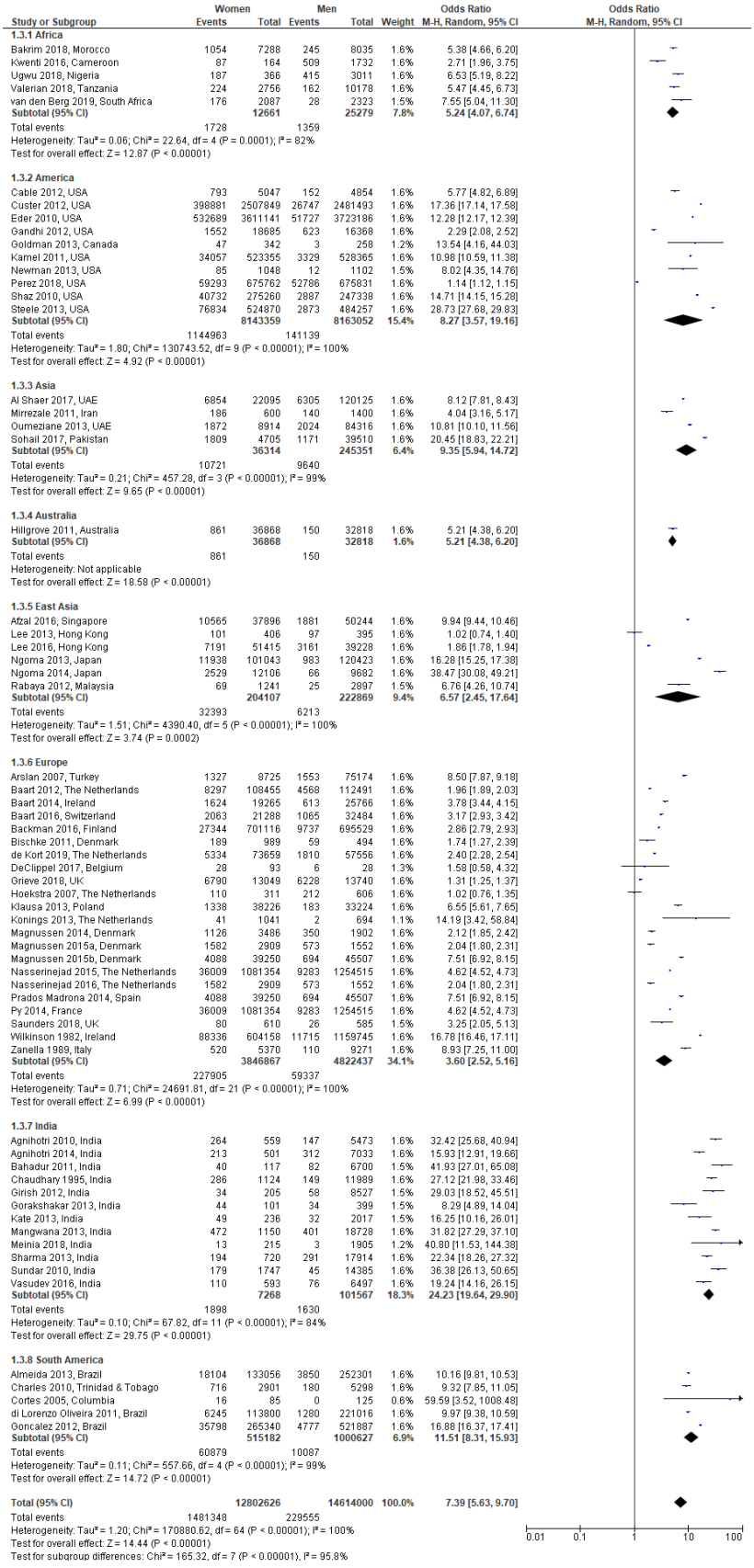


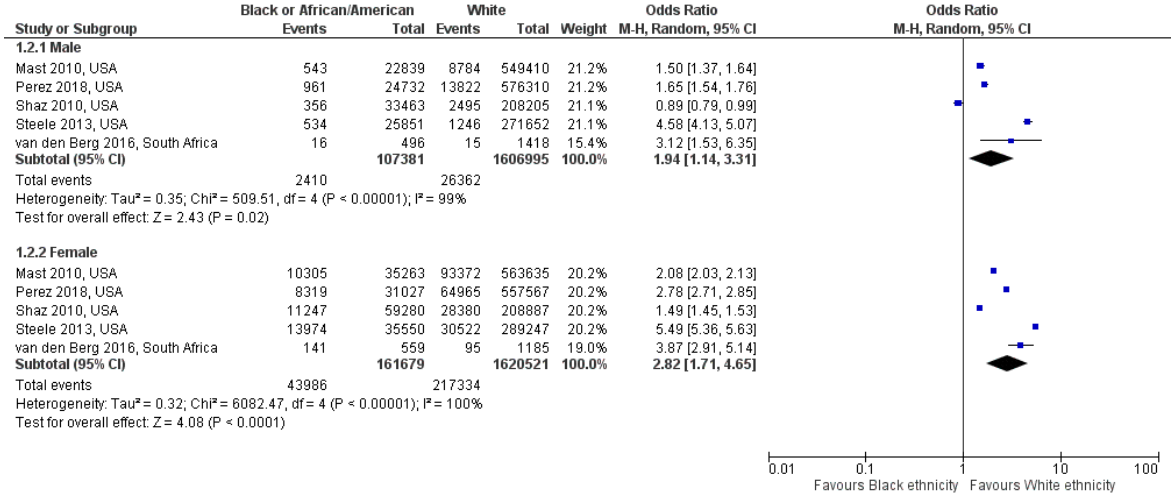

**Figure 2.4**: Meta-analyses of low Hb deferral for females compared with males stratified by study setting. In all locations, women had higher odds of deferral than men.

### 2.4.2 Ethnicity

Nine studies reported LHD by ethnicity, although there was a lack of consistency in the groups studied [72, 84-90]. In four studies, [72, 84, 85, 89] the non-white ethnicities reported were Asian, Black, Hispanic and not stated, whereas in one study [90] donors were categorized as White, Asian, African, unknown and "coloured" and in another study [88], donors were defined as African-American, Hispanic, White and "other". Another study [87] reported deferral rates for Black, Asian, Mixed, Native America, and Native Hawaiian. One study only compared white donors with non-white donors. Five studies reported Hb deferrals by ethnicity separately for male and female donors.

For male donors, four out of five studies observed a significantly higher risk of LHD in African-American/Black donors compared with White donors, with meta-analysis showing an approximate two-fold increased risk (OR 1.94, 95%CI 1.14-3.31) associated with African-American/Black ethnicity. In female donors, the risk of LHD associated with African-American/Black ethnicity was higher and found in all five studies (OR 2.82, 95%CI 1.71-4.65) (**Figure 2.5A**). In the comparison of Asian and White male donors, the combined risk across four studies was not significant (OR 1.17, 95%CI 0.77-1.78). However, a significantly higher risk of LHD was found in female Asian donors compared with female white donors, with an overall 63% increased risk associated with Asian ethnicity (OR 1.63, 95%CI 1.31-2.03) (**Figure 2.5B**). A similar increased risk of LHD was found in female Hispanic donors compared with female White donors (OR 1.43, 95%CI 1.22-1.68). However, in male donors, there was no evidence that Hispanic ethnicity was associated with an increased risk of LHD (OR 0.74, 95%CI 0.53-1.03) (**Figure 2.5C**). However, it should be noted that the degree of heterogeneity across studies was high ($I^2$>88% in all comparisons) and these ORs represent an average increased risk across studies.

A

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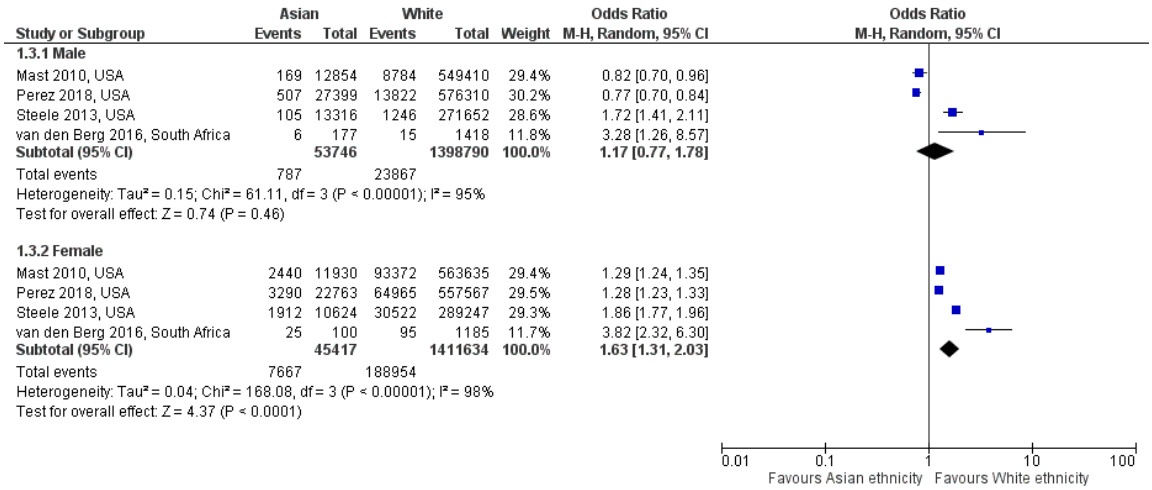



C

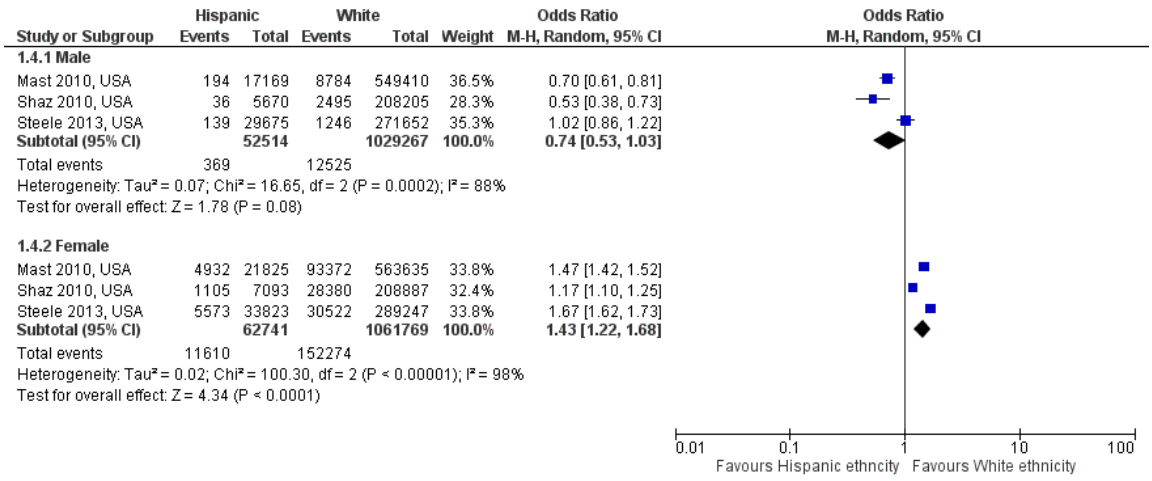



**Figure 2.5**: Meta-analysis of low haemoglobin deferral by ethnicity. A: Black or African-American vs White; B: Asian vs White; C: Hispanic vs White. White women had a lower deferral rate than other ethnicities, and black men had a higher deferral rate than black men.

### 2.4.3 Age

LHD was reported by age groups in 19 new studies [72, 84, 87, 90-105]. In 14 studies which reported results separately for men and women, the general trend across studies for men was for LHD rates to increase with age in men and to mostly decrease with age in women after 50 years (**Figure 2.6**). Some studies reported that the youngest groups of women had the highest deferral rates [84, 90] while others saw a higher deferral rate for women aged 30-50 [92, 101, 102].

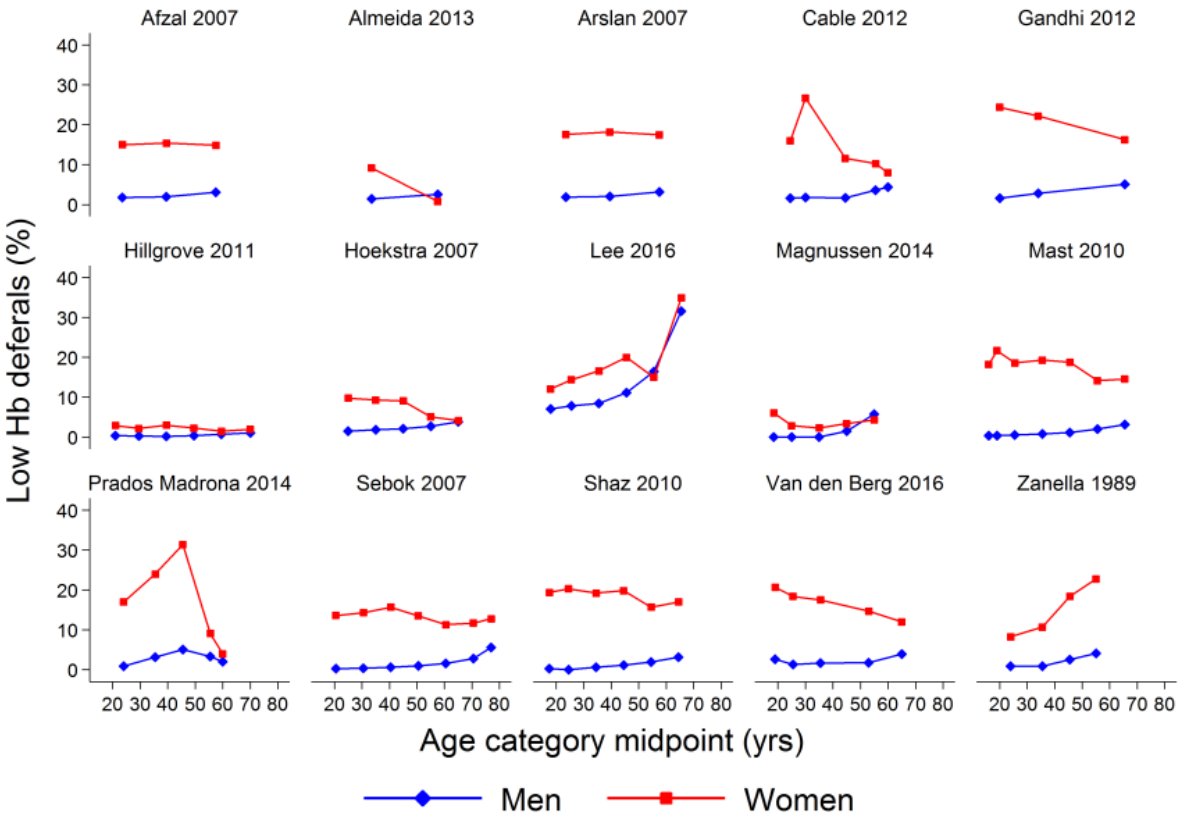



**Figure 2.6**: Graphs of deferral by age for men and women. Each tile represents a study, red lines indicate deferral percentage for men, and blue deferral percentage for men. In most studies, women were deferred less often as age increased, while the reverse was true of men.

One study from Africa [100] reported deferral rates by age for men and women separately but these were universally very high, with the lowest rate of LHD in any one age group being 27% (in men in their 30s) and the highest 71.4% (in women under 20). Two European studies found that male donors who were deferred for low Hb had a higher mean age (years) than those not deferred, (47 vs 43) [93] (50 vs 46) [94]. In contrast, both studies reported a lower mean age in female deferred donors (38 vs

41) [93], (39 vs 43) [94]. A study from the Netherlands [97] showed via a multivariable logistic regression model an increased risk of LHD for older men and younger women. A US study [72] compared different age groups to those aged 40-49 and found that older men were more likely to be deferred (50-59: OR 1.56; 60+: OR 2.96), and younger men less likely (<30: OR 0.57; 30-39: OR 0.74), with an inverse trend for women (<30: OR 1.09; 30-39: OR 1.00; 50-59: OR 0.73; 60+: OR 0.72). Finally, an Indian study [99] reported that more younger than older donors of both sexes were deferred.

### 2.4.4 Seasonality

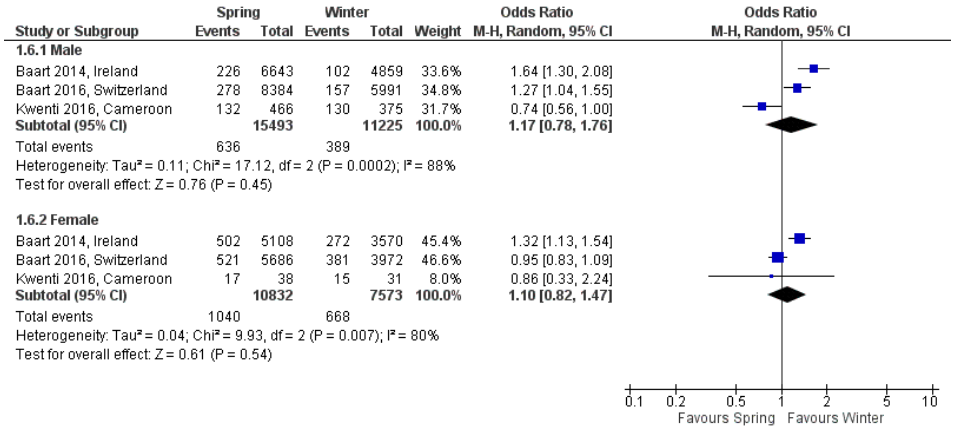
Seven studies reported the relationship between seasonal or temperature changes and LHD [93, 94, 97, 100, 106-108]. Three of these, two European [93, 94] and one African study [100], reported deferral numbers by season. Meta-analyses of the three studies showed no significant difference in LHD rates between spring and summer months. One other European study noted a significant increase in the LHD rate of donors from 2% in January to 3.5% in July [106]. In addition, a US study [107] reported a deferral rate of approximately 8% when the temperature was above 12 degrees Celsius, compared with 6% in winter. A Dutch study [97] performed logistic regression and found that higher LHD was associated with spring and summer compared with autumn and winter, which was more pronounced in men.

In studies that reported deferral numbers separately by sex [93, 94, 100], meta-analysis of each season versus winter showed no significant differences by season in male donors (**Figure 2.7**). In female donors, evidence from three studies showed a significantly higher risk of LHD in summer compared with winter (OR 1.18, 95%CI 1.07-1.30) (**Figure 2.7**), but no differences associated with either spring or autumn seasons.

An additional study from the USA [108] reported percentages of low Hb deferrals for men and women between 2002 and 2004. It found an overall increase in LHD in other seasons compared with winter, which was most pronounced in summer. Looking at sex and age specific deferral rates, older women

had a higher increase in deferrals by season compared to younger women, as did older men compared to younger men all be it at a lower magnitude of absolute deferral rate than for women.

**A**

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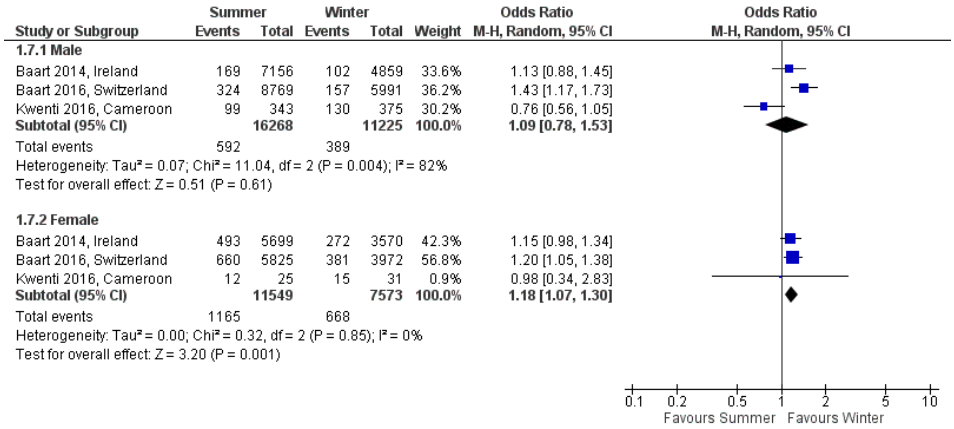


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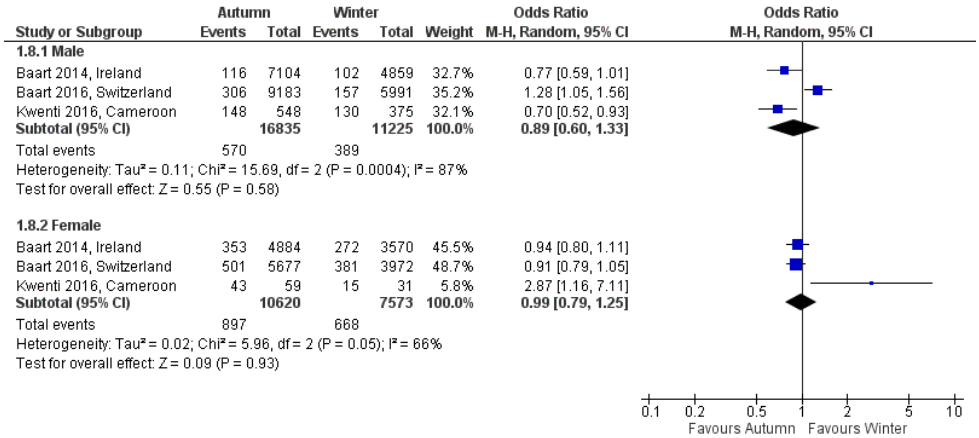



**Figure 2.7**: Meta-analysis of low haemoglobin deferral by season. A: Spring vs Winter; B: Summer vs Winter; Autumn vs Winter. Only for women in winter compared to summer were deferral rates higher between all studies.

### 2.4.5 Weight

Four studies [72, 84, 85, 109] reported ORs for LHD by weight and sex. One US study [72] found that, compared to people who weighed between 150 and 174lbs (68.0 to 79.3 kg), lighter men (OR 1.38) and women (OR 1.13) were deferred at a higher frequency, as were the heaviest women (equal to or over 200lbs weight (equal to or over 90.7 kg), OR 1.04), although no CIs were reported. Men who were between 175 and 199lbs (79.4 and 90.6 kg) (OR 0.79) and above 200lbs (equal to or over 90.7 kg) (OR 0.72) were less likely to be deferred, as were women between 175 and 199lbs (79.4 and 90.6 kg) (OR 0.98), although the statistical significance of these ORs were not reported. One US study [84] used the same weight categories as the previous study and showed a marginally significant increase in LHD for the heaviest women (>200lbs) (over 90.7 kg) (OR 1.5, 95%CI 1.0 -2.2). In a US study [85], the heaviest age group (>200lbs) (over 90.7 kg) was used as the reference group, and logistic regression showed significantly higher deferral rates for both sexes for donors in all lighter weight categories with the exception of female donors who weighed between 150 and 174lbs (68.0 to 79.3 kg), who had a marginally lower deferral rate (OR 0.95, 95%CI 0.93-0.97), and females between 175-199lbs (79.4 and 90.6 kg) (OR 0.92, 95% 0.89-0.94). A Dutch study [109] used age adjusted ORs and found that, compared to those in the lightest weight category (<60kg), deferral risk decreased with increasing weight, with the lowest risk for male donors over 100kg (OR 0.22, 95%CI 0.18-0.27).

### 2.4.6 Donation Characteristics

Donation history was reported using a number of different methods including donation intensity, inter-donation interval and repeat versus new donors (**Table 2.1**). Evaluation of the association of donation characteristics with low Hb deferral is difficult as there is an effect of selection and also an opposite effect of lowering of iron stores with repeated donation.

Twelve studies reported deferral by donation intensity, either the number of previous donations the donor had made in the past two years expressed as a categorical [72, 84, 85, 96, 110] or continuous [93, 94, 97, 111] variable, or the time since the donor's previous donation [93, 94, 96, 112-114].

Two European studies found that the number of previous donations was associated with lower LHD risk in both men and women, with a stronger association in men [93, 94]. However, the third study concluded that for men, LHD deferral increased as the number of donations increased. One US study [84] found no effect of donation history, while the other [72] reported ORs below 1 for donors who had seven or more donations in the past two years. A Brazilian study [92] found that, for women in particular, the percentage of donation attempts that resulted in low HCT deferrals increased to above 0.3% for those with seven donations in the past, compared with a proportion of around 0.1% or less for those with one or two previous donations. These results for women were in particular contrast with the earlier US studies, which found that women who donated more often were at a lower risk of deferral, and that only men who had donated once or twice in the past year had a significantly reduced risk of deferral [85, 96].

Eight studies reported an association between LHD and time since previous donation [84, 92-94, 96, 112-114]. Four European studies found that men had a reduced chance of LHD the longer they waited to donate, however the results for women were inconsistent [93, 94, 112, 114]. A US study [96] reported no significant association for donors who had returned to donate 24 weeks or less since their previous donation compared with those who returned between 24 and 36 weeks, however those who waited longer between donations were significantly less likely to be deferred. A South African study [113] found no significant effect of donation interval. A Brazilian study [92] found that in male donors aged >44y, the time since previous donation had little effect on LHDs, whilst younger male donors saw a decrease in low HCT deferrals until around 10 months since their previous donation. Results for female donors were similar, although the proportion of low HCT deferrals was higher for all age groups than for men. A US study [84] found that donors who waited 13 weeks or less were significantly more likely to be deferred for low Hb than those who waited 26 weeks or longer.

Two studies [115, 116] reported the effect of minimum inter-donation interval on LHDs. A US study [115] found that, after increasing the minimum inter-donation interval from eight to twelve weeks,

the LHD rate was reduced from 12.5% to 10.2% (p<0.0001). A Canadian study [116] also found that after raising its minimum donation interval from 56 to 84 days, deferral rates fell from 13% to 9.5%.

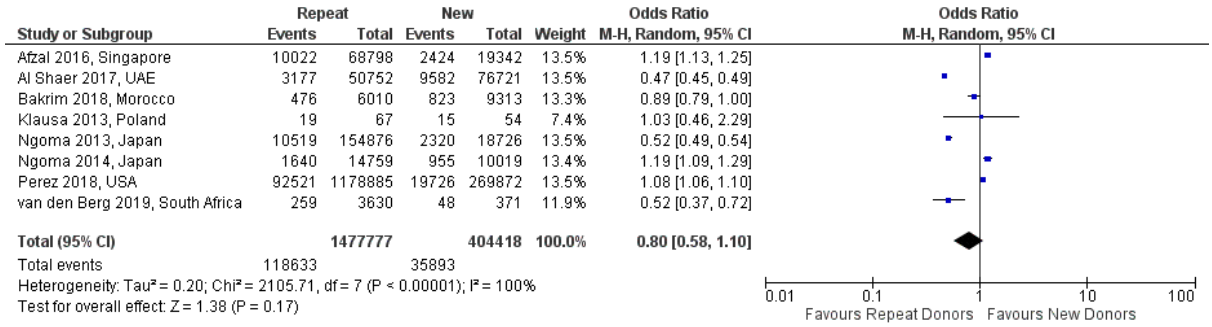



**Figure 2.8**: Meta-analysis of low haemoglobin deferral, repeat versus new donors. The pooled effect was non-significant, indicating that there is no difference in deferral rates by donor status.

Fifteen studies [87, 90, 95, 96, 104, 105, 117-125] reported LHD for new and repeat donors. Meta-analysis of 13 studies which reported LHD rates found no difference in the risk of LHD between new and repeat donors (OR 0.80, 95%CI 0.58-1.10) (**Figure 2.8**). In addition, a study from Thailand [121] reported only deferral percentages for repeat and new donors and found a higher percentage of LHDs for regular donors than new donors, whereas a study from the Caribbean [124] observed the opposite effect. The risk of LHD in new and repeat donors is likely to be confounded by the number and intensity of donations in individual studies which may contribute to the high heterogeneity observed across studies.

**Table 2.1**: Deferral results by donation characteristics.

| Study | Donation Group | Hb Deferral Rate (%) (males/females) | OR (95% CI) (male/female) |
|---|---|---|---|
| *Donation Intensity* | | | |
| Baart 2012 | Number of whole blood donations in past two years | | |
| | Continuous | n/r | 1.14 (1.12-1.15)/0.92 (0.9-0.93) |
| Baart 2014 | Number of whole blood donations in past two years | | |
| | Continuous | n/r | 0.97/0.9 |
| Baart 2016 | Number of whole blood donations in past two years | | |
| | Continuous | n/r | 0.97/0.9 |
| Cable 2012 | Number of whole blood donations in past two years | | |
| | 1-3 | 12.26 | 1 |
| | 4-6 | 11.23 | 0.9 (0.7-1.3) |
| | 7-9 | 9.07 | 1 (0.8-1.3) |
| | 10+ | 5.17 | 1 (0.9-1.4) |
| Custer et al 2012 and Mast et al 2010 | Number of whole blood donations during previous 12 months | | |
| | 0 | 1·3/18·2 | 1 |
| | 1 | 1·1/17·5 | 0·72 (0·67–0·77)/0·99 (0·98–1·01) |
| | 2 | 1·5/18·1 | 0·84 (0·79–0·90)/1·07 (1·05–1·09) |
| | 3 | 2·1/18·1 | 1·04 (0·97–1·11)/1·09 (1·06–1·12) |
| | 4 | 2·3/16·4 | 1·08 (1·01–1·15)/0·97 (0·94–1·00) |
| | 5 | 2·6/13·4 | 1·07 (0·99–1·16)/0·76 (0·73–0·80) |
| | ≥6 | 2·8/8·8 | 1·00 (0·89–1·13)/0·45 (0·40–0·51) |
| De Kort 2019 | Number of whole blood donations in past two years | | |
| | Continuous | n/r | 0.025/0.015 |
| Spencer 2016 | Number of whole blood donations in past two years | | |
| | 0 | n/r | 1 |
| | 1-3 | n/r | 1.02 |
| | 4-6 | n/r | 1 |
| | 7-9 | n/r | 0.83 |
| | 10+ | n/r | 0.59 |
| Zanella et al 1989 | Annual Rate of Donation | | |
| | M: <2/year | M: trend is to fall over the 16 donations. | |
| | F: <1.5/year | F: trend is to rise over the 18 donations. | |
| | M: 2–3/year | M: trend is to fall over 23 donations (slight rise in 18-21 year olds). | |
| | F: 1·5–2·5/year | F: stable over 11 donations then varies. | |
| | M: >3/year | M: falls over the 22 donations. | |
| | F: >2·5/year | F: falls to 12 donations then rises. | |
| *Inter-donation Interval* | | | |
| Baart 2014 | Time since previous donation per month smaller than one year | | |
| | Continuous | n/r | 0.89/0.9 |
| Baart 2016 | Time since previous donation per month smaller than one year | | |
| | Continuous | n/r | 0.87/1.11 |
| Custer 2014 | Time since previous donation in weeks | | |
| | 8-16 | n/r | 1.5 (1.4-1.6) |
| | 16-20 | n/r | 1.2 (1.05-1.3) |
| | 20-24 | n/r | 1.2 (1.1-1.3) |
| | 24-36 | n/r | 1 |
| | 36-52 | n/r | 0.8 (0.7-0.9) |
| | 52+ | n/r | 0.7 (0.7-0.8) |
| Muon 2018 | Interdonation Interval | | |
| | <3 months | 4.4/40.4 | 3.58 (3.22-3.99)/8.48 (7.95-9.06) |
| | 3-4 months | 1.8/13 | 1.39 (1.26-1.54)/1.87 (1.76-1.99) |
| | 4-5 months | 1.6/9.9 | 1.23 (1.12-1.36)/1.38 (1.32-1.43) |
| | 5-6 months | 1.5/8.9 | 1.16 (1.07-1.25)/1.22 (1.18-1.26) |
| | ≥6 months | 1.3/7.4 | 1/1 |
| Van den Berg 2019 | Interdonation Interval | | |
| | ≤3 months | 1.98/13.95 | 1.18 (0.52-2.69)/0.94 (0.63-1.41) |
| | 3 to 6 months | 2.37/16.88 | 1.41 (0.65-3.05)/1.14 (0.83-1.56) |
| | >6 months | 1.68/14.78 | 1/1 |
| Zeimann et al 2006 | Interdonation Interval | | |
| | <6 months | 6·3 | 0·70 (0·56–0·87) |

| | | | |
|---|---|---|---|
| | 6 to 11 months | 6·2 | 0·68 (0·54–0·86) |
| | 12 to 23 months | 7·7 | 0·87 (0·67–1·13) |
| | ≥24 months | 8·8 | 1 |
| *New vs Repeat* | | | |
| Afzal 2016 | New | 1.04/41.99 | 1/1 |
| | Repeat | 4.77/37.82 | 4.58 (3.8-5.52)/0.85 (0.9-0.95) |
| Al Shaer 2017 | New | 14.27 | 1 |
| | Repeat | 6.68 | 0.47 (0.45-0.49) |
| Bakrim 2018 | New | 9.69 | 1 |
| | Repeat | 8.6 | 0.89 (0.79-1) |
| Custer 2004 | New | 0·7/12·6 | 1/1 |
| | Repeat | 0·5/10·3 | 0·69 (0·47–1·03)/0·80 (0·76–0·85) |
| Custer 2012 | New | 8·8 | 1 |
| | Repeat | 8·1 | 0·87 (0·86–0·88) |
| Goncalez 2013 | New | 7·9 | 1 |
| | Repeat | 3·9 | 0·47 (0·47–0·48) |
| Klausa 2013 | New | 38.46 | 1 |
| | Repeat | 39.58 | 1.03 (0.46-2.29) |
| Kouao 2012 | New | 2·6 | 1 |
| | Repeat | 4·0 | 1·61 (1·23–2·11) |
| Ngoma 2013 | New | 14.14 | 1 |
| | Repeat | 7.29 | 0.52 (0.49-0.55) |
| Ngoma 2014 | New | 10.54 | 1 |
| | Repeat | 12.5 | 1.09 (0.99-1.19) |
| Perez 2018 | New | 1/14 | 1/1 |
| | Repeat | 2.76/15.27 | 2.75 (2.6/2.91)/1.09 (1.07/1.11) |
| Van den Berg 2019 | New | 1.55/19.01 | 1/1 |
| | Repeat | 1.98/13.28 | 1.28 (0.31-5.34)/0.65 (0.46-0.93) |
| Wilkinson 1982 | New | 1·2/17·6 | 1/1 |
| | Repeat | 1·0/13·2 | 0·78 (0·75–0·81)/0·71 (0·70–0·72) |

### 2.4.7 Previous Haemoglobin Levels

Six studies reported the relationship between a donor's previous Hb or HCT level and the likelihood of LHD [92-94, 96, 110, 126]. Two European studies [93, 94] applied logistic regression and showed a reduced risk of LHD in donors who were not deferred at their previous visit. A third study [111] found that men whose Hb increased between visits were less likely to be deferred than those whose Hb levels had decreased. In another study from the Netherlands [126] there was a higher risk of LHD for donors whose Hb levels were stable across visits, while donors in classes III and IV (whose initial Hb levels were higher but experienced a sharper decline) had a lower risk of deferral. In a US study [96], donors who had previous Hb levels below the reference group of ≥145 g/L had a higher risk of LHD, while previously deferred donors (Hb <125 g/L) were more likely to be deferred due to low Hb. This relationship held across all three blood donation types (RBC, double RBC and multicomponent). Finally, a Brazilian study [92] compared initial HCT levels with those in the visit immediately before a low HCT

deferral over a maximum 11 years of study and found that women whose initial HCT was <41 were three times more likely to be deferred than those who had initial HCT>43, and men with low initial HCT were almost six times more likely to be deferred. There was a similar relationship when examining the donor's HCT level at their previous visit.

### 2.4.8 Iron Status Interventions

Four interventional studies [127-130] investigated whether interventions to improve a donor's iron status affected their likelihood to be deferred for low Hb.

One Indian study [127] gave deferred donors information on diet, and recommended oral iron supplementation. Of the 68.8% of donors who returned to donate, 85% were successful. A Danish study [128] directed iron supplementation to those that were considered to potentially benefit and saw a significant decrease in the male LHD percentage from 0.92 to 0.55 (p=0.03).

A randomized trial from the USA of iron intervention strategies [129] found that the LHD rate in donors who received daily oral iron (19mg or 38mg) was lower (2.7%) than in those who received placebo (6.1%). Furthermore, the LHD rate in donors who received a ferritin status letter which either recommended they continue donation (Ferritin>26ug/L) or took iron supplementation/delayed donation (Ferritin≤26ug/L) was lower (4.1%) than in those who received a letter with no such information/advice (9.8%).

Another randomized trial from Germany [130] comparing 50mg iron, 20mg food supplement or no iron found a higher rate of LHD in those who did not take iron supplements compared to those who did (OR 5.03, 95%CI 1.52-16.69). A significant effect was found from the combined treatment groups (OR 6.56, 95%CI 2.32-18.53).

### 2.4.9 Donation Setting

Four studies [96, 131-133] compared donor deferral rates at fixed and mobile collection points. A study from the UAE [133] reported LHD rates for fixed centres versus mobile sites of 25.3% and 14.4%

respectively. Meta-analysis of the remaining three studies showed a significant increase in the risk of LHD for those who donated at fixed compared to mobile sites (OR 1.14, 95%CI 1.03-1.27) (**Figure 2.9**).

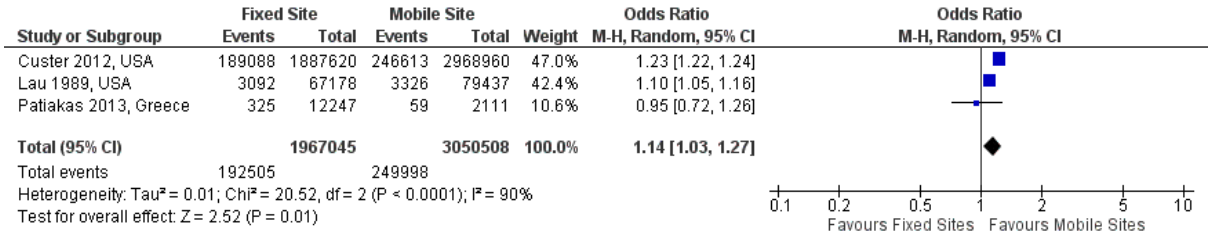



**Figure 2.9**: Meta-analysis of low haemoglobin deferral, fixed versus mobile donation sites. There was a significantly higher deferral rate in fixed sites compared to mobile sites.

Three studies [72, 84, 105] reported deferral by donor centre. The US studies found that in some centres the deferral rates were significantly different, with a maximum adjusted odds ratio of 1.87 [72]. The Japanese study [105] reported deferral numbers by centre and an odds ratio for low haemoglobin deferrals in Miyagi compared with Fukushima was calculated as 1.71 (95% CI 1.65–1.77). Variation in deferral rates by location was also reported in a Brazilian study [120]. In addition, another Brazilian study [134] found that people resident in larger cities were less likely to be accepted for donation ($p<0.0001$).

### 2.4.10 Blood Group

LHD by blood group was analysed in three studies [87, 97, 100]. An African study [100] found a significant association between LHD and AB blood group (AB vs O: OR 4.12, 95%CI 1.81-9.4) but not A or B blood groups. A US study [87] reported ORs by blood group compared to O negative, both before and after changes to the criteria for blood donation in the USA. In both time periods, there was a significantly higher risk of deferral for those in blood group A+, while blood groups AB+ and B+ had a lower risk of deferral. Finally, a Dutch study [97] analysed blood group O- compared to others and did not find a significant difference in deferral rates.

### 2.4.11 Other Factors

There were additional factors that were analysed by only one study in the review. One study [126] found a relationship between donors' haemoglobin recovery time (defined as constant and non-constant) affected the likelihood of low haemoglobin deferral. For both men (OR 6.99 95% CI 5.05-9.67) and women (OR 2.35 95% CI 2-2.76), there was a significant increase in the number of donors with at least one low haemoglobin deferral amongst those who had a non-constant haemoglobin recovery time compared to those with a constant recovery time.

Another study [135] looked at deferral numbers of voluntary and replacement donors and found an odds ratio of 10.2 (95% CI 8.23-12.65) of deferral for replacement donors compared to voluntary donors.

A Dutch study [106] examined the relationship between the time of day and low haemoglobin deferral. It found that more donors who donated in the afternoon and evening were deferred than those who donated in the morning.

A study from Denmark [136] developed a low haemoglobin deferral prediction model including genetic data, and found that in 6 SNPs, for each additional copy of the C-allele in the HFE rs1799945 a male donor's risk of low haemoglobin deferral increased (OR 1.3 95% CI 1.1-1.76), and for women there was an increase of a smaller magnitude (OR 1.2 95% CI 1.02-1.41). As well as this, each additional G allele in HFE rs1800562 increased the risk of deferral for male donors by 50% (OR 1.52 95% CI 1.07-2.17), and the T-allele TMPRSS6 increased the risk of deferral in female donors (OR 1.21 95% CI 1.08-1.35).

Other factors which were investigated but not found to be significantly associated with LHD included smoking [84], diet [122], and Rh-positive blood group [100].

### 2.4.12 Publication Bias

As there was strong evidence of heterogeneity in the odds of low Hb deferral in females as compared to males by geographic regions, with studies in Europe generally showing smaller associations than other regions (**Figure 2.4**), region-specific funnel plots were used to further assess potential publication bias. There was little overall evidence of publication bias within each geographic region (**Figure 2.10**).

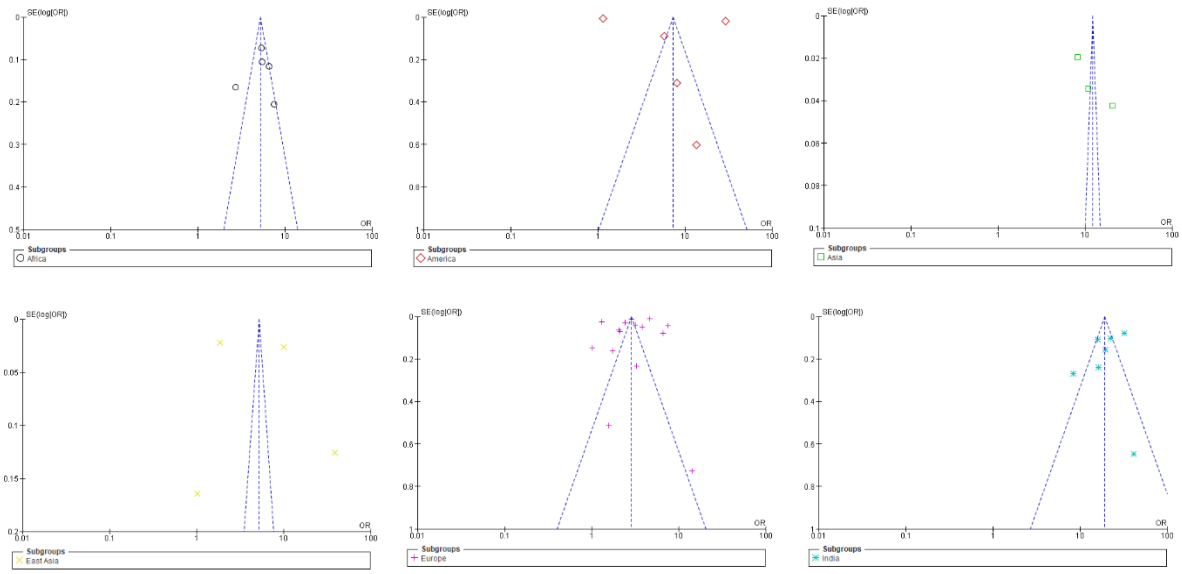



**Figure 2.10**: Funnel plots of studies used in meta-analysis stratified by study setting to highlight the geographical differences in effect size. While there are differences, there is no evidence of publication bias by region.

## 2.5 Discussion

This review has identified 116 published studies that investigated the association of various variables with deferral of donors from donation due to failure to meet minimum Hb thresholds. Random effects meta-analyses and qualitative syntheses of results have shown that female sex, ethnicity, age, weight, seasonality, donation intensity, iron interventions and historical Hb levels can all affect a donor's risk of being deferred for low Hb.

All blood donor deferrals have cost and healthcare implications and have a negative effect on donor motivation to donate in the future. If blood collection services can reduce deferral rates, especially

those due to low Hb, they may maximize donor return and reduce costs. It is well known that clear differences exist in the rates of deferral of donors due to low Hb between men and women, with women significantly more likely to be deferred than men. Our meta-analysis confirms that clear differences exist in deferral rates between male and female donors irrespective of whether minimum Hb thresholds for donation are defined universally for male and female donors, or whether sex-specific thresholds are used. Reasons for these differences in deferral rates between males and females have been suggested to include both physiological and social causes. There are also differences in deferral rates by sex among geographical regions. The odds ratio for deferral of females compared to males was highest in developing countries, which could reflect differences in healthcare systems, practices, or guidance afforded to blood donors.

Premenopausal female donors have lower iron stores than post-menopausal resulting from the effects of menstruation and pregnancy [137]. In contrast, men have increased testosterone levels which are associated with higher Hb levels [138]. In most populations, males are also more likely to be cigarette smokers [139] and cigarette smoking increases carboxyhaemoglobin resulting in increased Hb levels [140]. The need for different Hb donation thresholds for male and female donors therefore is clearly warranted. Indeed, the US Food and Drug Administration recently changed the Hb threshold for donation from a universal threshold of 125 g/L to sex-specific thresholds of 130 g/L for men and 125 g/L for women [141]. However, sex-specific Hb thresholds only provide a benchmark and cannot account for the many other factors may be differentially associated with LHD between male and female donors. The associations of other factors therefore can only reliably be evaluated by stratification of donors by sex. However, the lack of consistent reporting within individual studies prohibits accurate quantification of the magnitude of sex-specific associations. Separate analyses for male and female donors are clearly needed in future studies, as is controlling for other risk factors, for example by adjustment or stratification in multivariable analyses.

### 2.5.1 Ethnicity and sex as major risk factors for Low Hb Deferral

The association of ethnicity and low Hb deferral has been described previously [72, 84-90]. Our results confirm that both male and female African-American donors are at higher risk of LHD than White donors, although the differential risk of deferral appears to be higher in female than male donors. Female donors of either Asian or Hispanic ethnicity are also at higher risk of LHD than their white counterparts, although the evidence in male Asian or Hispanic donors is inconclusive. The low haemoglobin deferral rates observed across different populations show wide variation (**Appendix B**). Although these absolute rates are not directly comparable due to the different Hb thresholds applied and other differences in eligibility criteria, the LHD rates in female donors compared to males in studies that use identical Hb thresholds show wide variation according to the country of study, again suggesting that a donor's ethnicity may affect their ability to meet minimum Hb thresholds for donation. Differences in Hb levels between Black and White populations are well established [142]. Different minimum Hb thresholds for male and female donors according to ethnic origin have also been suggested [143].

### 2.5.2 Factors Affecting Low Hb Deferrals

Genetic factors may explain some of the differences between donors of different ethnic groups in their susceptibility to LHD, although few studies have investigated the effect of genotypes on LHD. Different Hb genotypes which occur at varying frequencies in different populations may be associated with lower Hb levels, for example α-thalassemia traits that occur at high frequencies of 20% or more in some populations [144]. Polymorphisms in the HFE and TMPRSS6 genes have been shown to be associated with higher and lower Hb levels [136] and ethnic variation in the frequency of mutations in both genes has been identified [145, 146]. Ethnic variation may also arise from nutritional differences. For example, a vegetarian diet has been suggested to occur more often in the Indian population [147] with particularly high frequencies in certain religious groups. The European Vegetarian Union have reported vastly different proportions of vegetarians in different countries [148]. The effect of blood

group on LHD is inconclusive with conflicting results obtained from three trials. However, analysis of low Hb deferral by blood group is confounded by different frequency of donation as many blood services recall donors with RH negative groups and O and A ABO groups more often than RH positive and A and AB ABO blood group donors. In addition, the study populations were diverse and so may not be directly comparable.

### 2.5.3 Age

There is considerable evidence that increasing age is associated with a higher risk of LHD deferrals in males. Some explanation can be gained from the decreasing testosterone levels and thus reduced Hb levels with age [138, 149, 150]. The effect of age of LHD in women is less clear, with some studies reporting high LHD rates in younger women whilst others found an association between LHD and age. This is likely explained by the combined effects of menstruation and pregnancy in younger women alongside menopausal effects in older women. A large population-based US study found a pronounced increase in the prevalence of anaemia with increasing age and suggested that key causes for this were likely to be nutritional deficiencies and chronic disease [149].

### 2.5.4 Seasonality

The increased risk of LHD observed in warmer months observed in several studies may be attributed to transient haemodilution as blood flow to the skin increases as an element of the heat balance mechanism [151]. Other indirect factors influencing Hb level have been proposed, including nutrition, physical activity and viral infections [109]. It may be that a change in minimum Hb thresholds for donation according to ambient temperature could be possible, but a detailed understanding of the degree of change in Hb associated with different seasons/temperatures and the relationship between these changes and iron stores would be required before this can be considered further.

### 2.5.5 Weight

A lower risk of LHD has also been observed with increasing body weight [72, 84, 85, 109]. It has been suggested that heavier individuals might be expected to have a greater absolute blood volume and so would donate proportionally less of their total iron stores than a lighter person, with a lesser impact of loss of iron through donation [85]. However, the effect of weight on LHD is likely to be confounded by gender and age as well as lower iron stores as BMI increases [74] which may explain some inconsistency in the findings of individual studies.

### 2.5.6 Donation Characteristics

Donation characteristics associated with LHD include previous donation [87, 95, 96, 104, 105, 113, 117-125], frequency of donations [92-94, 96, 112, 114-116], and previous deferral due to low Hb [93, 94] although results are conflicting and apparent differences exist between male and female donors. Evaluation of the association of donation characteristics with low Hb deferral is difficult as there may be confounding related to selection biases related to donation frequency and an opposite consequence of lowering of iron stores with repeated donation. Shorter inter-donation intervals have been associated with higher frequency of iron deficiency, lower Hb, and higher rates of deferral [72, 152, 153]. As males have typically two to four times greater iron stores than females [137], it would be expected that males are better suited to high intensity donation than women. The historical 8-week inter-donation interval used in the USA has recently been brought into question. An AABB Association Bulletin recommended that consideration should be given to increasing inter-donation intervals in some circumstances, in particular for young and/or female donors, in order to reduce iron deficiency in blood donors [154], although no formal change has been implemented [141]. INTERVAL randomized donors to 8, 10, or 12 week (men) or 12, 14, or 16 week (women) inter-donation intervals over two years found that although shorter inter-donation intervals increased the risk of LHD, shorter inter-donation intervals were associated with a higher mean number of successful donations in both men and women over the trial duration [74].

### 2.5.7 Confounding

Interpretation of the factors that determine LHD in relation to age, donation frequency and total number of units donated is confounded by many differences that may exist between the study populations selected. For example, donors who have given blood at higher than average frequencies over many years are a selected population and may have; genetic or environmental factors that predispose to high iron stores and/or rapid loading of iron; and/or learnt behaviour to maintain donations at higher than average frequency without breaching the haemoglobin threshold; and/or taken over-the-counter iron supplementation. These confounding factors add to the difficulty of comparing and combining studies of low haemoglobin deferral and suggest that large-scale studies incorporating measurement and analysis of these factors may be required for each donor population.

### 2.5.8 Iron Supplementation

Oral iron supplementation has been shown to reduce the risk of LHD, elevating Hb and iron stores in blood donors [155]. However, the prospect of implementing iron supplementation in blood donors is a matter of some controversy [156, 157]. Depleted iron stores are more common in female and young donors as well as those who donate regularly [158], and it has been suggested that low iron is associated with cognitive impairment [159], although there was no evidence of any cognitive impairment in the INTERVAL study across randomised groups donating a different intervals and with different proportions of donors with iron deficiency [74]. Targeted iron supplementation in these high risk groups has been recommended [160]. However, this comes with cost implications, associated adverse effects and compliance issues as well as possible health risks associated with regular iron intake [157]. Alternative strategies have been suggested [160]. These include extending inter-donation intervals, limiting donations in young donors (as implemented in Australia where the minimum age for donation has been increased from 16 to 18 years [161], and introducing serum ferritin testing as part of donor eligibility to donate in order to identify those individuals with low iron stores, as implemented in donors aged 16-18 years by Vitalant and the American Red Cross in the US

[162, 163]. It remains to be seen which approach can best balance the health of donors against maintaining adequate blood supplies. This will be further investigated in **Chapter 5**.

### 2.5.9 Limitations of the Analysis

This analysis has a number of key limitations. Firstly, the association estimates presented in this review are unadjusted for potential confounding factors. The random-effects meta-analyses results are therefore only average estimates and should be interpreted with caution. Clearly, individually-tailored inter-donation criteria will be most effective when considering a number of characteristics simultaneously. A number of studies have performed multivariable analysis and report results adjusted for a range of potential confounding factors including age, sex, ethnicity, weight, season, ambient temperature, blood group, inter-donation interval, number of prior donations and use of iron supplementation [72, 74, 84, 85, 87, 93, 97, 108, 109, 136, 164]. However, the disparity in characteristics adjusted for between these individual studies precluded meta-analysis of these adjusted results. A more flexible approach might be gained through meta-regression, allowing individual study characteristics to be included as covariates, thus assessing the effect of study level characteristics on effect size estimates. Adjustment for confounding factors at study level can be incorporated into the regression model, thus allowing interpretation of the extent to which such factors affect the effect size. However, the most powerful approach is the large trial which measures a wide range of factors to be evaluated in a multivariate analysis approach. The factors identified in this review provide a basis for the design of such trials, giving due attention given to gender, age and ethnicity in particular.

Secondly, a high degree of heterogeneity exists across studies and care should be taken over the interpretation and reporting of summary estimates given this high heterogeneity. Key sources of heterogeneity include minimum Hb donation thresholds and differences in the ratio of male to female donors in individual studies. The determination of Hb levels in prospective donors included a variety of methods which included both venous and capillary blood measurements, leading to varying levels

of imprecision and bias across studies. Thirdly, the differential number of donations included in each study, as well as donation and deferral history, is likely to introduce further heterogeneity in the likelihood of deferral at the point of study. Multiple donation attempts, whether successful or unsuccessful, could lead to differences in the underlying distribution of Hb levels, and thus the risk of LHD. Moreover, it is possible that there is a variation in the amount of blood collected in one donation session between countries. Finally, despite the clear evidence for differences in the risk of LHD between male and female donors, many studies reported results for male and female donors combined, limiting the ability to assess the sex-specific effect of other factors.

### 2.5.10 Personalised Eligibility Criteria

Given the vast number of characteristics which appear to affect a donor's ability to meet Hb thresholds, individually tailored donation criteria may help improve the retention of donors and increase blood supply overall. However, tailoring inter-donation frequency will require the development of sophisticated mathematical models in prospective studies and this could be an important area for donor research in the future. We suggest that future prospective studies of LHD should incorporate the factors identified in this study, and appropriate statistical modelling methods should be used. This may lead to the identification of specific sub-groups of prospective donors which can then be subject to tailored donation criteria and/or iron supplementation.

### 2.5.11 Conclusions

This systematic review has highlighted a number of factors which affect a donor's ability to meet minimum Hb donation thresholds. A donor's sex, age, ethnicity, weight, donation history and inter-donation interval as well as ambient temperature and donation setting affect the risk of LHD. Other potential factors which may influence LHD include diet, smoking, blood group and genetic factors but further evidence is required. In conclusion, large prospective studies are needed, with an emphasis on collecting a wide spectrum of data on participant demographics, ethnicity and donation characteristics and using appropriate statistical models to establish the combined effect of these multiple factors on

LHD in blood donors. Further analyses of these factors, including an assessment of the combined effect of multiple factors on LHD will be conducted in **Chapter 4**.

# Chapter 3 - Data Sources

## Summary

The primary data source for the analyses in this thesis was the INTERVAL trial [7, 73, 74], a large multi-centre trial which randomised 45,267 blood donors to different inter-donation intervals for two years at one of the 25 static blood donation centres in the UK. My aim is to identify characteristics of donors able to safely give blood more frequently than current NHS guidelines.

Data were collected from participants via self-reported questionnaires at baseline and every six months for two years, and haemotology analysis of blood samples provided at baseline and after two years of follow-up. Additional data were obtained from the PULSE database of all blood donors registered with NHSBT.

This chapter describes the INTERVAL trial's design and methodology, as well as the data that were collected and used in my subsequent analyses. The INTERVAL data were used to examine associations between donor characteristics (demographics, dietary and lifestyle factors, and biomarkers) and several outcomes including the number of blood donations, low haemoglobin deferrals, and haemoglobin and ferritin levels. Randomisation of donors to different inter-donation intervals throughout the trial enabled the assessment of the effect of inter-donation interval on these outcomes including identifying characteristics of donors who may be able to safely donate more frequently than current NHSBT guidelines without compromising their wellbeing, as well as an assessment of the effects of increased donation frequency on post-donation symptoms.

## 3.1 The INTERVAL Trial

INTERVAL was a large, randomised trial that assigned men and women to donate at various inter-donation intervals – some being more frequently than current NHS guidelines, with a control group donating at the current inter-donation intervals. The shorter donation intervals trialled were 8 and 10 weeks for men, and 12 and 14 weeks for women. The trial aimed to maximise the blood supply and maintain donor wellbeing by determining optimal inter-donation intervals, and to explore how donor characteristics such as demographics, lifestyle traits, and genetics may be useful in the assignment of an optimal inter-donation interval [7].

The trial ran for two years, between 2014 and 2016, and randomised 45,263 donors (22,466 men and 22,797 women) to donate blood at one of the donation intervals in a 1:1:1 split between donation groups. Randomisation was designed to balance key donor characteristics such as age, weight, and new donor status. Donors donated in one of the 25 fixed donation centres in England (UK) during the trial period.

Participants were recruited initially by post, with invitations sent only to those registered at one of the fixed blood donation centres. However additional recruitment strategies were adopted in 2013 to include those donating in mobile centres who had expressed a willingness to give platelets at a static donor centre, lived within ten miles of one, and later who lived within 30 miles, as it was thought that these sets of people may be more willing to attend a fixed donation centre for the trial despite not using one at the time of recruitment. Information was also given to donors at blood donation appointments.

Inclusion criteria for the trial were that participants had to be at least 18 years old, fulfil normal criteria for blood donation, be willing to be allocated any of the three possible donation intervals, and attend a static donor centre for the duration of the trial. The main exclusion criteria were related to data collection, namely that those without a stable internet connection, or those unwilling or unable to provide a contact e-mail address, could not participate as questionnaires sent via e-mail were a key

method of data collection [7]. A flowchart of the recruitment process can be seen in **Figure 3.1**. Once recruited, donors were randomised into one of three inter-donation groups using a minimisation algorithm to balance key characteristics at baseline between the inter-donation interval groups, including weight, age, and new/repeat donor status [7]. This was stratified by donation centre and gender and attempts to minimise confounding due to key variables on the observed effect of inter-donation interval.

Data quality control (QC) in the trial was ensured through the Independent Data Monitoring committee, the Research Ethics Committee, Trial Steering Committee, and the Trial Management Group. The trial data manager and statistician oversaw the daily accrual of data and periodically reported to the preceding trial management groups. Furthermore, of relevance to the current work were QC conducted on Sysmex haematology measurements to monitor and correct for any drift in analysers, which involved a team of lab technicians, statisticians, and haematology experts. The INTERVAL trial sample size calculations aimed to have 80% power to detect a 5% difference in the number of donations given over two years, and a 3% difference or more change in quality of life measurements [7].

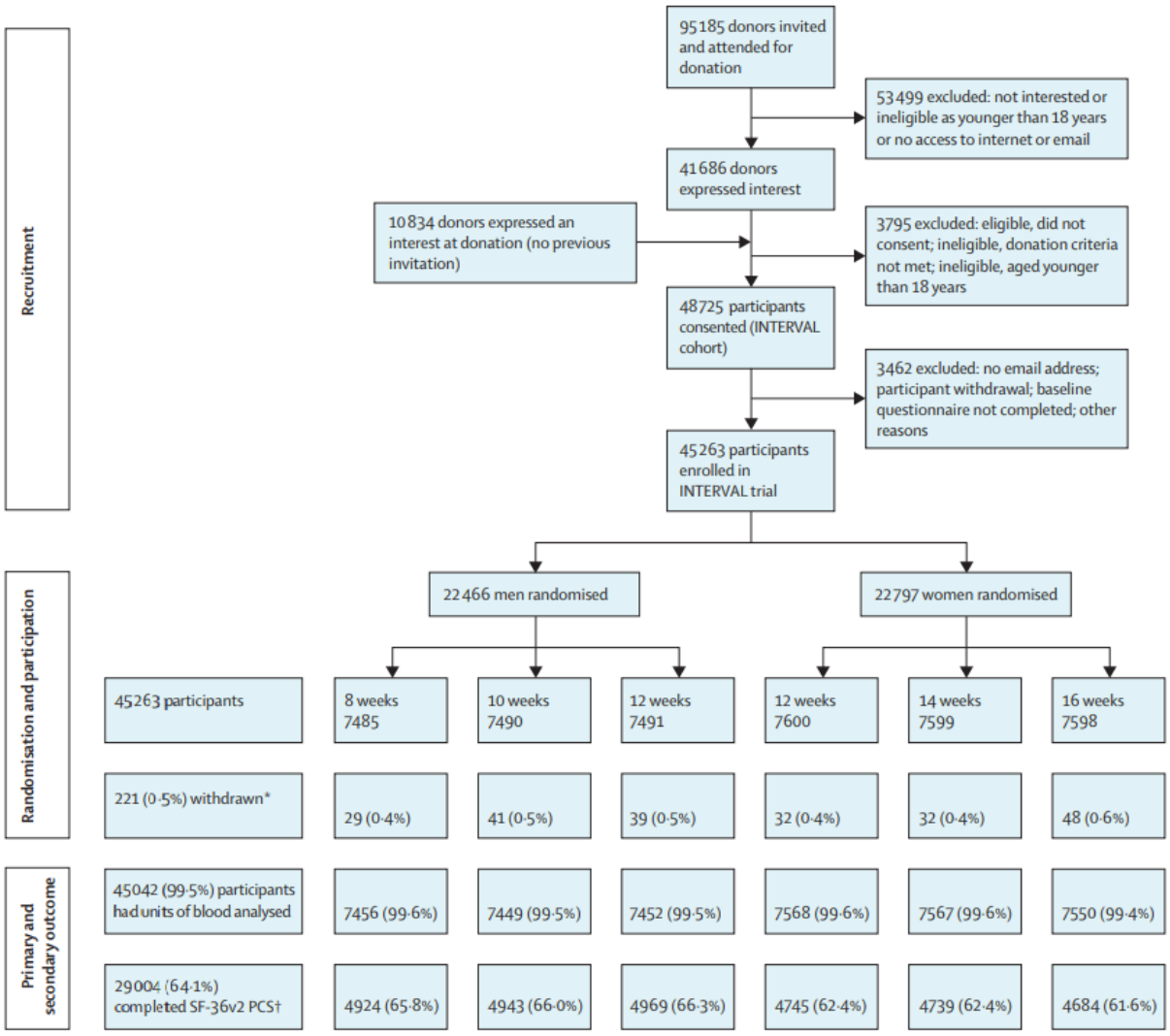



**Figure 3.1**: INTERVAL Recruitment flowchart, taken from [74]. Roughly 50% of those invited to participate in the trial enrolled, and completeness of data was not differential by inter-donation interval group.

## 3.2 The INTERVAL Trial Participants

Compared to the general NHSBT population, which consists of all donors who give blood in England and Wales, the INTERVAL trial sample had a higher proportion of men than the general donor population (50% vs 44%), and the sample were older in age (mean age 43.1 versus 42.3 years). There were also differences in past donation history, with the INTERVAL sample both having a longer history of donation (mean of 10.7 years vs 8.6 years) and a higher number of donations given in the previous two years (mean of 3.2 donations vs 2.1 donations) than the general NHSBT donor population. There was also a lower proportion of new donors in INTERVAL (3% vs 22%). Aside from these differences,

there was broad similarity between the INTERVAL donors and the general donor population, so results should be generalisable to the NHSBT donor population. However, it is possible that the effect of different inter-donation intervals on donation rates could be greater in INTERVAL than the general population of blood donors, owing both to the trial setting and the fact that the donors in the trial were already a more committed group of donors. On the other hand, as NHSBT aims to have a greater proportion of donations made at static donor centres by 2020/21 (rising from 15% to 25%), it is possible that the INTERVAL cohort may be more representative of future NHSBT blood donor populations [73].

## 3.3 Data Collection in INTERVAL

### 3.3.1 Self-Reported Data Collection in INTERVAL

Information on INTERVAL participants was mainly collected with self-reported questionnaires. Participants were e-mailed the baseline questionnaire a few days after enrolment, which contained the following sections:

1. Compulsory questions on month and year of birth, sex, height, and weight, to verify that the intended person was the one who completed the questionnaire.
2. Well-being assessments using the SF-36v2 questionnaire. This comprises 36 questions that are used to generate summary scores of a participant's physical and mental well-being. It has been reported to differentiate well between health benefits produced my treatments in trials, regardless of age, disease condition, and treatment group [165-168].
3. History of iron deficiency such as whether the participant had ever been diagnosed with anaemia by a doctor.
4. Lifestyle information including diet, alcohol and smoking status, and physical activity.

Participants were also asked to complete follow-up questionnaires every six months during the trial. These questionnaires asked the following:

1. Compulsory questions on month and year of birth, sex, height, and weight, to verify that the intended person was the one who completed the questionnaire.
2. Well-being assessments using the abbreviated Sf-12v2. This was chosen over the full Sf-36v2 because it takes just five minutes to complete, reducing the time burden on participants.
3. Reporting of adverse events and serious adverse events. These included heart problems, falls, accidents when driving, new illnesses, diagnoses of low iron, low haemoglobin, and prescriptions of iron supplements, as well as nine post-donation symptoms: tiredness, dizziness, feeling faint, fainting, fit or seizure, breathlessness, palpitations, chest pain, and restless leg syndrome [169].

A final questionnaire was sent to participants upon their two-year anniversary of involvement in the trial (and thus completion). This was a longer questionnaire which asked:

1. Month and year of birth, and sex, to validate the person completing the questionnaire was the participant
2. The Sf-36v2 questionnaire
3. Reporting of adverse events and serious adverse events as above, with additions – severity of breathlessness, palpitations and chest pain, headaches, sleep disturbances, irritability, concentration, restlessness, and pica.
4. Medication and supplement use such as glucose-lowering, antihypertensive, and lipid-lowering supplements, as well as over the counter dietary supplements, with an additional question as to whether these contained iron.
5. Cognitive function tests, designed to measure a range of attributes: attention and reaction times (using Stroop Test), executive function (using Trail Making Test), episodic memory (using Pairs Test), and intelligence (using Reasoning Test). These are a series of tests designed for cognitive testing in an epidemiological setting [170], and also contain a mood questionnaire to adjust for effects that may skew these tests [171, 172].

6. Beliefs on blood donation, and particularly questions asking if increased donation frequency raised difficulties or concerns.
7. An invitation to take part in physical activity monitoring
8. Recent physical activity questionnaire which measures activity in the past four weeks across a participant's leisure time, occupation, commuting, and domestic life [173, 174].

### 3.3.2 The PULSE database

An additional source of data collection in INTERVAL is the PULSE database. PULSE is an electronic database that covers all aspects of blood donation and donor management, with codes added to donor records describing the type of donation they gave, and communications sent to donors. Special codes were added to the records of INTERVAL donors in PULSE during the trial to facilitate identification and exchange of information with the trial academic co-ordinating centre. The PULSE database includes information on donors' sex, age, ethnicity, ABO and Rhesus D blood groups, and information on donation history from donors which were used in INTERVAL analyses. These data were confidentially retrieved by NHSBT the day after donors enrolled into INTERVAL and were securely transferred to the academic coordinating centre.

### 3.3.3 Biomarker Data Collection in INTERVAL

Research blood samples were collected from donors at their baseline donation visit. A full blood count was performed on these using a SYSMEX XN-2000 haemotology analyser (Sysmex UK Limited, Milton Keynes, UK). Indices obtained included haemoglobin levels, red and white blood cell counts, and mean corpuscular volume and haemoglobin. In addition, one of the research samples collected was used to create buffy coat, from which DNA was extracted using a Kleargene method (LGC Genomics, Teddington UK). Ferritin concentrations were also measured using an immunoturbidimetric assay (Roche/Hitachi chemistry analyser, Stichting Huisartsen Laboratorium, Etten-Leur, Netherlands). This research blood sample collection was then repeated during a participant's final donation before their two-year involvement in the trial.

## 3.4 INTERVAL Primary and Secondary Outcomes

### 3.4.1 Primary Outcome of the INTERVAL Trial

The primary outcome of the trial was the number of blood donations given during the two years of the trial. This was defined as donating one full unit of blood of 470 mL [74].

### 3.4.2 Secondary Outcomes of the INTERVAL Trial

#### 3.4.2.1 Low Haemoglobin Deferrals

Before donating blood, all donors must pass a haemoglobin test, to both protect them from iron deficiency and associated consequences, and to ensure that any blood collected is fit for purpose. In the UK, haemoglobin cut-off points are 135 g/L for men and 125g/L for women. At the time of the INTERVAL trial, haemoglobin was measured using the copper sulphate test only. The exception to this was during the donor's baseline donation visit. On this occasion, if a donor failed the copper sulphate test they had their haemoglobin concentration measured using a Hemocue test [7].

#### 3.4.2.2 Haemoglobin and Ferritin Concentrations

Haemoglobin and ferritin levels in donors were measured at their two year involvement in the trial.

#### 3.4.2.3. Self-reported symptoms

Every six months during the trial, and upon the trial's completion, participants were asked if they had experienced the following symptoms: being more tired than usual, feeling more breathless than usual, palpitations, dizziness, chest pain, fainting or feeling faint, restless legs syndrome, and pica (the desire to eat non-nutritious foods, usually ice) [74].

#### 3.4.2.4 PCS and MCS Quality of Life Measures

The trial's key secondary outcomes included well-being (quality of life) measures, in particular the physical and mental component scores of the Sf-36 questionnaire (PCS and MCS). These are summary scores taken from either the Sf-36 or Sf-12 questionnaire, and are used to measure the person's well-being. They give a numerical value, with higher indicating a better score, as a measure of the

participant's physical and mental well-being. The PCS and MCS are reported on a population-norm standardised scale, with mean 50, standard deviation 10, transformed from a raw score of 0-100 [175]. This has the advantage that the mean and standard deviation of these scores is always known and so they are easier to interpret.

The scores are constructed from the answers to questions in the questionnaire which fall under multi-item dimensions of health: physical functioning (10 items) social functioning (2 items) role limitations due to physical problems (4 items), role limitations due to emotional problems (3 items), mental health (5 items), energy/vitality (4 items), pain (2 items), and general health perception (5 items) [176]. There is also a question on general health assessment ("Compared to one year ago, how would you rate your health in general now?") [177]. The PCS and MCS are then derived from these results [176]. Studies have shown that there is a gender difference in PCS and MCS scores, with women having lower scores than men in all aspects of the questionnaire aside from self-reported health [176, 177]. There was also found to be a difference in scores by age [177].

## 3.5 INTERVAL Results

### 3.5.1 Descriptive Statistics of Key Baseline Characteristics

Baseline variables had similar distributions and levels of missingness across all randomised groups in both sexes. A much higher proportion of women than men indicated a diagnosis of anaemia (24% vs 4%), and women had lower haemoglobin and ferritin levels than men. Dietary variables were comparable between sexes, as were the baseline PCS and MCS scores. Women were more likely to have had previous low haemoglobin deferrals and had donated less than men in the past two years (**Tables 3.1 and 3.2**).

**Table 3.1**: Summary of the key baseline variables for men by trial arm, presented as mean (standard deviation) or frequency (%), and level of missingness

| | Trial arm: 8 weeks N = 7456 | | Trial arm: 10 weeks N = 7446 | | Trial arm: 12 weeks N =7452 | |
|---|---|---|---|---|---|---|
| **Variable** | **Mean (SD) or N (%)** | **Missing (%)** | **Mean (SD) or N (%)** | **Missing (%)** | **Mean (SD) or N (%)** | **Missing (%)** |
| **Demographics** | | | | | | |
| Height (m) | 1.79 (0.08) | 0 | 1.79 (0.079) | 0 | 1.79 (0.08) | 0 |
| Weight (kg) | 85.2 (16.42) | 0 | 85.16 (16.67) | 0 | 85.15 (16.47) | 0 |
| Ethnicity – White British (Y/N)[1] | 5840 (78%) | 902 (12) | 5861 (79%) | 910 (12) | 5819 (78%) | 901 (12) |
| Age (years approx.) | 45.22 (14.14) | 92 (1) | 45.24 (14.22) | 96 (1) | 45.22 (14.19) | 108 (1) |
| PCS | 56.79 (4.61) | 58 (1) | 56.89 (4.54) | 52 (1) | 56.84 (4.51) | 39 (1) |
| MCS | 54.62 (6.02) | 58 (1) | 54.48 (6.26) | 51 (1) | 54.53 (6.07) | 36 (1) |
| **Iron Status** | | | | | | |
| Anaemia Ever (Y/N) | 319 (4%) | 111 (1) | 325 (4%) | 105 (1) | 327 (4%) | 124 (2) |
| Iron Prescription Use (Y/N) | 8 (<1%) | 109 (1) | 13 (<1%) | 117 (2) | 27 (<1%) | 117 (2) |
| Iron Multivitamin Use (Y/N) | 922 (12%) | 118 (2) | 937 (13%) | 120 (2) | 950 (13%) | 105 (1) |
| Iron Supplement Use (Y/N) | 53 (1%) | 144 (2) | 79 (1%) | 140 (2) | 70 (1%) | 153 (2) |
| **Diet/Lifestyle** | | | | | | |
| Non-Vegetarian (Y/N) | 7177 (96%) | 49 (1) | 7167 (96%) | 46 (1) | 7148 (96) | 49 (1) |
| Liver (Portions per Week) | 0.19 (0.72) | 158 (2) | 0.2 (0.93) | 162 (2) | 0.19 (0.81) | 173 (2) |
| Red Meat (Portions per Week) | 2.59 (1.84) | 69 (1) | 2.58 (1.82) | 67 (1) | 2.58 (1.88) | 73 (1) |
| Poultry (Portions per Week) | 2.43 (1.62) | 76 (1) | 2.46 (1.64) | 73 (1) | 2.42 (1.62) | 78 (1) |
| White Fish (Portions per Week) | 1.19 (1.07) | 94 (1) | 1.21 (1.06) | 83 (1) | 1.22 (1.09) | 92 (1) |
| Oily Fish (Portions per Week) | 0.81 (1.03) | 123 (2) | 0.82 (1.14) | 106 (1) | 0.81 (1.02) | 119 (2) |
| Vegetable Consumption (Y/N) | 7216 (97%) | 64 (1) | 7225 (97%) | 58 (1) | 7197 (97%) | 63 (1) |
| Fruit Consumption (Y/N) | 6560 (88%) | 82 (1) | 6542 (88%) | 72 (1) | 6541 (88%) | 81 (1) |
| Juice Consumption (Y/N) | 4723 (63%) | 117 (2) | 4706 (63%) | 88 (1) | 4771 (64%) | 108 (1) |
| Smoothie Consumption (Y/N) | 989 (13%) | 172 (2) | 922 (12%) | 152 (2) | 1012 (14%) | 154 (2) |
| Tea Consumption (Y/N) | 5664 (76%) | 65 (1) | 5570 (75%) | 70 (1) | 5552 (75%) | 65 (1) |
| Alcohol Ever (Y/N) | 7214 (97%) | 54 (1) | 7211 (97%) | 48 (1) | 7208 (97%) | 61 (1) |
| Smoking Ever (Y/N) | 3100 (41%) | 96 (1) | 3065 (41%) | 90 (1) | 3173 (43%) | 95 (1) |
| **Donation History** | | | | | | |
| Donations in Past 2 Years (n) | 3.58 (1.87) | 0 | 3.59 (1.86%) | 0 | 3.57 (1.85) | 0 |
| Low Hb Deferral Rate in Past 2 Years | 1.09 | 0 | 1.03 | 0 | 1.03 | 0 |
| Other Deferrals in Past 2 Years (n) | 0.32 (0.69) | 0 | 0.32 (0.68) | 0 | 0.32 (0.69) | 0 |
| Under-Donations in Past 2 Years (n) | 0.045 (0.22) | 0 | 0.048 (0.23) | 0 | 0.043 (0.22) | 0 |
| **Biomarkers** | | | | | | |
| Ferritin (µg/L) [Median (IQR)] | | 420 (6) | | 423 (6) | | 468 (6) |
| Haemoglobin (g/dL) | 14.98 (1.01) | 118 (2) | 14.96 (0.99) | 131 (2) | 14.98 (0.99) | 131 (2) |
| Mean Corpuscular Haemoglobin (g/dL) | 29.72 (1.71) | 118 (2) | 29.72 (1.68) | 131 (2) | 29.76 (1.79) | 131 (2) |
| Mean Corpuscular Volume (fL) | 92.24 (4.63) | 118 (2) | 92.27 (4.66) | 131 (2) | 92.31 (4.67) | 131 (2) |
| Red Blood Cell Count ($10^{12}$/L) | 5.05 (0.39) | 118 (2) | 5.04 (0.38) | 131 (2) | 5.04 (0.38) | 131 (2) |
| White Blood Cell Count ($10^{9}$/L) | 6.17 (1.68) | 118 (2) | 6.17 (1.53) | 131 (2) | 6.18 (1.52) | 131 (2) |
| Platelet Count ($10^{9}$/L) | 230.02 (56.78) | 118 (2) | 230.96 (57.13) | 131 (2) | 229.99 (57.72) | 131 (2) |

[1] Y/N – indicates yes/no, with the table corresponding to "yes" responses

**Table 3.2**: Summary of the key baseline variables for women by trial arm, presented as mean (standard deviation) or frequency (%), and level of missingness

| | Trial arm: 12 weeks N = 7567 | | Trial arm: 14 weeks N = 7565 | | Trial arm: 16 weeks N =7548 | |
|---|---|---|---|---|---|---|
| **Variable** | **Mean (SD) or N (%)** | **Missing (%)** | **Mean (SD) or N (%)** | **Missing (%)** | **Mean (SD) or N (%)** | **Missing (%)** |
| **Demographics** | | | | | | |
| Height (m) | 1.65 (0.073) | 0 | 1.65 (0.076) | 0 | 1.65 (0.075) | 0 |
| Weight (kg) | 71.33 (14.43) | 0 | 71.94 (18.87) | 0 | 71.83 (14.85) | 0 |
| Ethnicity – White British (Y/N)[1] | 6479 (86%) | 788 (10) | 6474 (86%) | 811 (11) | 6460 (86%) | 790 (11) |
| Age (years approx.) | 41.29 (14.02) | 122 (2) | 41.38 (13.88) | 133 (2) | 41.45 (13.98) | 136 (2) |
| PCS | 57.03 (4.69) | 61 (1) | 57 (4.69) | 52 (1) | 57 (4.61) | 54 (1) |
| MCS | 53.49 (6.68) | 59 (1) | 53.51 (6.61) | 51 (1) | 53.51 (6.53) | 51 (1) |
| Hormone Replacement Therapy | 199 (3%) | 1901 (25) | 236 (3) | 1858 (25) | 245 (3) | 1814 (24) |
| Contraceptive Pill Use | 1668 (22%) | 432 (6) | 1649 (22%) | 448 (6) | 1600 (21%) | 459 (6) |
| Menopause – Yes | 1796 (24%) | 85 (1) | 1786 (24%) | 70 (1) | 1802 (24%) | 68 (1) |
| **Iron Status** | | | | | | |
| Anaemia Ever (Y/N) | 1804 (24%) | 215 (3) | 1813 (24%) | 229 (3) | 1793 (24%) | 223 (3) |
| Iron Prescription Use (Y/N) | 47 (1%) | 208 (3) | 42 (1%) | 156 (2) | 43 (1%) | 204 (3) |
| Iron Multivitamin Use (Y/N) | 1384 (18%) | 140 (2) | 1314 (17%) | 124 (2) | 1352 (18%) | 139 (2) |
| Iron Supplement Use (Y/N) | 211 (3%) | 254 (3) | 191 (3%) | 216 (3) | 201 (3%) | 248 (3) |
| **Diet/Lifestyle** | | | | | | |
| Non-Vegetarian (Y/N) | 7098 (94%) | 69 (1) | 7106 (94%) | 50 (1) | 7081 (94%) | 46 (1) |
| Liver (Portions per Week) | 0.11 (0.58) | 208 (3) | 0.11 (0.64) | 178 (2) | 0.1 (0.63) | 200 (3) |
| Red Meat (Portions per Week) | 1.97 (1.54) | 95 (1) | 1.98 (1.57) | 73 (1) | 1.96 (1.54) | 72 (1) |
| Poultry (Portions per Week) | 2.3 (1.52) | 96 (1) | 2.27 (1.55) | 74 (1) | 2.32 (1.56) | 74 (1) |
| White Fish (Portions per Week) | 1.16 (1.04) | 124 (2) | 1.14 (1.04) | 108 (1) | 1.14 (1.04) | 103 (1) |
| Oily Fish (Portions per Week) | 0.81 (1.05) | 137 (2) | 0.78 (0.97) | 131 (2) | 0.81 (1.01) | 117 (2) |
| Vegetable Consumption (Y/N) | 7373 (97%) | 87 (1) | 7376 (98%) | 62 (1) | 7381 (98%) | 54 (1) |
| Fruit Consumption (Y/N) | 6888 (91%) | 94 (1) | 6887 (91%) | 75 (1) | 6912 (92%) | 73 (1) |
| Juice Consumption (Y/N) | 3922 (52%) | 124 (2) | 3897 (52%) | 97 (1) | 39.35 (52%) | 98 (1) |
| Smoothie Consumption (Y/N) | 1128 (15%) | 161 (2) | 1060 (14%) | 150 (2) | 1094 (14%) | 143 (2) |
| Tea Consumption (Y/N) | 5502 (73%) | 89 (1) | 5540 (73%) | 65 (1) | 5529 (73%) | 61 (1) |
| Alcohol Ever (Y/N) | 7312 (97%) | 72 (1) | 7331 (97%) | 53 (1) | 7327 (97%) | 51 (1) |
| Smoking Ever (Y/N) | 3035 (40%) | 121 (2) | 3070 (41%) | 110 (1) | 2948 (39%) | 99 (1) |
| **Donation History** | | | | | | |
| Donations in Past 2 Years (n) | 2.87 (1.69) | 0 | 2.88 (1.64) | 0 | 2.85 (0.34) | 0 |
| Low Hb Deferral Rate in Past 2 Years | 3.58 | 0 | 3.51 | 0 | 3.7 | 0 |
| Other Deferrals in Past 2 Years (n) | 0.36 (0.68) | 0 | 0.34 (0.68) | 0 | 0.34 (0.68) | 0 |
| Under-Donations in Past 2 Years (n) | 0.052 (0.23) | 0 | 0.051 (0.23) | 0 | 0.056 (0.24) | 0 |
| **Biomarkers** | | | | | | |
| Ferritin (µg/L) [Median (IQR)] | | 505 (7) | | 503 (7) | | 484 (7) |
| Haemoglobin (g/dL) | 13.39 (0.94) | 180 (2) | 13.4 (0.91) | 164 (2) | 13.38 (0.9) | 170 (2) |
| Mean Corpuscular Haemoglobin (g/dL) | 29.38 (1.93) | 180 (2) | 29.38 (1.83) | 164 (2) | 29.35 (1.86) | 170 (2) |
| Mean Corpuscular Volume (fL) | 93.16 (5) | 180 (2) | 93.22 (4.96) | 164 (2) | 93.18 (5.07) | 170 (2) |
| Red Blood Cell Count ($10^{12}$/L) | 4.57 (0.36) | 180 (2) | 4.57 (0.35) | 164 (2) | 4.57 (0.35) | 170 (2) |
| White Blood Cell Count ($10^{9}$/L) | 6.76 (1.68) | 180 (2) | 6.77 (1.69) | 164 (2) | 6.83 (1.75) | 170 (2) |
| Platelet Count ($10^{9}$/L) | 261.48 (66.75) | 180 (2) | 261.86 (66.47) | 164 (2) | 263.93 (67.4) | 170 (2) |

### 3.5.2 Outcome Summary Statistics

The main results from the INTERVAL trial showed that allocation of donors to shorter inter-donation intervals increased units of blood collected during the trial period for both sexes. No difference in quality of life, physical activity, or cognitive function was observed across randomised groups, however those on shorter inter-donation intervals experienced an increase in symptoms and low haemoglobin deferrals, and a decrease in their two year haemoglobin and ferritin levels.

### 3.5.3 Number of Donations and Low Haemoglobin Deferrals

The maximum number of donations possible during two years of follow-up were 12, 10, and 8 for men allocated to the 8, 10, and 12 respectively, and were 6, 7, and 8 donations for women in the 16, 14, and 12 week groups respectively. However, around 2,750 men (12%) of men and 5,000 women (22%) did not return to donate during the trial. Most participants, regardless of sex, received no low haemoglobin deferrals during the trial. (**Figures 3.2 and 3.3**).

Considering these outcomes by randomised trial arm (**Table 3.3**), one can see that the mean number of donations decreased as the randomised inter-donation interval lengthened in both sexes, although this effect was less pronounced in women than in men. The number of low haemoglobin deferrals decreased as the interval was lengthened, with the male 12 week group having a particularly low number, and the two-year haemoglobin and ferritin levels were also higher for donors on the longer intervals. This is likely due to the longer intervals allowing the body more time for recovery before donating again, and cumulatively losing less during the trial than those on shorter inter-donation intervals (**Table 3.3**).

### 3.5.4 Haemoglobin and Ferritin Levels

Men generally had higher 2-year haemoglobin and ferritin levels than women (**Figures 3.2 and 3.3**). In both sexes, those in the shortest randomised group had the lowest haemoglobin and ferritin, and those in the longest interval had the highest (**Table 3.3**).

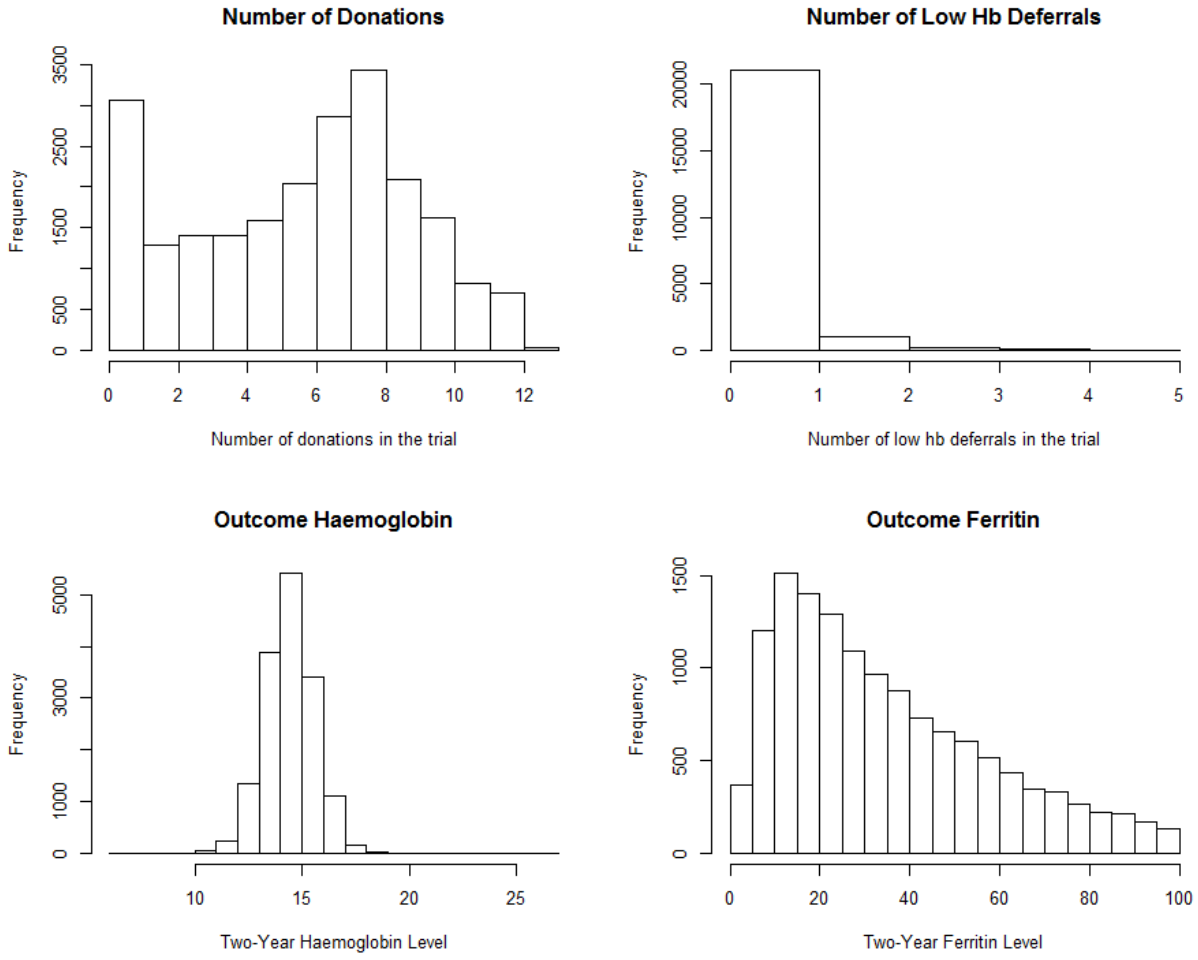



**Figure 3.2**: Histograms of the number of donations over the two year trial period, low hb deferrals, and haemoglobin and ferritin levels at the end of the trial for men (ferritin capped at 100)

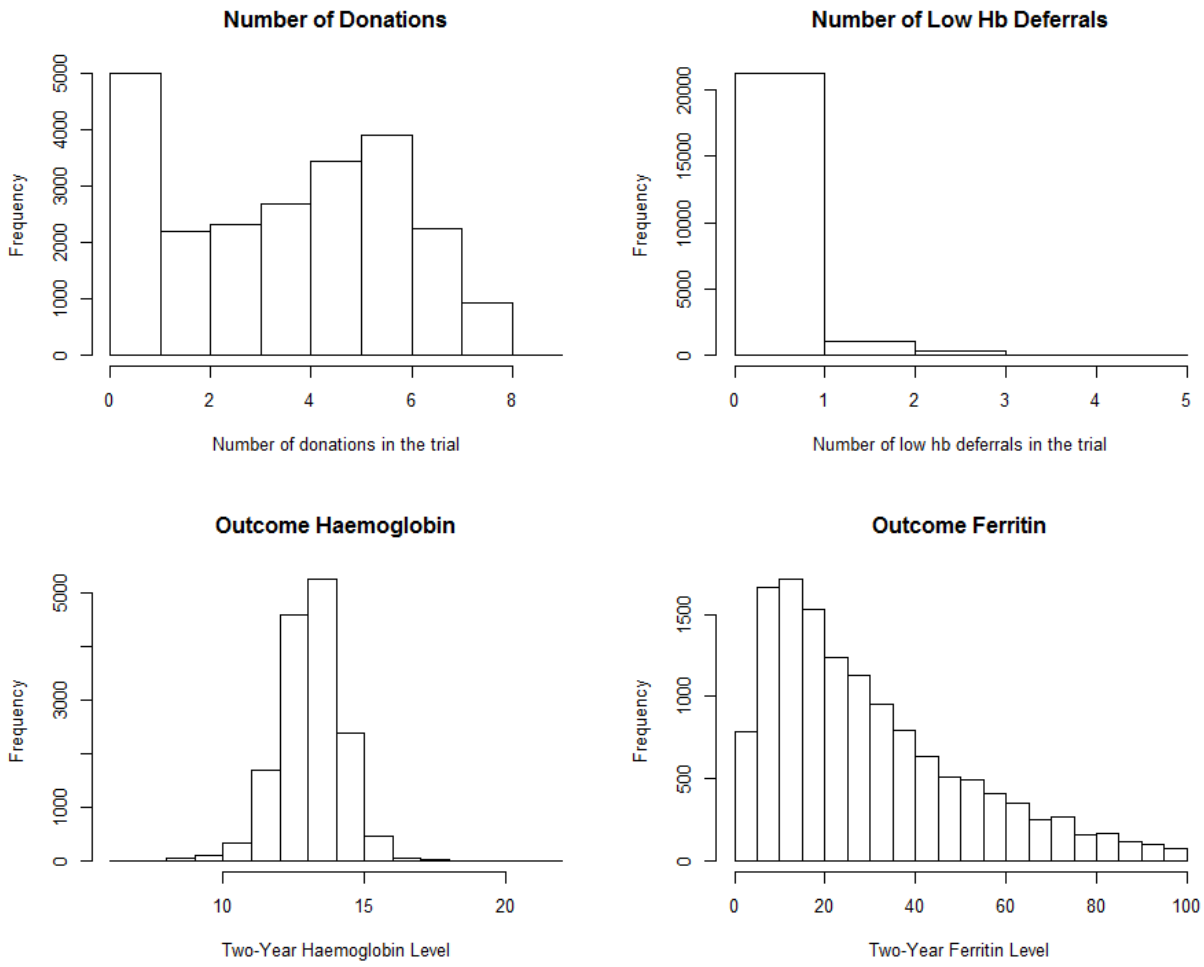



**Figure 3.3**: Histograms of number of donations over the two-year trial period, low hb deferrals, and haemoglobin and ferritin levels at the end of the trial for women (ferritin capped at 100)

**Table 3.3**: Mean (SD) by donation group of the two-year number of donations, low hb deferrals, and haemoglobin and ferritin levels.

| Gender | Group (N) | Donations | Low Hb Deferrals | Haemoglobin (g/dL) | Ferritin (µg/L) |
|---|---|---|---|---|---|
| **Men** | 8 weeks (7456) | 6.88 (3.75) | 0.44 (0.77) | 14.13 (1.21) | 40.61 (53.17) |
| | 10 weeks (7446) | 5.97 (3.18) | 0.25 (0.58) | 14.46 (1.16) | 44.49 (40.01) |
| | 12 weeks (7452) | 5.19 (2.66) | 0.14 (0.45) | 14.64 (1.14) | 51.1 (64.63) |
| **Women** | 12 weeks (7567) | 4.28 (2.63) | 0.40 (0.73) | 13.07 (1.13) | 31.98 (33.34) |
| | 14 weeks (7565) | 3.9 (2.34) | 0.30 (1.15) | 13.16 (1.15) | 33.72 (36.3) |
| | 16 weeks (7548) | 3.45 (2.07) | 0.20 (0.51) | 13.21 (1.1) | 36.85 (36.02) |

### 3.5.5 PCS and MCS

The individual participants' two year PCS and MCS scores were mostly in the range of 55-60 (**Figure 3.4**) indicating better physical and mental well-being in relation to the population mean of 50. This may have been expected, as blood donors are typically healthier than the general population (**Figure**

**3.4**). For approximately one third of male respondents, the outcome PCS and/or MCS score was missing. This was anticipated in the trial design, and INTERVAL was designed to account for this while retaining statistical power [3]. There were slightly more missing data for women than men (**Table 3.4**). It is possible that those with lower PCS and MCS scores in the intermediate Sf-12 surveys given at baseline, 6, 12, and 18 months were more likely to not complete the final survey.

The frequency of missing data was comparable across the randomised inter-donation groups and thus assessment of the effect of inter-donation frequency on well-being measures would be unbiased (i.e. limited selective attrition). In men, the median PCS was 57.7 (IQR 54.7 – 59.8, and the median MCS was 55.7 (IQR 51.6 – 58.2). In women, the median PCS was 57.7 (IQR 54.3 – 60.0), and the median MCS was 54.8 (IQR 49.9 – 57.6). In all of these summary scores, there was a negative skew. The assigned inter-donation group had little impact on the PCS and MCS 2-year outcomes.

**Table 3.4**: Summary statistics by donation group of the outcome PCS and MCS scores in INTERVAL

| | | PCS | | MCS | |
|---|---|---|---|---|---|
| **Gender** | **Group (N)** | **Mean (SD)** | **Missing (%)** | **Mean (SD)** | **Missing (%)** |
| **Men** | 8 weeks (7456) | 56.51 (5.20) | 2531 (33.65) | 53.96 (6.87) | 2530 (33.58) |
| | 10 weeks (7446) | 56.59 (4.99) | 2503 (33.62) | 53.89 (6.79) | 2500 (33.58) |
| | 12 weeks (7452) | 56.52 (5.07) | 2483 (33.32) | 53.82 (6.83) | 2482 (33.31) |
| **Women** | 12 weeks (7567) | 56.48 (5.54) | 2823 (37.31) | 52.47 (7.65) | 2822 (37.29) |
| | 14 weeks (7565) | 56.48 (5.56) | 2826 (37.36) | 52.63 (7.55) | 2824 (37.33) |
| | 16 weeks (7548) | 56.27 (5.77) | 2865 (37.96) | 52.61 (7.53) | 2864 (37.94) |

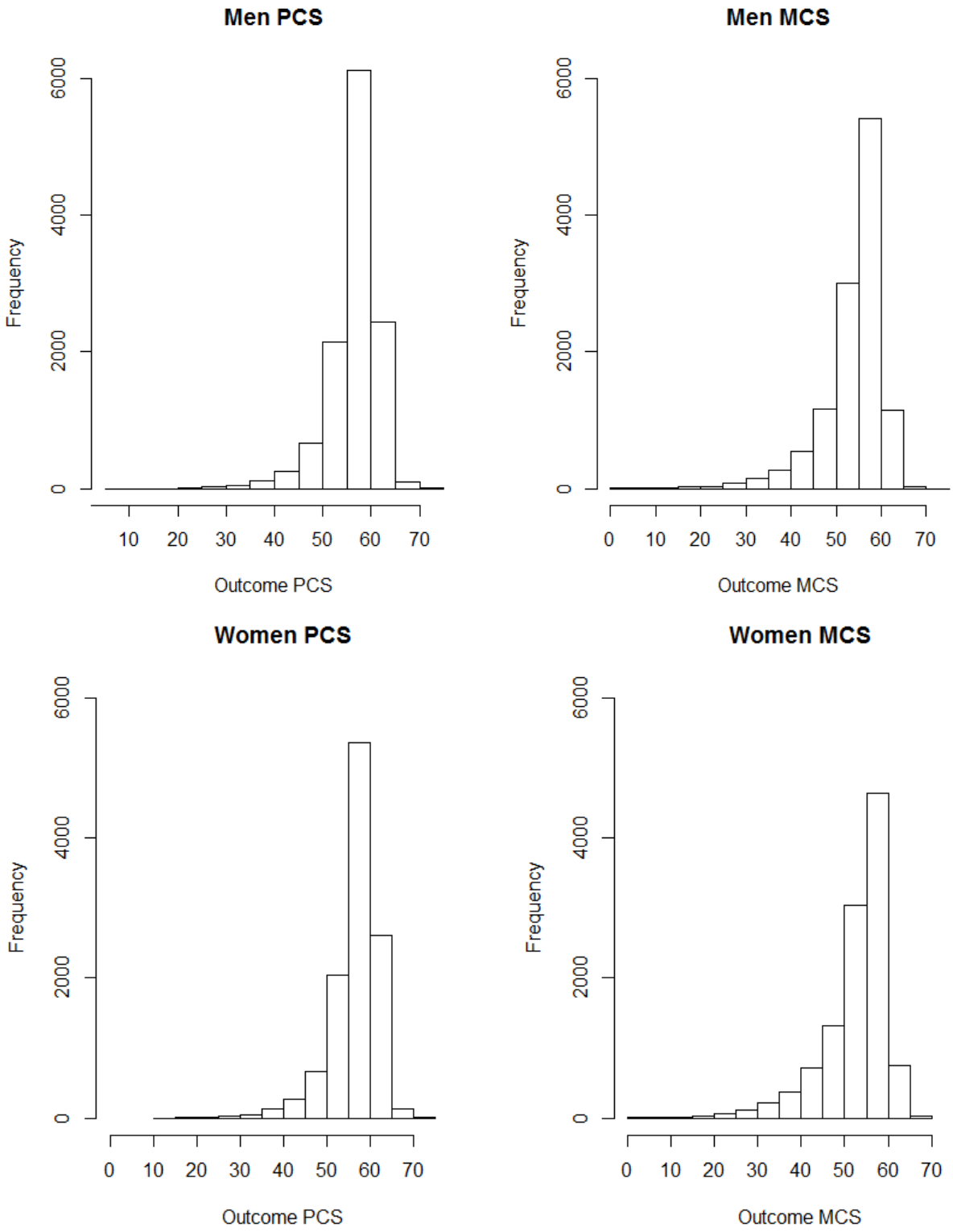



**Figure 3.4**: Histograms of the PCS and MCS scores in men and women. Scores were centred between 50 and 60, indicating above average physical and mental wellbeing in INTERVAL participants.

## 3.6 Discussion

This chapter has described the design and procedures of the INTERVAL trial, the main data source used for further analyses presented in subsequent chapters. It has described the trial's protocol and

aims. INTERVAL randomised donors to donate blood at different inter-donation intervals, and is the first trial of its kind. It provides a powerful basis for conducting unbiased inferences on the effects of randomised inter-donation intervals. Furthermore, given the breadth of information collected and randomisation, it enables further detailed study of blood donor health than previously done.

The strengths of INTERVAL are that, by design as a randomised control trial, results are not as susceptible to uncontrolled confounding which may be present in observational studies. The large sample size of 45,000 participants also gives high statistical power for inferences, including subgroup analyses. The trial was successful in randomising donors to different inter-donation intervals and had excellent follow up outcomes and other assessments, with a clear difference shown in the number of donations given in each group. In addition, recruitment spanned the geographical breadth of England and as such results should be more generalisable to the English blood donor population as a whole [74].

There are, however, limitations. Data were self-reported, and this can be unreliable and susceptible to bias. The main biases inherent to self-reported questionnaire data include misclassification bias from participants having a subjective view of the questions, particularly for post-donation symptoms, and recall bias due to the questionnaires only being given every six months, so it is possible that donors may not remember all symptoms that they experienced. In addition, the differing length of the questionnaires could induce bias as participants may stop answering longer questionnaires to the best of their ability. Moreover, it is possible that the repeated Sf-12 questionnaires and their corresponding questions in the Sf-36 could be subject to learned answers, as participants may fill them in on autopilot after multiple times answering in the same format.

As well as this, the results may also not be generalisable to the entire English blood donor population as one of the trial's requirements was regular internet access. Furthermore, it is likely that the trial only attracted more motivated donors from the NHSBT population, and the structured trial setting with more regular reminders about donation than routine practice could result in the trial

overestimating the potential impact of reducing inter-donation intervals on the blood supply in practice. The trial also may not be generalisable to countries outside of England, which can have different administrative systems for blood donation to the UK, such as non-scheduled appointments.

INTERVAL analyses previously published have been limited in scope, focussing on a subset of key variables on the outcome of number of donations. A comprehensive analysis of low haemoglobin deferrals, haemoglobin and ferritin levels, symptoms experienced during the trial, and donor wellbeing has not been conducted. These will be addressed in **Chapters 4, 5 and 6**.

# Chapter 4 – Effect of Randomised Inter-Donation Intervals on Blood Donations, Low Haemoglobin Deferrals, and Iron-Related Biomarkers: Extended Analyses of Main Effects and Interactions

## Summary

Previous published results from the INTERVAL trial assessing interactions with randomised inter-donation interval focused on a small subset of pre-specified key variables including age and HFE carrier status, in relation to the primary outcome of donations given during the trial. Interactions in relation to the number of low haemoglobin deferrals experienced, and the associated biomarkers have not been assessed, yet could be informative for personalised assignment of inter-donation intervals.

In this chapter I performed variable selection on a larger subset of baseline variables including demographics, dietary and lifestyle variables, donation history and biomarkers with two key aims: (i) to assess associations of baseline characteristics with outcomes post-randomisation, and (ii) to assess interactions of baseline characteristics and randomised inter-donation interval on outcomes. Four outcomes were analysed: number of donations, low haemoglobin deferral, and levels of haemoglobin and ferritin.

For both sexes, inter-donation interval had a significant effect on all four outcomes, with a stronger effect in men, likely due to the shorter intervals to which they were assigned. Higher baseline ferritin and haemoglobin, and more donations in the two years preceding the trial were all associated with more donations given during the trial. Number of previous donations and baseline ferritin had differential effects by randomised group for both sexes. Baseline haemoglobin was significantly associated with higher haemoglobin after two-years for both sexes, with a significant interaction with inter-donation interval for both sexes. For haemoglobin, other significant interactions were due to biomarkers in men and donation history in women. Concerning ferritin, for both sexes there were significant interactions between inter-donation interval and baseline log ferritin and red blood cell

count, and previous donations. Donation history and blood group were differentially associated with the number of low haemoglobin deferrals during the trial by randomised inter-donation interval in both sexes. For men there were also interactions between inter-donation interval and age, baseline haemoglobin and ferritin in relation to low haemoglobin deferrals.

These analyses suggest that there exist variables with convincing evidence of associations with the four outcomes studied, including variables significantly modifying the effect of randomised inter-donation interval on the outcomes. These four outcomes have routinely been of interest to the blood service, and thus the current results may be useful to inform personalisation of inter-donation intervals to maximise blood collection safely. A few blood-based biomarkers not routinely collected, particularly ferritin, were also identified as important, often with a higher magnitude of association than routinely collected variables, and thus may prompt reconsideration of routinely assessed biomarkers.

## 4.1 Introduction

### 4.1.1 Rationale

The UK's blood service relies on donations from unpaid, voluntary donors. New donor numbers are falling [71, 178], and while the current demand for blood is steadily decreasing [179] owing to better transfusion practices, it is possible that this demand could increase in the future for a variety of reasons, such as the aging population [71].

Currently, the minimum length of time between blood donations in England and Wales is twelve weeks for men and sixteen weeks for women [71] (**Chapter 3**). Decreasing the length of time between blood donations (i.e. inter-donation interval) could be one approach to mitigate shortfalls in blood supply related to the declining number of new donors. Furthermore, assignment to different inter-donation intervals based on the individual donor characteristics constitutes a more personalised donation experience, which could make donors feel more engaged with the service.

### 4.1.2 The INTERVAL Trial

As detailed in **Chapter 3**, the INTERVAL trial was a large randomised trial conducted between June 2012 and June 2016 that assigned 45,263 donors (22,466 men and 22,797 women) to donate more frequently than existing NHS guidelines, with a control group donating at the usual inter-donation intervals. The shorter donation intervals trialled were 8 and 10 weeks (vs. 12 weeks usual) for men, and 12 and 14 weeks (vs. 16 weeks usual) for women [7]. The trial aimed to identify characteristics of donors who were able to safely donate blood more frequently than existing guidelines, maximising the number of units of blood collected from the decreasing donor population in England.

Donors had to donate in one of the 25 fixed donation centres in England (**Chapter 3**). Recruitment was staggered by centre between June 2012 and June 2014, and participants were individually randomised to the sex-specific inter-donation intervals in the ratio 1:1:1, stratified by centre and minimising differences in age, weight, and new donor status. Participants were followed for outcomes over two years ending in June 2016 [73].

### 4.1.3 Outcomes of Interest

Four outcomes were investigated in this chapter, namely the number of donations given by donors during the trial, the number of low haemoglobin deferrals experienced, and donors' two year haemoglobin and ferritin levels. The number of donations given was the INTERVAL trial's primary outcome, and the number of low haemoglobin deferrals was a secondary outcome. As low haemoglobin deferrals depend on an individual's haemoglobin levels, monitoring would be of interest. Ferritin is a measure of iron stores, and quantification of the effect of more frequent donation on ferritin levels, including potential moderators, could be useful in monitoring a donor's risk of iron deficiency and associated symptoms.

### 4.1.4 Donor Characteristics of Interest

The analyses in this chapter looked beyond the characteristics that were prioritised for analyses in the INTERVAL trial main paper [74]. Based on findings of the INTERVAL trial main paper [74], donor age,

baseline haemoglobin, baseline log ferritin, weight, and number of donations in the previous two years were expected to be important covariates for the number of donations given in the trial and low haemoglobin deferrals. From the systematic review conducted in **Chapter 2**, the donor's status as a new or returning donor, donor centre, ethnicity and blood group were also identified as potentially important characteristics. However, there have been few or no studies providing evidence on the association of many other variables collected in the INTERVAL trial and four outcomes prioritised for evaluation in this chapter, thus a wide range of variables assessed at baseline were considered for analyses (**Table 4.1**) on the basis of their potential relevance to the prioritised outcomes, including components of dietary and lifestyle habits advised or followed by donors.

**Table 4.1**: List of variables considered for inclusion in each model. Asterisked variables were considered for women only.

| Questionnaire Variables | Dietary Variables | Biomarker Variables |
|---|---|---|
| Height | Vegetarianism | Red Blood Cell Count |
| Weight | Liver Consumption Per Week | White Blood Cell Count |
| Baseline PCS | Red Meat Consumption Per | Platelet Count |
| Baseline MCS | Week | Mean Corpuscular Volume |
| Ethnicity | Poultry Consumption Per | Mean Corpuscular Baseline |
| Anaemia | Week | Haemoglobin |
| Iron Prescription Use | White Fish Consumption Per | Baseline Log Ferritin |
| Iron Supplement Use | Week | |
| Iron Multivitamin Use | Oily Fish Consumption Per | |
| Contraceptive Pill Use* | Week | |
| Menopausal Status* | Fruit Consumption | |
| O Blood Group | Juice Consumption | |
| Rare Blood Group | Vegetable Consumption | |
| Smoking Status | Smoothie Consumption | |
| Alcohol Status | Tea Consumption | |
| Type of Work | | |
| Leisure Activity | | |
| Occupation Status | | |

## 4.2 Statistical Methods

Cross-sectional correlates of baseline haemoglobin and log-transformed ferritin levels were assessed using a linear model adjusted for age, sex, and centre. Continuous explanatory variables were divided into groups based on sex-specific distributions. This approach allowed assessment of the shape of any association with haemoglobin without imposing any particular shape on the association a priori [180,

181]. Categorical variables were modelled similarly to the risk-factor groups, except that dummy variables were used since there was no natural monotonic ordering of the categories. All other models were adjusted for age, sex, weight, new donor status, and donor centre. From each fitted linear model, overall adjusted mean values and 95 percent confidence intervals for each outcome by sex and categories of explanatory variables (ie tenths of continuous markers, or within category for categorical variables), were obtained with age fixed at 50 years. These adjusted mean values were used to summarise the shape of the association by plotting the mean outcome level against the mean marker value within each category.

Linear regression models were used to assess the associations between baseline donor characteristics and the post-baseline outcome variables including (i) number of donations given over 2 years, (ii) 2-year haemoglobin deferrals (iii) 2-year haemoglobin levels, and (iv) 2-year ferritin levels. Ferritin values were $\log_e$ transformed and presented as geometric means and relative differences (i.e. taking exponents of estimated regression coefficients). Linear regression was chosen as the outcomes were continuous and a Gaussian distribution could be assumed due to the large sample size by the central limit theorem, including in the case of counts of number of donations given [182]. A Poisson model was used to assess the associations between baseline characteristics and number of post-baseline low haemoglobin deferrals over 2 years as low haemoglobin deferrals were relatively uncommon and thus more appropriately modelled as discrete count data. The Poisson distribution assumption of equal mean and variance could be relaxed with use of robust standard errors, which often gives similar inferences as alternative statistical models as negative binomial regression that include modelling of over-dispersion. Data for men and women were analysed separately by the intention-to-treat principle according to their randomised inter-donation interval. All models were adjusted for inter-donation interval, age, sex, weight, and new donor status.

Donor characteristics investigated further as potential modifiers of the effect of randomised inter-donation interval on outcomes were first prioritised on the basis of having shown significant

associations with outcomes using a backwards-forwards selection procedure with p-value thresholds for exclusion and inclusion set to 0.1 and 0.09 respectively.

Interactions between the selected baseline donor characteristics and the randomised inter-donation interval were then assessed in the regression models for the four post-baseline outcomes. Only the interaction effects between inter-donation interval and the prioritised donor characteristics were included as candidate variables. Interactions retained in the final multivariable adjusted models were identified using a backwards-forwards selection procedure with p-value thresholds for exclusion and inclusion set to 0.05 and 0.049 respectively. Those characteristics without a significant interaction effect were removed from the final model.

Throughout analyses, inter-donation interval was analysed as a continuous, rather than a categorical variable as was the case in previous analyses of the INTERVAL trial [74]. This has the advantage of greater statistical power when the assumption of linear trend is reasonable, as well as allowing inference of the effect per one week shorter donation interval, which allows interpolation over a possible range of inter-donation intervals to be investigated than assigned during the trial (i.e. inferences between 8 and 12 weeks for men, and between 12 and 16 weeks for women). To facilitate graphical visualisation of interaction effects in the final multivariable models, the models were refitted with interacting continuous variables divided into quintiles one at a time and the interaction with inter-donation interval modelled as a categorical variable to calculate group-specific marginal means of the outcomes, which were then plotted against mean values of the covariates to visualise. These plots are referred to as marginal effect plots in the results. Statistical significance was based on $p < 0.05$ throughout. Analysis was performed in Stata version 14.

## 4.3 Results

### 4.3.1 Number of Donations

#### 4.3.1.1 Baseline Variable Associations with Number of Donations

Randomisation to shorter inter-donation intervals significantly increased the number of donations given over 2 years of the trial in both men and women, with quantitatively twice as large an effect in men than women per week shorter inter-donation interval (**Table 4.1**). Other baseline covariates significantly associated with number of donations given after adjusting for the randomised inter-donation interval and randomisation minimisation variables (i.e. age, weight, centre and new donor status) included, in both men and women, positive associations with age, PCS, MCS, iron multivitamin supplement use, red meat consumption, vegetable consumption, current alcohol consumption, number of donations given in the past two years, and four iron-related biomarkers (i.e. ferritin, haemoglobin, MCH, MCV). In women only, donations were also higher with higher reported levels of leisure activity. In men only, donations were higher with higher weight and in non-smokers compared to smokers.

Significant negative associations were found in both men and women for first time donor status compared to returning donor, Asian ethnicity compared to white ethnicity, and two biomarkers (white blood cell count and platelet count), moreover, donations were also lower in part time workers and the unemployed compared to full time workers, and in those with a previous diagnosis of anaemia compared to those without. Compared to engagement in sitting work, engagement in other types of work (standing/walking, manual labour, heavy manual labour, and not working) was also associated with decreased units of blood collected during the trial. Ethnic differences were only evident in men, with the black ethnic group having lower donations than the white ethnic group (**Table 4.2**).

**Table 4.2**: Univariate associations with number of donations over two years adjusted for baseline age, weight, centre and new donor status

| | Men | | Women | |
|---|---|---|---|---|
| **Variable** | **Coefficient (SE)** | **P** | **Coefficient (SE)** | **P** |
| **Inter-donation interval (1 week decrease)** | **0.42 (0.013)** | **<0.001** | **0.21 (0.0090)** | **<0.001** |
| **Demographics** | | | | |
| Height (m)[2] | 0.048 (0.023) | 0.034 | 0.075 (0.015) | <0.001 |
| Weight (kg)[2] | 0.089 (0.021) | <0.001 | 0.0043 (0.015) | 0.77 |
| Ethnicity (compared to White) | | <0.001 | | |
| Mixed | -0.40 (0.20) | 0.048 | -0.26 (0.13) | 0.041 |
| Asian | -1.12 (0.14) | <0.001 | -0.87 (0.14) | <0.001 |
| Black | -0.64 (0.24) | 0.009 | -0.28 (0.17) | 0.094 |
| Chinese | 0.67 (0.40) | 0.096 | -0.091 (0.26) | 0.73 |
| Other | -0.30 (0.34) | 0.39 | -0.42 (0.29) | 0.15 |
| Age (years)[2] | 0.60 (0.022) | <0.001 | 0.72 (0.015) | <0.001 |
| PCS (score)[2] | 0.23 (0.022) | <0.001 | 0.18 (0.015) | <0.001 |
| MCS (score)[2] | 0.20 (0.021) | <0.001 | 0.15 (0.015) | <0.001 |
| Non-O Blood Group vs O | -0.13 (0.043) | 0.002 | | |
| Contraceptive Pill Use (Y vs N) | - | - | 0.22 (0.40) | <0.001 |
| Menopause – Yes vs No | - | - | 0.17 (0.055) | 0.003 |
| Menopause – Unsure vs No | - | - | 0.064 (0.073) | 0.32 |
| **Iron Status** | | | | |
| Anaemia Ever (Y vs N) | -0.98 (0.10) | <0.001 | -0.42 (0.035) | <0.001 |
| Iron Prescription Use (Y vs N) | -0.74 (0.45) | 0.10 | -0.28 (0.19) | 0.15 |
| Iron Multivitamin Use (Y vs N) | 0.20 (0.063) | <0.001 | 0.25 (0.039) | <0.001 |
| Iron Supplement Use (Y vs N) | -0.0043 (0.22) | 0.98 | 0.18 (0.092) | 0.052 |
| **Diet/Lifestyle** | | | | |
| Vegetarian (Y vs N) | -0.47 (0.12) | <0.001 | -0.25 (0.065) | <0.001 |
| Liver (Portions per Week) [2] | -0.042 (0.022) | 0.06 | -0.060 (0.015) | <0.001 |
| Red Meat (Portions per Week) [2] | 0.076 (0.022) | <0.001 | 0.069 (0.015) | <0.001 |
| Poultry (Portions per Week) [2] | 0.022 (0.022) | 0.31 | 0.019 (0.016) | 0.22 |
| White Fish (Portions per Week) [2] | -0.025 (0.021) | 0.25 | 0.12 (0.015) | 0.45 |
| Oily Fish (Portions per Week) [2] | -0.018 (0.022) | 0.41 | -0.17 (0.016) | 0.27 |
| Vegetable Consumption (Y vs N) | 0.70 (0.14) | <0.001 | 0.41 (0.12) | 0.001 |
| Fruit Consumption (Y vs N) | 0.10 (0.067) | 0.13 | 0.14 (0.056) | 0.014 |
| Juice Consumption (Y vs N) | -0.035 (0.044) | 0.42 | 0.015 (0.030) | 0.63 |
| Smoothie Consumption (Y vs N) | -0.10 (0.064) | 0.12 | -0.14 (0.043) | 0.001 |
| Tea Consumption (Y vs N) | -0.13 (0.50) | 0.008 | -0.12 (0.034) | <0.001 |
| Alcohol Status – Ex vs Never | 0.21 (0.12) | 0.075 | 0.031 (0.078) | 0.69 |
| Alcohol Status – Current vs Never | 0.71 (0.085) | <0.001 | 0.32 (0.057) | <0.001 |
| Smoking Status – Ex vs Never | -0.24 (0.046) | <0.001 | -0.079 (0.033) | 0.017 |
| Smoking Status – Current vs Never | -0.067 (0.079) | <0.001 | -0.18 (0.054) | 0.001 |
| **Lifestyle** | | | | |
| Type of Work (compared to sitting) | | <0.001 | | |
| Standing/Walking | -0.44 (0.062) | <0.001 | -0.28 (0.038) | <0.001 |
| Manual Labour | -0.40 (0.065) | <0.001 | -0.26 (0.053) | <0.001 |
| Heavy Manual Labour | -0.49 (0.11) | <0.001 | -0.55 (0.13) | <0.001 |
| Do Not Work | -0.63 (0.069) | <0.001 | -0.22 (0.048) | <0.001 |
| Leisure Activity (compared to very inactive) | | 0.015 | | |
| Moderately Inactive | 0.043 (0.21) | 0.84 | 0.36 (0.14) | 0.008 |
| Moderately Active | 0.21 (0.21) | 0.30 | 0.57 (0.13) | <0.001 |
| Very Active | 0.28 (0.21) | 0.18 | 0.67 (0.14) | <0.001 |
| Occupation – Part Time vs Full Time | -0.54 (0.074) | <0.001 | -0.097 (0.037) | 0.009 |
| Occupation – Do Not Work vs Full Time | -0.71 (0.056) | <0.001 | -0.25 (0.040) | <0.001 |
| **Donation History** | | | | |
| New Donor vs Returning Donor | -1.16 (0.079) | <0.001 | -0.65 (0.49) | <0.001 |
| Donations in Past 2 Years (n) [2] | 0.69 (0.024) | <0.001 | 0.55 (0.017) | <0.001 |
| Low Hb Deferrals in Past 2 Years (n)[2] | -0.25 (0.021) | <0.001 | -0.14 (0.015) | <0.001 |
| **Biomarkers** | | | | |
| Ferritin (µg/L) [2] | 0.27 (0.023) | <0.001 | 0.29 (0.016) | <0.001 |
| Haemoglobin (g/dL) [2] | 0.33 (0.022) | <0.001 | 0.28 (0.015) | <0.001 |
| Mean Corpuscular Haemoglobin (g/dL) [2] | 0.32 (0.021) | <0.001 | 0.27 (0.015) | <0.001 |
| Mean Corpuscular Volume (fL) [2] | 0.21 (0.022) | <0.001 | 0.19 (0.015) | <0.001 |
| Red Blood Cell Count ($10^{12}$/L) [2] | 0.025 (0.022) | 0.25 | 0.025 (0.015) | 0.11 |
| White Blood Cell Count ($10^9$/L) [2] | -0.17 (0.022) | <0.001 | -0.048 (0.016) | 0.002 |
| Platelet Count ($10^9$/L) [2] | -0.18 (0.021) | <0.001 | -0.096 (0.015) | <0.001 |

[2] Per standard deviation increase. SDs reported in **Table 3.1** and **Table 3.2**

Variable selection amongst men resulted in the selection of seven baseline variables with significant associations with number of donations, including baseline MCS, MCV, haemoglobin, ferritin, iron multivitamin use, occupation status, and the number of donations in the previous two years, their corresponding interaction effects with inter-donation interval, adjusted for age, inter-donation interval, weight, donor status, and donor centre (**Table 4.3**). The selection procedure (**Section 4.2**) initially resulted in red blood cell count, MCH and haemoglobin selected for men, but examination of regression model diagnostics (specifically, variance inflation factors) indicated high collinearity, and thus the variable selection procedure was repeated to force retaining of haemoglobin only on the basis of its clinical relevance. The significant associations with an increase in blood donations were (in order of magnitude) higher number of donations in the past two years, inter-donation interval, age, baseline log ferritin, haemoglobin, MCS and MCV. Conversely, occupation status (part time or not working vs full time work), and first time donor status, were significantly associated with fewer donations (**Table 4.3**).

Variable selection amongst women resulted in the selection of five baseline variables with significant associations with number of donations, including baseline log ferritin, the number of donations in the previous two years, baseline white blood cell count, PCS, and red blood cell count. The significant associations with more donations were (in order of magnitude) higher number of donations in the past two years, age, baseline log ferritin, inter-donation interval, and baseline PCS (**Table 4.4**).

**Table 4.3**: Coefficients from the final selected model for number of donations in the trial for men (N = 20353).

| Main Effects | | |
|---|---|---|
| **Variable** | **Coefficient (95% CI)** | **P Value** |
| Inter-donation interval (1 week decrease) | 0.44 (0.41, 0.47) | <0.001 |
| 2-Year Donations (n, 1 SD higher) | 0.67 (0.59, 0.75) | <0.001 |
| Age (years, 1 SD higher) | 0.42 (0.37, 0.47) | <0.001 |
| Occupation – Part Time vs Full Time | -0.26 (-0.49, -0.032) | 0.025 |
| Occupation – Do Not Work vs Full Time | -0.45 (-0.62, -0.28) | <0.001 |
| Donor Status (New vs Returning) | -0.19 (-0.36, -0.014) | 0.034 |
| Iron Multivitamin Use (Yes vs No) | -0.10 (-0.30, 0.093) | 0.31 |
| MCS (score, 1 SD higher) | 0.073 (0.0058, 0.14) | 0.033 |
| Weight (kg, 1 SD higher) | -0.041 (-0.084, 0.0024) | 0.064 |
| Log Ferritin (µg/L, 1 SD higher) | 0.18 (0.096, 0.26) | <0.001 |
| Haemoglobin (g/dL, 1 SD higher) | 0.12 (0.054, 0.19) | 0.10 |
| MCV (fL, 1 SD higher) | 0.059 (-0.011, 0.13) | 0.001 |
| **Interaction with Inter-donation interval** | | |
| Iron Multivitamin Use (Yes vs No) | 0.12 (0.048, 0.20) | 0.001 |
| 2-Year Donations (n, 1 SD higher) | 0.11 (0.078, 0.14) | <0.001 |
| Occupation – Part Time vs Full Time | -0.10 (-0.19, -0.015) | 0.021 |
| Occupation – Do Not Work vs Full Time | -0.11 (-0.18, -0.045) | 0.001 |
| MCS (score, 1 SD higher) | 0.041 (0.015, 0.067) | 0.002 |
| Log Ferritin (µg/L, 1 SD higher) | 0.14 (0.10, 0.17) | <0.001 |
| Haemoglobin ((g/dL, 1 SD higher) | 0.055 (0.029, 0.082) | <0.001 |
| MCV (fL, 1 SD higher) | 0.026 (-0.0007, 0.053) | 0.056 |

**Table 4.4**: Coefficients from the final selected model for number of donations in the trial for women (N = 20886)

| Main Effects | | |
|---|---|---|
| **Variable** | **Coefficient (95% CI)** | **P Value** |
| Inter-donation interval (1 week decrease) | 0.21 (0.19, 0.22) | <0.001 |
| 2-Year Donations (n, 1 SD higher) | 0.53 (0.47, 0.58) | <0.001 |
| Age (years, 1 SD higher) | 0.39 (0.34, 0.45) | <0.001 |
| PCS (score, 1 SD higher) | 0.093 (0.046, 0.14) | <0.001 |
| Donor Status (New vs Returning) | 0.020 (-0.087, 0.13) | 0.72 |
| Weight (kg, 1 SD higher) | -0.0081 (-0.039, 0.023) | 0.61 |
| Log Ferritin (1 SD higher) | 0.27 (0.22, 0.32) | <0.001 |
| White Blood Cell Count ($10^9$/L, 1 SD higher) | -0.11 (-0.16, -0.065) | <0.001 |
| Red Blood Cell Count ($10^{12}$/L, 1 SD higher) | -0.0015 (-0.048, 0.046) | 0.95 |
| **Interaction with Inter-donation interval** | | |
| Age (years, 1 SD higher) | 0.050 (0.030, 0.070) | <0.001 |
| 2-Year Donations (n, 1 SD higher) | 0.052 (0.033, 0.072) | <0.001 |
| PCS (score, 1 SD higher) | 0.028 (0.0010, 0.046) | 0.003 |
| Log Ferritin (µg/L, 1 SD higher) | 0.075 (0.056, 0.093) | <0.001 |
| White Blood Cell Count ($10^9$/L, 1 SD higher) | 0.033 (0.015, 0.051) | <0.001 |
| Red Blood Cell Count ($10^{12}$/L, 1 SD higher) | 0.020 (0.0018, 0.038) | 0.031 |

#### 4.3.1.2 Effect Modification by Randomised Group

The final multivariable model for men retained seven baseline characteristics as significant modifiers of the average positive effect of randomised inter-donation interval on the number of donations over two years (**Table 4.3**). These interactions included significantly more donations than average with higher values of iron multivitamin supplement use, higher number of donations in the two years prior to baseline, MCS, log ferritin, and haemoglobin (**Table 4.3, Figure 4.1**). Conversely the model indicated significantly fewer donations than average with working part-time or not at all as compared to full time employment (**Table 4.3, Figure 4.1**). Comparing the standardised coefficients, the strongest effect modifier among the continuous variables was baseline log ferritin levels which was more than twice as large as the estimated independent interaction effects of other standardised continuous variables. Considering the categorical variables, the difference according to iron multivitamin use and inter-donation interval was mostly apparent in the eight week group (**Figure 4.1**). The interaction with MCV had been selected in the smaller complete-case dataset of all variables considered (n = 20,231), but was not as convincing when refitted in the larger complete-case dataset of the seven retained variables (n = 20,353). In women, all of the interaction effects with inter-donation interval, namely higher age, baseline PCS, red blood cell count, white blood cell count, number of donations in the previous two years, and baseline log ferritin, were positive (**Figure 4.2**), but all were of low magnitude (**Table 4.4**).

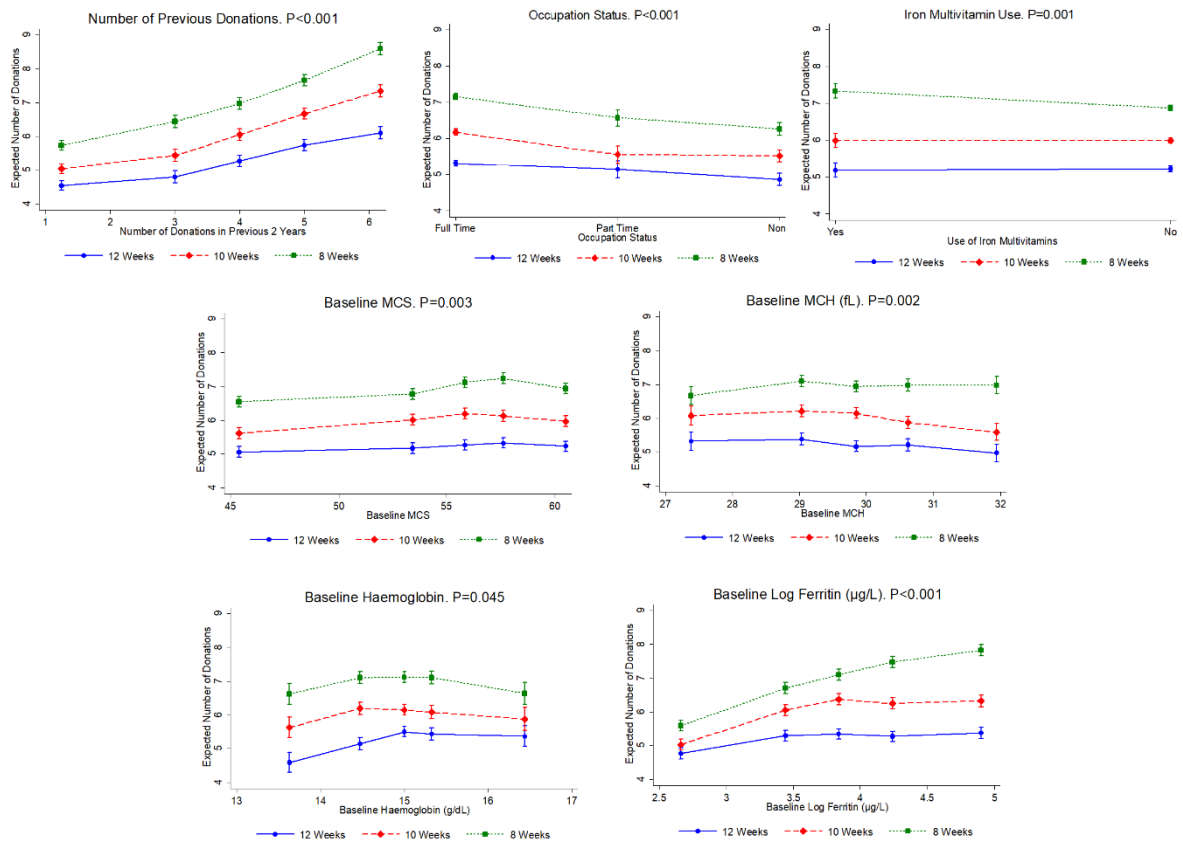



**Figure 4.1**: Marginal effects of number of donations in the past two years, occupation status, iron multivitamin use, baseline MCS, baseline MCH, haemoglobin levels, and log ferritin levels on number of donations for men. Green lines indicate the eight week group, red the 10 week group, and blue the 12 week group.

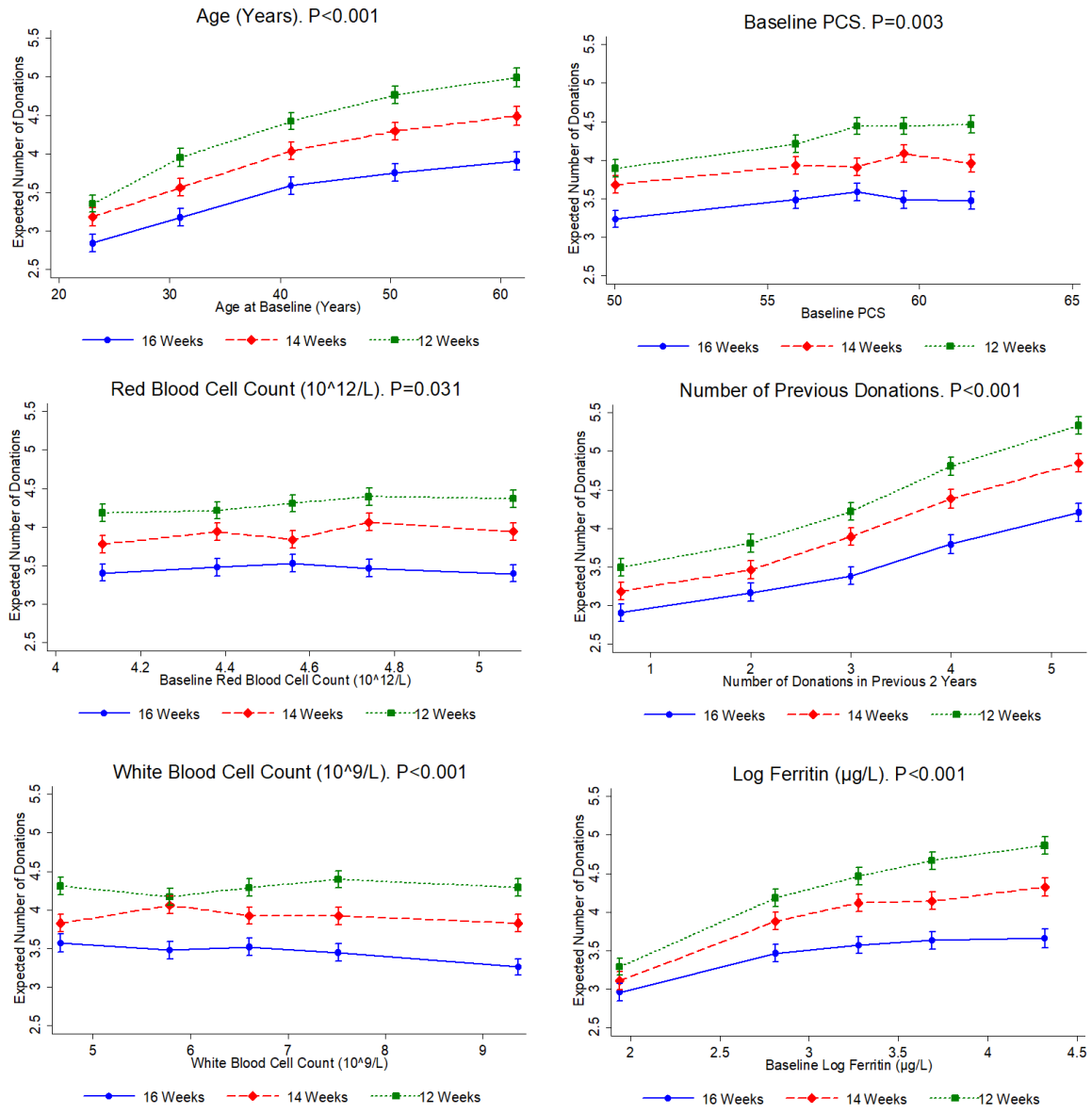



**Figure 4.2**: Marginal effects of age, baseline PCS, red blood cell count, white blood cell count, number of previous donations and baseline log ferritin on number of donations for women. Green lines indicate the 12 week group, red the 14 week group, and blue the 16 week group.

#### 4.3.1.3 Performance of Models for Number of Donations

Considering the performance of the models, the proportion of variance explained, as measured by the $R^2$ values, increased marginally (<1%) when interaction effects were added to the model containing selected main effects with biomarkers, implying that the addition of interactions did not explain much variation in the number of donations given by donors during the trial. Comparing the models with and without biomarkers, there was a difference of approximately 3%. The results for women were similar although biomarkers provided less of an increase to the $R^2$ values (**Table 4.5**).

**Table 4.5**: $R^2$ values for models of number of donations in the trial for men

| | Men (N=16004) | | Women (N=14605) | |
|---|---|---|---|---|
| **No Biomarkers** | **$R^2$** | **$R^2$ Difference** | **$R^2$** | **$R^2$ Difference** |
| Saturated Model | 15.98% | - | 18.94% | - |
| Selected Main Effects & Interactions | 13.77% | 2.21% | 16.62% | 2.32% |
| Selected Main Effects Only | 13.57% | 0.02% | 16.36% | 0.026% |
| **Add Biomarkers** | **$R^2$** | **$R^2$ Difference** | **$R^2$** | **$R^2$ Difference** |
| Saturated Model | 18.97% | - | 21.82% | - |
| Selected Main Effects & Interactions | 17.01% | 1.96% | 19.5% | 1.32% |
| Selected Main Effects Only | 16.09% | 0.093% | 19% | 0.050% |

### 4.3.2 Haemoglobin Levels

#### 4.3.2.1 Cross-Sectional Correlates of Haemoglobin

Examining the cross-sectional correlates of baseline haemoglobin, for all variables women had an equal or lower baseline haemoglobin than men. There was a positive association between baseline red blood cell count and baseline haemoglobin, and a negative association between the number of previous low haemoglobin deferrals and baseline haemoglobin. There was no association between other variables and baseline haemoglobin (**Figure 4.3**). For both sexes, black donors had the lowest baseline haemoglobin of all ethnic groups, and donors who had never been previously diagnosed with anaemia had higher haemoglobin than those who had reported history of anaemia diagnosis. There was no difference in haemoglobin levels by dietary and lifestyle factors (**Figure 4.4**).

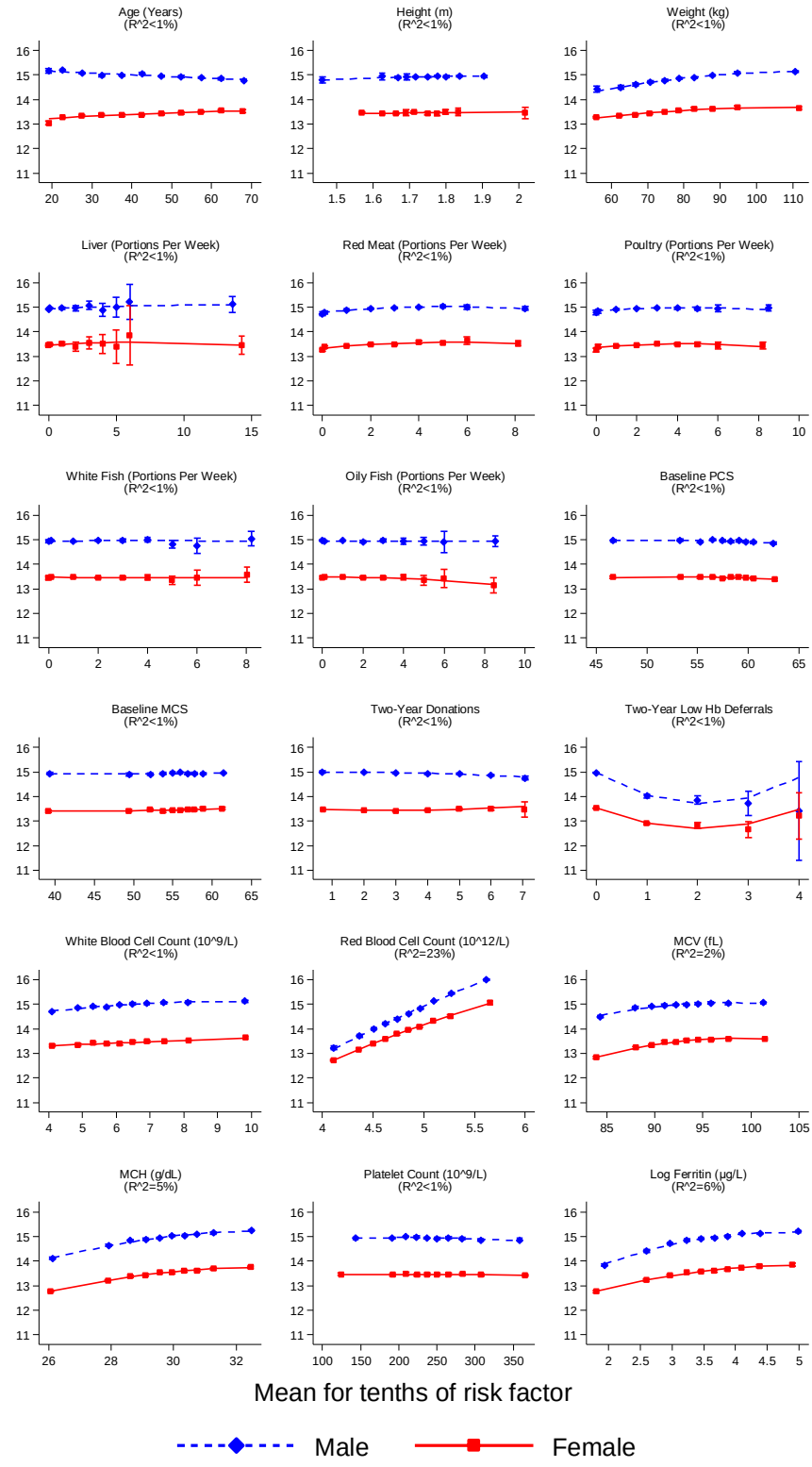



**Figure 4.3**: Cross-sectional correlates of baseline haemoglobin and continuous variables (partial $R^2$ difference in brackets). Panels indicate a separate continuous variable, plotted against haemoglobin on the y axis.

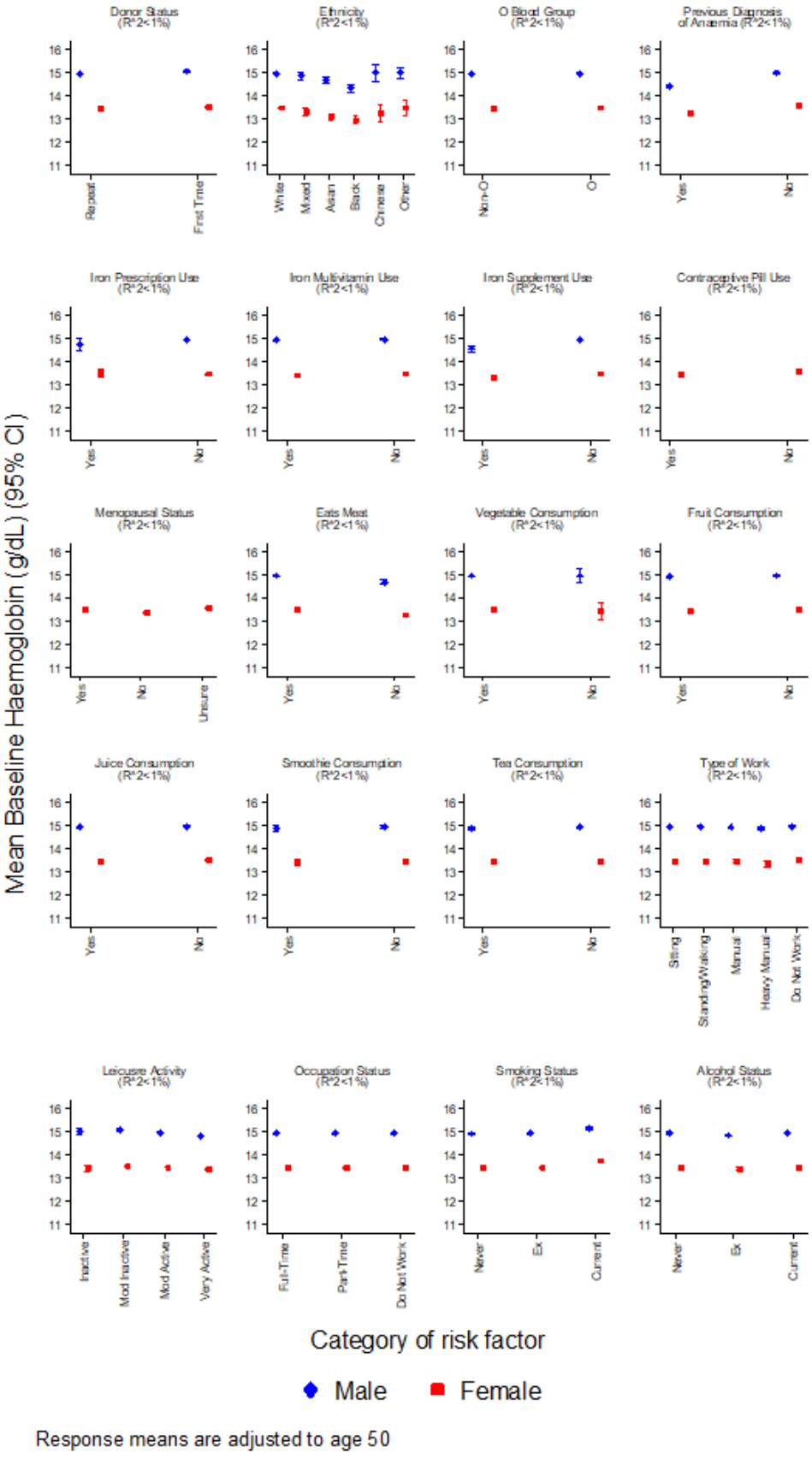



**Figure 4.4**: Cross-sectional correlates of baseline haemoglobin and categorical variables (partial $R^2$ difference in brackets). Panels indicate a separate categorical variable, with levels plotted against mean haemoglobin.

#### 4.3.2.2 Baseline Variable Associations with 2-Year Haemoglobin

Variable selection amongst men resulted in the selection of three baseline variables with significant associations with two-year haemoglobin – haemoglobin, platelet count, and MCV, their corresponding interaction effects with inter-donation interval, adjusted for age, inter-donation interval, weight, donor status, and donor centre (**Table 4.6**). The significant associations with higher haemoglobin after two years were (in order of magnitude) higher baseline haemoglobin, weight, and MCV. Conversely, inter-donation interval, platelet count, age, and new donor status were significantly associated with lower haemoglobin (**Table 4.6**).

**Table 4.6**: Coefficients from the final selected model for two year haemoglobin in the trial for men (N=15,431)

| **Main Effects** | | |
|---|---|---|
| **Variable** | **Coefficient (95% CI)** | **P Value** |
| Inter-donation interval (1 week decrease) | -0.085 (-0.095, -0.075) | <0.001 |
| Weight (kg, 1 SD higher) | 0.098 (0.081, 0.11) | <0.001 |
| Age (years, 1 SD higher) | -0.050 (-0.069, -0.030) | <0.001 |
| Donor Status (New vs Returning) | -0.049 (-0.13, 0.032) | 0.24 |
| Haemoglobin (g/dL, 1 SD higher) | 0.58 (0.55, 0.61) | <0.001 |
| Platelet Count ($10^9$/L, 1 SD higher) | -0.052 (-0.078, -0.025) | <0.001 |
| MCV (fL, 1 SD higher) | 0.013 (-0.014, 0.041) | 0.33 |
| **Interaction with Inter-donation interval** | | |
| Haemoglobin (g/dL, 1 SD higher) | -0.048 (-0.058, -0.037) | <0.001 |
| Platelet Count ($10^9$/L, 1 SD higher) | 0.011 (0.0004, 0.021) | 0.041 |
| MCV (fL, 1 SD higher) | 0.010 (0.0001, 0.021) | 0.048 |

Variable selection amongst women resulted in two baseline variables with significant associations with two-year haemoglobin: baseline haemoglobin and the number of donations in the previous two years. The significant associations with higher two-year haemoglobin were (in order of magnitude) higher baseline haemoglobin, number of donations in the previous two years, weight, and age. Only inter-donation interval was associated with lower haemoglobin at two years. (**Table 4.7**).

**Table 4.7**: Coefficients from the final selected model for two year haemoglobin in the trial for women (N=14652)

| Main Effects | | |
|---|---|---|
| **Variable** | **Coefficient (95% CI)** | **P Value** |
| Inter-donation interval (1 week decrease) | -0.032 (-0.042, -0.021) | <0.001 |
| Age (years, 1 SD higher) | 0.036 (0.017, 0.054) | <0.001 |
| Weight (kg, 1 SD higher) | 0.048 (0.031, 0.065) | <0.001 |
| Donor Status (New vs Returning) | 0.037 (-0.033, 0.11) | 0.30 |
| Haemoglobin (g/dL, 1 SD higher) | 0.49 (0.46, 0.52) | <0.001 |
| 2-Year Donations (n, 1 SD higher) | 0.16 (0.13, 0.19) | <0.001 |
| **Interaction with Inter-donation interval** | | |
| Haemoglobin (g/dL, 1 SD higher) | -0.029 (-0.040, -0.019) | <0.001 |
| 2-Year Donations (n, 1 SD higher) | -0.019 (-0.029, -0.0089) | <0.001 |

#### 4.3.2.3 Effect Modification by Randomised Group

The only significant selected interaction effect in the model for men was with baseline haemoglobin. Higher baseline haemoglobin was associated with less of an increase in haemoglobin at two years on shorter inter-donation intervals (**Table 4.6**). In addition, higher platelet count and MCV were associated with a higher haemoglobin level after two years on shorter inter-donation intervals. The marginal effect plots show that higher baseline haemoglobin was associated with a higher two year haemoglobin level, most apparent in those with higher baseline haemoglobin levels (**Figure 4.5**).

Both interaction effects selected in women – baseline haemoglobin and the number of donations given in the past two years - were negative with a low magnitude, indicating that the effect of these is lessened on shorter inter-donation intervals. (**Table 4.7, Figure 4.6**).

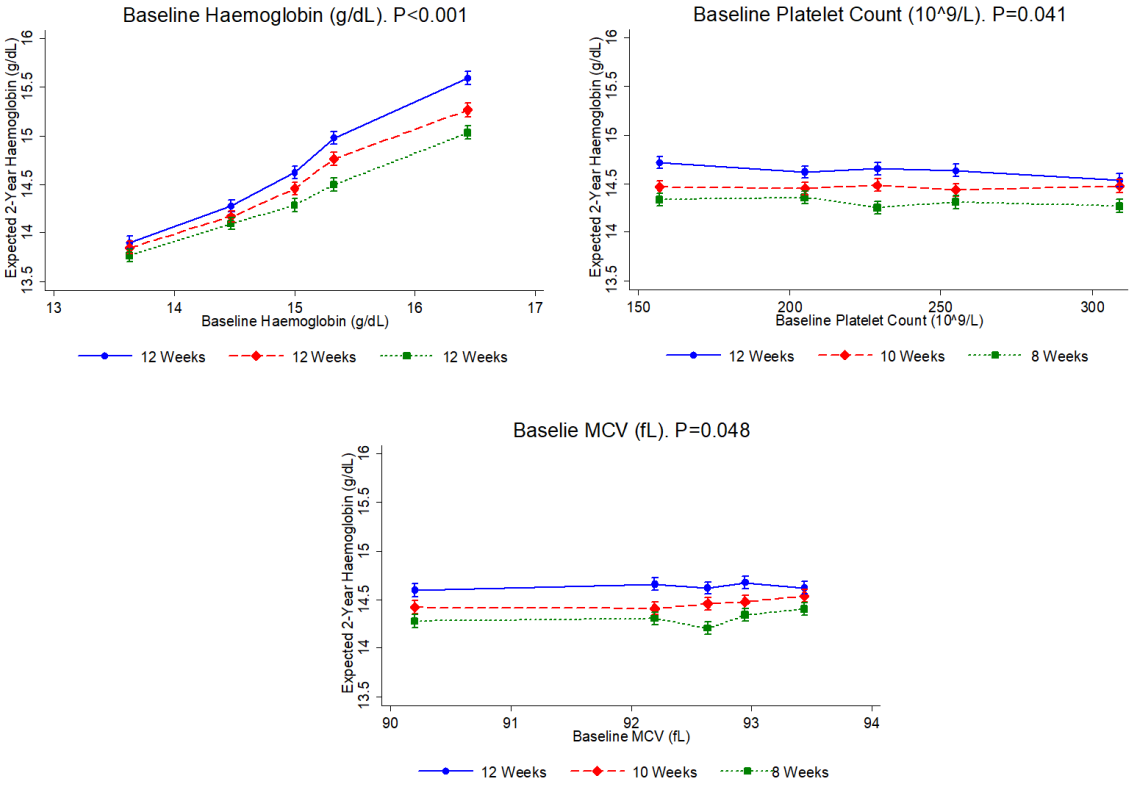



**Figure 4.5**: Marginal effects of baseline haemoglobin, platelet count, and MCV on two year haemoglobin for men. Green lines indicate the eight week group, red the 10 week group, and blue the 12 week group.

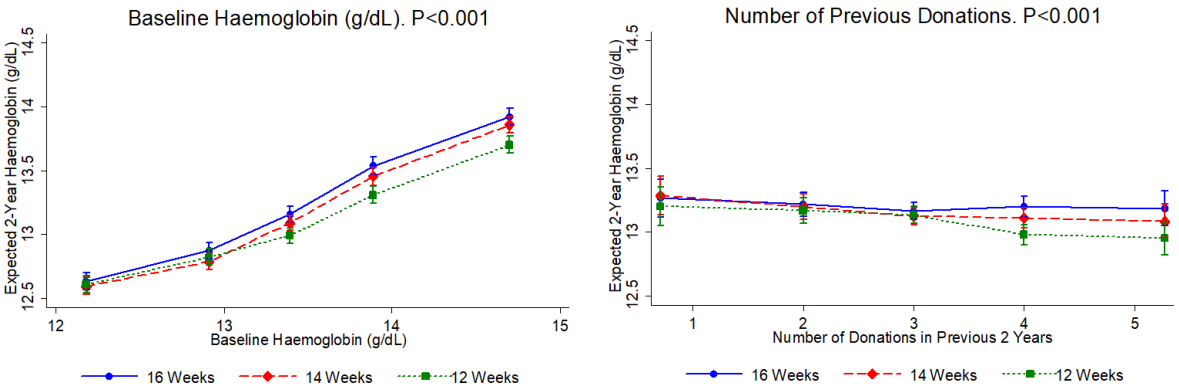



**Figure 4.6**: Marginal effects of baseline haemoglobin and number of previous donations on two year haemoglobin for women. Green lines indicate the 12 week group, red the 14 week group, and blue the 16 week group.

#### 4.3.2.4 Performance of Models for Haemoglobin

Considering the performance of the models, the proportion of variance explained, as measured by the $R^2$ values, increased marginally (<1%) when interaction effects were added to the model containing selected main effects with biomarkers, implying that the addition of interactions did not explain much variation in the number of donations given by donors during the trial. Comparing the models with and without biomarkers, there was a difference of approximately 13% in women and 17% in men. (**Table 4.8**).

**Table 4.8**: $R^2$ values for the haemoglobin models for men and women

| | **Men (N=11369)** | | **Women (N=9773)** | |
|---|---|---|---|---|
| **No Biomarkers** | **$R^2$** | **$R^2$ Difference** | **$R^2$** | **$R^2$ Difference** |
| Saturated Model | 7.09% | - | 7.79% | - |
| Selected Main Effects & Interactions | - | - | 3.32% | 4.47% |
| Selected Main Effects Only | 5.01% | 2.08% | 3.25% | 0.07% |
| **Add Biomarkers** | **$R^2$** | **$R^2$ Difference** | **$R^2$** | **$R^2$ Difference** |
| Saturated Model | 24.27% | - | 20.65% | - |
| Selected Main Effects & Interactions | 21.2% | 3.07% | 18.04% | 2.61% |
| Selected Main Effects Only | 20.65% | 5.5% | 17.78% | 0.26% |

### 4.3.3 Ferritin Levels

#### 4.3.3.1 Cross-Sectional Correlates of Ferritin

Examining the cross-sectional correlates of baseline ferritin, for most variables women had a lower baseline haemoglobin level than men. There was a positive association between height, weight, white blood cell count, baseline haemoglobin, MCV, and MCH and baseline ferritin, and a negative association between the number of previous donations and low haemoglobin deferrals and baseline ferritin. There was no association between other variables and baseline ferritin (**Figure 4.7**). For both sexes, first time donors had higher ferritin than repeat donors. In addition, those without a previous diagnosis of anaemia had higher baseline ferritin than those with a diagnosis. There was no difference in ferritin levels by dietary and lifestyle factors (**Figure 4.8**).

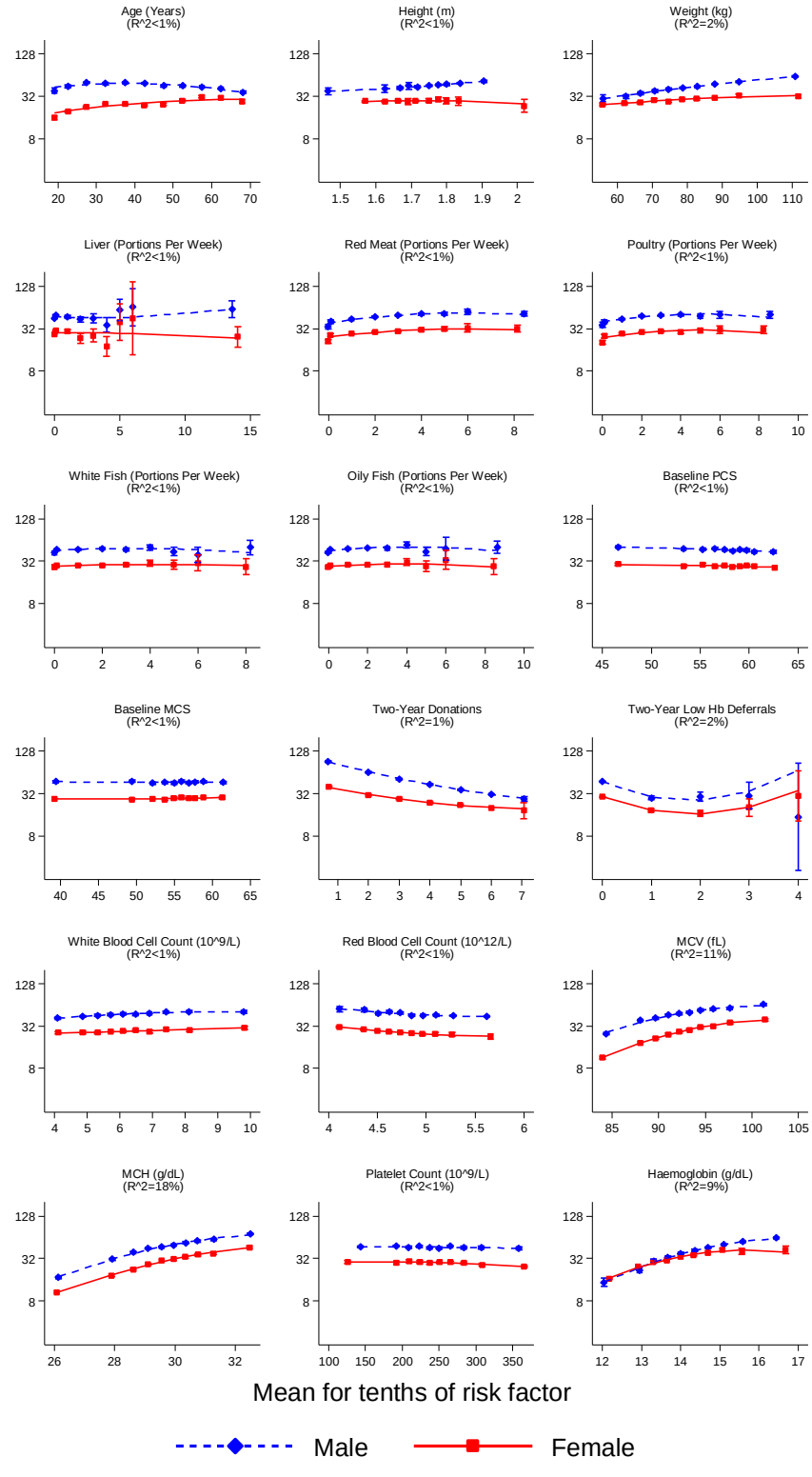



**Figure 4.7**: Cross-sectional correlates of baseline ferritin and continuous variables (partial $R^2$ difference in brackets). Panels indicate a separate continuous variable, plotted against ferritin on the y axis.

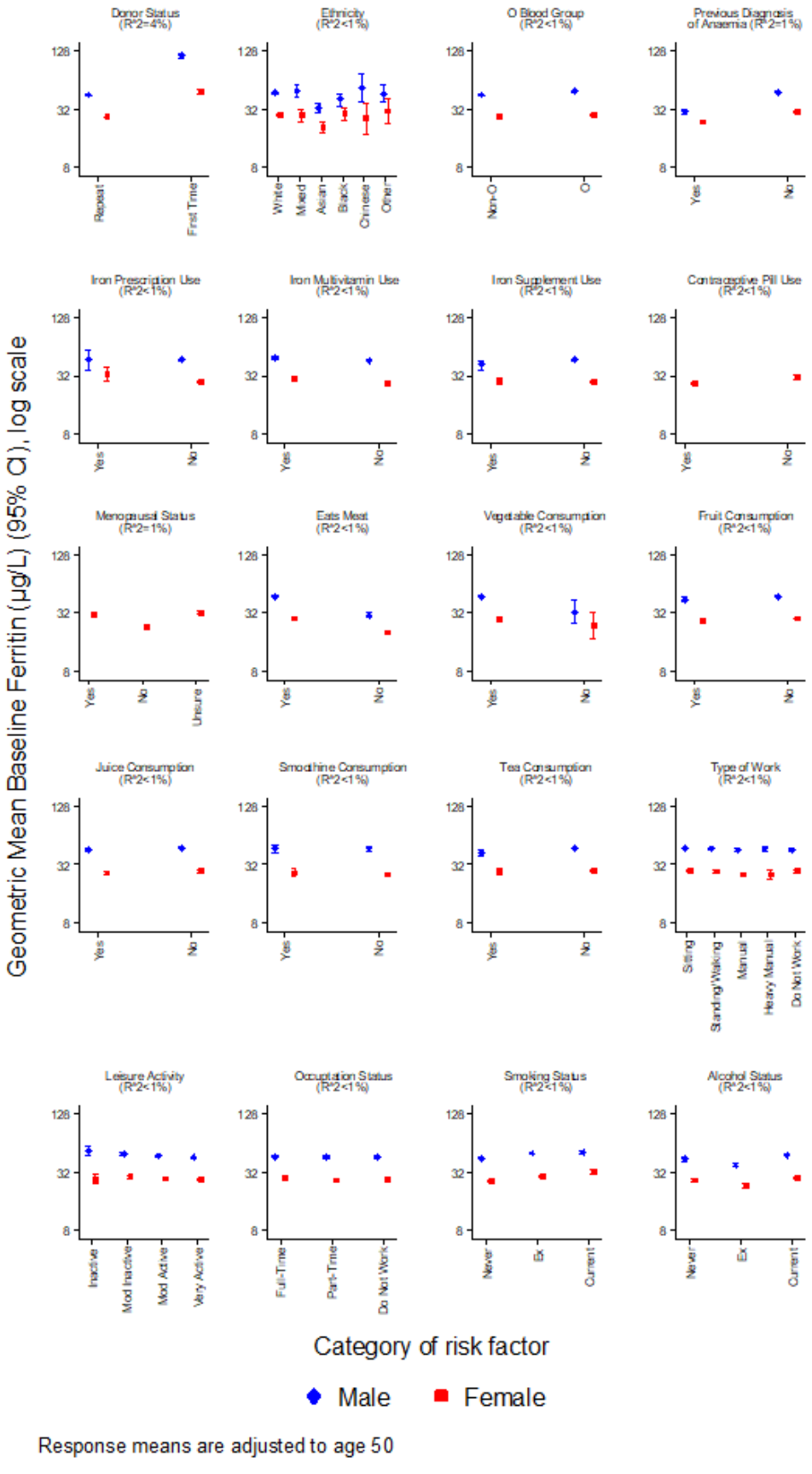



**Figure 4.8**: Cross-sectional correlates of baseline ferritin and categorical variables (partial $R^2$ difference in brackets). Panels indicate a separate continuous variable, plotted against ferritin on the y axis.

#### 4.3.3.2 Baseline Variable Associations with Ferritin Levels

Variable selection amongst men resulted in four main effects, baseline log ferritin, MCH, and red blood cell count, and the number of donations in the previous two years and their corresponding interaction effects with inter-donation interval, the interaction effect between age and inter-donation interval, and fixed effects of age, inter-donation interval, weight, donor status, and donor centre. Log ferritin was used as the outcome, with geometric means presented to show the multiplicative change in two year ferritin levels. Characteristics associated with proportionally higher ferritin were (in order of magnitude) higher baseline log ferritin, the number of donations in the previous two years, and weight. Conversely, new donor status, inter-donation interval, higher red blood cell count and MCH were significantly associated with lower ferritin at two years (**Table 4.9**).

**Table 4.9**: Proportional changes from the final selected model for two year log ferritin in the trial for men. (N=13,402)

| **Main Effects** | | |
|---|---|---|
| **Variable** | **Coefficient** | **P Value** |
| Inter-donation interval (1 week decrease) | 0.92 (0.91,0.93) | <0.001 |
| 2-Year Donations (n, 1 SD higher) | 1.15 (1.13, 1.18) | <0.001 |
| Weight (kg, 1 SD higher) | 1.05 (1.03, 1.06) | <0.001 |
| Donor Status (New vs Returning) | 0.88 (0.83, 0.93) | <0.001 |
| Age (years, 1 SD higher) | 0.99 (0.97, 1.01) | 0.342 |
| Log Ferritin (µg/L, 1 SD higher) | 1.70 (1.65, 1.74) | <0.001 |
| Red Blood Cell Count ($10^{12}$/L, 1 SD higher) | 0.94 (0.92, 0.97) | <0.001 |
| MCH (g/dL, 1 SD higher) | 0.97 (0.95, 1.00) | 0.062 |
| **Interaction with Inter-donation interval** | | |
| 2-Year Donations (n, 1 SD higher) | 0.98 (0.97, 0.99) | <0.001 |
| Age (years, 1 SD higher) | 1.01 (1.01, 1.02) | 0.001 |
| Log Ferritin (µg/L, 1 SD higher) | 0.96 (0.95, 0.97) | <0.001 |
| Red Blood Cell Count ($10^{12}$/L, 1 SD higher) | 0.98 (0.97, 0.99) | <0.001 |
| MCH (g/dL, 1 SD higher) | 0.98 (0.97, 0.990 | <0.001 |

Variable selection amongst women resulted in four main effects and their corresponding interaction effects added to the model, including baseline log ferritin, the number of donations in the previous two years, baseline red blood cell count, and iron supplement use. The significant associations with higher ferritin at two years (in order of magnitude) were higher baseline log ferritin, donations in the

previous two years, weight, and age. Conversely, higher baseline red blood cell count was significantly associated with lower ferritin at two years (**Table 4.10**).

**Table 4.10**: Proportional changes from the final selected model for two year log ferritin in the trial for women (N=12,409)

| **Main Effects** | | |
|---|---|---|
| **Variable** | **Coefficient** | **P Value** |
| Inter-donation interval (1 week decrease) | 0.96 (0.95, 0.97) | <0.001 |
| 2-Year Donations (n, 1 SD higher) | 1.14 (1.11, 1.16) | <0.001 |
| Donor Status (New vs Returning) | 0.95 (0.90, 1.01) | 0.113 |
| Weight (kg, 1 SD higher) | 1.04 (1.03, 1.06) | <0.001 |
| Iron Supplement Use (Yes vs No) | 0.97 (0.85, 1.10) | 0.621 |
| Age (years, 1 SD higher) | 1.02 (1.00, 1.04) | 0.01 |
| Log Ferritin (µg/L, 1 SD higher) | 1.60 (1.56, 1.63) | <0.001 |
| Red Blood Cell Count ($10^{12}$/L, 1 SD higher) | 0.97 (0.94, 0.99) | 0.003 |
| **Interaction with Inter-donation interval** | | |
| Iron Supplement Use (Yes vs No) | 1.06 (1.01, 1.11) | 0.023 |
| 2-Year Donations (n, 1 SD higher) | 0.99 (0.98, 0.99) | 0.001 |
| Log Ferritin (µg/L, 1 SD higher) | 0.97 (0.96, 0.98) | <0.001 |
| Red Blood Cell Count ($10^{12}$/L, 1 SD higher) | 0.99 (0.98, 1.00) | 0.006 |

#### 4.3.3.3 Effect Modification by Randomised Group

In men, the rises in two year ferritin associated with two-year donations and baseline log ferritin were less on the shorter inter-donation intervals, and there was a greater negative association with red blood cell count and MCH on shorter inter-donation intervals (**Table 4.9**). The marginal effect plots showed that there was little difference between two-year ferritin levels at the lowest levels of donations in the past two years, baseline ferritin, red blood cell count and MCH, and amongst the oldest donors (**Figure 4.9**).

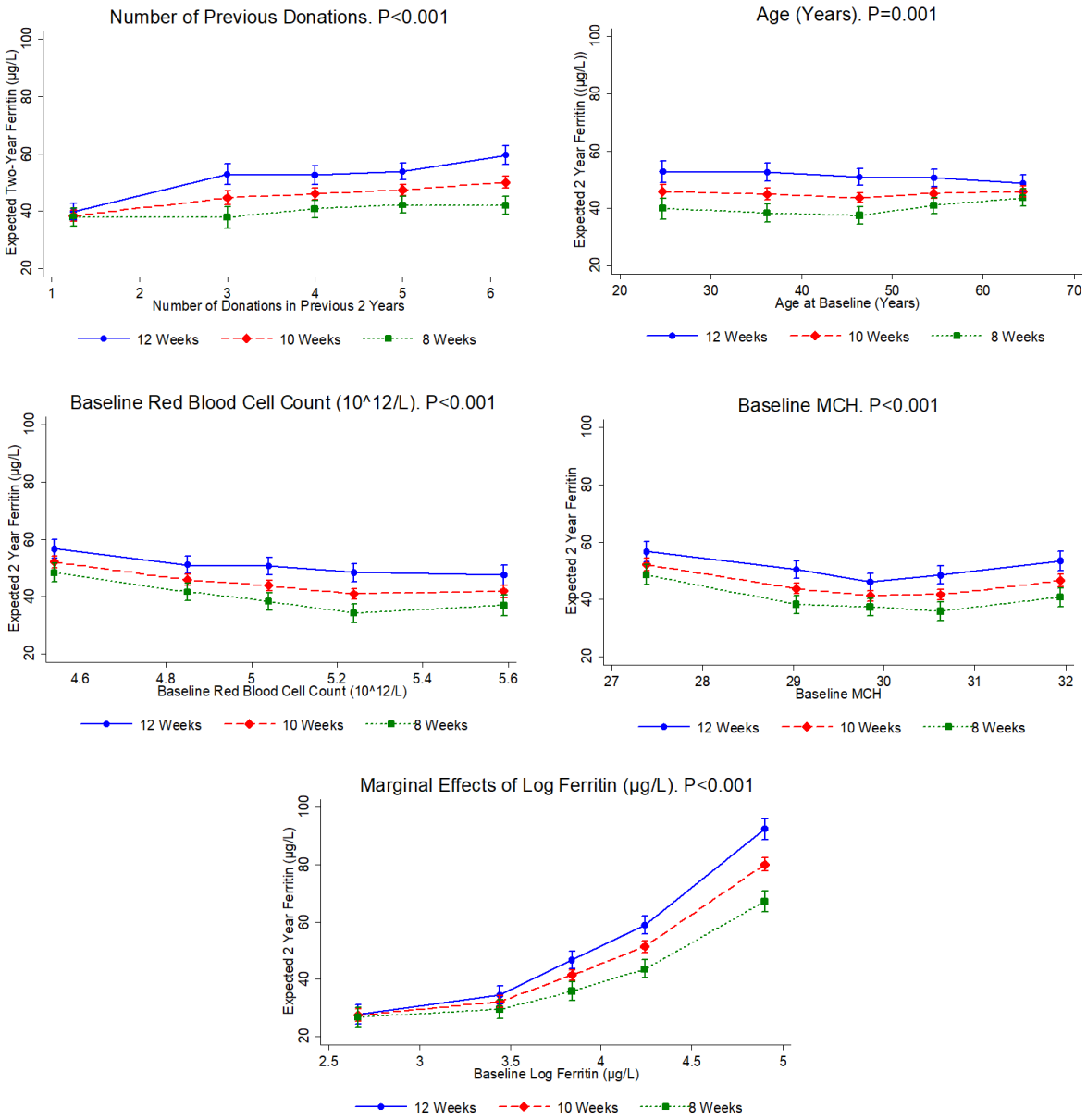



**Figure 4.9**: Marginal effects of number of donations in the previous two years, age at baseline, baseline red blood cell count, MCH and baseline ferritin on two year ferritin levels for men. Green lines indicate the eight week group, red the 10 week group, and blue the 12 week group.

In women, those who used iron supplements had 6% higher two year ferritin per week shorter inter-donation interval than those who did not take supplements. Similarly to men, for women the decrease in ferritin levels with shorter inter-donation intervals was attenuated in those with a higher baseline log ferritin or red blood cell count, and more donations in the previous two years, had lower ferritin proportionally on shorter inter-donation intervals (**Table 4.10**). Only for women with baseline ferritin

above the median was there a notable difference in two year ferritin levels by inter-donation interval (**Figure 4.10**).

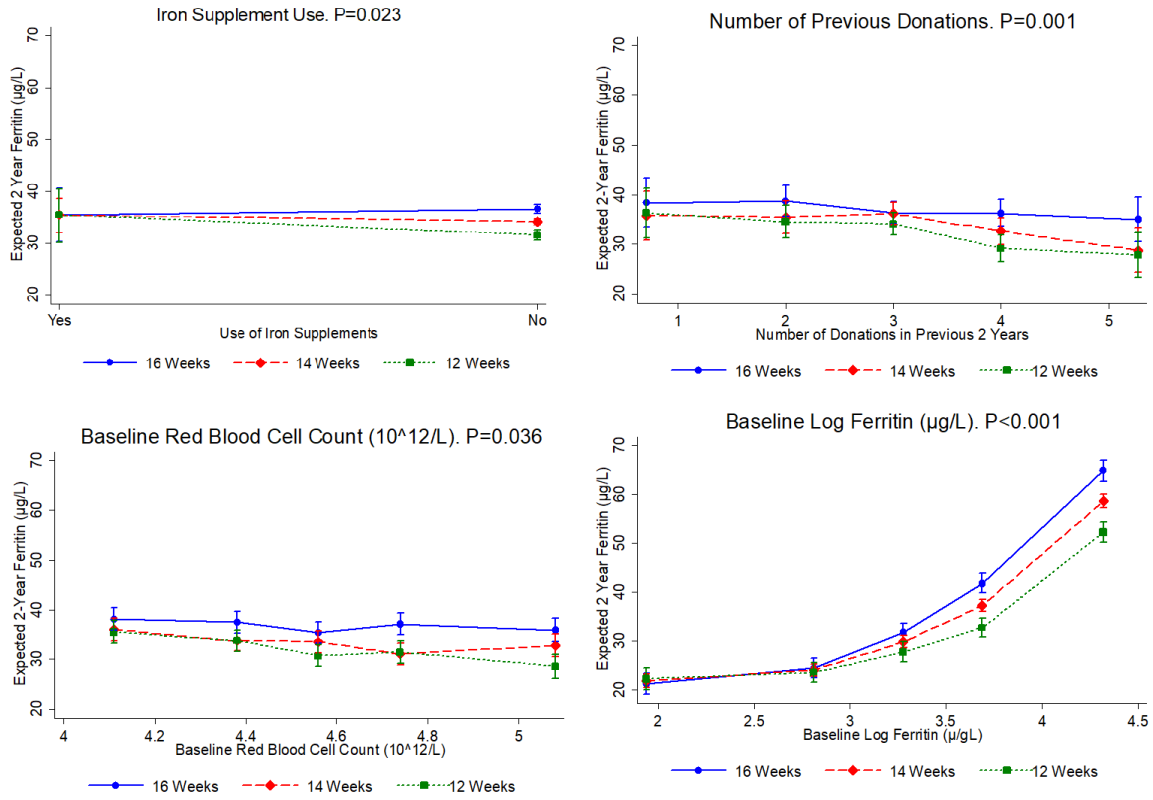



**Figure 4.10**: Marginal effects of iron supplement use, number of donations in the previous two years baseline red blood cell count and log ferritin on two year ferritin for women. Green lines indicate the 12 week group, red the 14 week group, and blue the 16 week group.

### 4.3.3.4 Performance of Models for Ferritin

Considering the performance of the models, the proportion of variance explained, as measured by the $R^2$ values, increased marginally (<1%) when interaction effects were added to the model containing selected main effects with biomarkers, implying that the addition of interactions does little to explain variation in two-year ferritin levels. Comparing the models with and without biomarkers, in men adding biomarkers contributed an extra 19% to $R^2$. The $R^2$ values were lower for the models in women containing biomarkers, however addition of biomarkers still added 10% to the $R^2$ values (**Table 4.11**).

**Table 4.11**: $R^2$ values for the ferritin models for men and women

| | Men (N=10,503) | | Women (N=9,035) | |
|---|---|---|---|---|
| **No Biomarkers** | **$R^2$** | **$R^2$ Difference** | **$R^2$** | **$R^2$ Difference** |
| Saturated Model | 8.79% | - | 7.57% | - |
| Selected Main Effects & Interactions | 5.79% | 3% | 4.43% | 3.14% |
| Selected Main Effects Only | 5.72% | 0.0007 | 4.29% | 0.14% |
| **Add Biomarkers** | **$R^2$** | **$R^2$ Difference** | **$R^2$** | **$R^2$ Difference** |
| Saturated Model | 27.47% | - | 17.65% | - |
| Selected Main Effects & Interactions | 25.97% | 1.5% | 16.62% | 1.03% |
| Selected Main Effects Only | 25.32% | 0.65% | 16.35% | 0.27% |

### 4.3.4 Low Haemoglobin Deferrals

#### 4.3.4.1 Baseline Variable Associations with Number of Low Haemoglobin Deferrals

Randomisation to shorter inter-donation intervals significantly increased the number of low haemoglobin deferrals over 2 years of the trial in both men and women, with a quantitatively larger effect in men than women per week shorter inter-donation interval (**Table 4.12**). Other baseline covariates significantly associated with the number of low haemoglobin deferrals after adjusting for the randomised inter-donation interval and randomisation minimisation variables (i.e. age, weight, centre and new donor status) included, in both men and women, positive associations with black and Asian ethnicity compared with white, higher age, non-O blood group, previous diagnosis of anaemia, vegetarianism, tea consumption, and the number of previous donations and low haemoglobin deferrals. In women only, deferrals were also higher with higher height, mixed ethnicity compared to white, those who consumed fruit compared to those who did not, and women who were pre-menopausal or unsure of their menopausal status compared to post-menopausal. In men only, deferrals were higher in ex-smokers compared to non-smokers, and those who used iron supplements compared to non-use.

Significant negative associations with low haemoglobin deferrals were found in both men and women for weight, iron multivitamin use, four dietary variables (red meat, poultry, white and oily fish consumption), new donor status, and seven iron-related biomarkers (i.e. ferritin, haemoglobin, MCH, MCV, red blood cell, white blood cell, and platelet counts). Only in men were deferrals significantly

lower among those who consumed vegetables, juice, and smoothies compared to those who did not, and in women only were deferrals less with higher levels of MCS. In addition, in women only were deferrals lower with current alcohol status compared to never, ex smoking status compared to never, and for those not in employment compared to full time work (**Table 4.12**).

**Table 4.12**: Univariable risk ratios for number of low hb deferrals adjusted for age, weight, centre and new donor status

| | Men | | Women | |
|---|---|---|---|---|
| **Variable** | **Coefficient (SE)** | **P** | **Coefficient (SE)** | **P** |
| **Inter-donation interval (1 week decrease)** | **1.33 (0.11)** | **<0.001** | **1.18 (0.090)** | **<0.001** |
| **Demographics** | | | | |
| Height (m)[3] | 0.99 (0.014) | 0.71 | 1.04 (0.013) | 0.004 |
| Weight (kg)[3] | 0.68 (0.11) | <0.001 | 0.80 (0.012) | <0.001 |
| Ethnicity (compared to White) | | <0.001 | | <0.001 |
| Mixed | 0.97 (0.13) | 0.79 | 1.28 (0.12) | 0.009 |
| Asian | 1.52 (0.12) | <0.001 | 1.53 (0.14) | <0.001 |
| Black | 1.77 (2.2) | <0.001 | 1.70 (0.19) | <0.001 |
| Chinese | 0.87 (0.24) | 0.61 | 0.99 (0.20) | 0.95 |
| Other | 0.91 (0.20) | 0.67 | 1.15 (0.25) | 0.51 |
| Age (years)[3] | 1.34 (0.18) | <0.001 | 0.96 (0.012) | <0.001 |
| PCS (score)[3] | 1.00 (0.013) | 0.92 | 1.00 (0.013) | 0.72 |
| MCS (score)[3] | 0.98 (0.013) | 0.14 | 0.94 (0.011) | <0.001 |
| Non-O Blood Group vs O | 1.14 (0.029) | <0.001 | 1.09 (0.026) | <0.001 |
| Contraceptive Pill Use (Y vs N) | - | - | 0.94 (0.030) | 0.071 |
| Menopause – Yes vs No | - | - | 1.66 (0.075) | <0.001 |
| Menopause – Unsure vs No | - | - | 1.17 (0.066) | 0.007 |
| **Iron Status** | | | | |
| Anaemia Ever (Y vs N) | 2.00 (0.086) | <0.001 | 1.73 (0.044) | <0.001 |
| Iron Prescription Use (Y vs N) | 1.52 (0.36) | 0.079 | 0.99 (0.16) | 0.93 |
| Iron Multivitamin Use (Y vs N) | 0.71 (0.031) | <0.001 | 0.81 (0.027) | <0.001 |
| Iron Supplement Use (Y vs N) | 1.33 (0.14) | 0.007 | 0.99 (0.073) | 0.94 |
| **Diet** | | | | |
| Vegetarian (Y vs N) | 1.62 (0.093) | <0.001 | 1.40 (0.063) | <0.001 |
| Liver (Portions per Week)[3] | 1.01 (0.013) | 0.49 | 1.01 (0.013) | 0.68 |
| Red Meat (Portions per Week)[3] | 0.91 (0.013) | <0.001 | 0.90 (0.012) | <0.001 |
| Poultry (Portions per Week)[3] | 0.93 (0.014) | <0.001 | 0.93 (0.013) | <0.001 |
| White Fish (Portions per Week)[3] | 0.97 (0.014) | 0.045 | 0.96 (0.013) | 0.003 |
| Oily Fish (Portions per Week)[3] | 0.95 (0.014) | <0.001 | 0.95 (0.013) | <0.001 |
| Vegetable Consumption (Y vs N) | 0.82 (0.068) | 0.016 | 0.92 (0.087) | 0.37 |
| Fruit Consumption (Y vs N) | 0.99 (0.042) | 0.78 | 1.14 (0.055) | 0.005 |
| Juice Consumption (Y vs N) | 0.93 (0.024) | 0.003 | 0.96 (0.023) | 0.098 |
| Smoothie Consumption (Y vs N) | 0.89 (0.038) | 0.006 | 1.01 (0.035) | 0.85 |
| Tea Consumption (Y vs N) | 1.18 (0.039) | <0.001 | 1.13 (0.032) | <0.001 |
| **Lifestyle** | | | | |
| Alcohol Status – Ex vs Never | 1.16 (0.077) | 0.023 | 1.06 (0.061) | 0.35 |
| Alcohol Status – Current vs Never | 0.92 (0.046) | 0.11 | 0.79 (0.034) | <0.001 |
| Smoking Status – Ex vs Never | 0.97 (0.027) | 0.33 | 0.89 (0.024) | <0.001 |
| Smoking Status – Current vs Never | 0.76 (0.043) | <0.001 | 0.56 (0.031) | <0.001 |
| Type of Work (compared to sitting) | | 0.037 | | <0.001 |
| Standing/Walking | 1.00 (0.039) | 0.99 | 1.02 (0.031) | 0.50 |
| Manual Labour | 1.13 (0.044) | 0.002 | 1.04 (0.044) | 0.41 |
| Heavy Manual Labour | 1.06 (0.069) | 0.36 | 0.86 (0.095) | 0.17 |
| Do Not Work | 1.03 (0.039) | 0.46 | 0.82 (0.034) | <0.001 |
| Leisure Activity (compared to very inactive) | | 0.90 | | 0.20 |
| Moderately Inactive | 1.05 (0.14) | 0.72 | 0.91 (0.11) | 0.44 |
| Moderately Active | 1.03 (0.14) | 0.82 | 0.95 (0.11) | 0.68 |
| Very Active | 1.02 (0.14) | 0.89 | 0.90 (0.11) | 0.38 |
| Occupation – Part Time vs Full Time | 1.01 (0.043) | 0.90 | 1.04 (0.031) | 0.22 |
| Occupation – Do Not Work vs Full Time | 0.98 (0.033) | 0.54 | 0.88 (0.30) | <0.001 |
| **Donation History** | | | | |
| New Donor vs Returning Donor | 0.54 (0.037) | <0.001 | 0.84 (0.035) | <0.001 |
| Donations in Past 2 Years (n)[3] | 1.08 (0.016) | <0.001 | 0.91 (0.013) | <0.001 |
| Low Hb Deferrals in Past 2 Years (n)[3] | 1.19 (0.0075) | <0.001 | 1.29 (0.0096) | <0.001 |
| **Biomarkers** | | | | |
| Ferritin (µg/L)[3] | 0.50 (0.0060) | <0.001 | 0.53 (0.0061) | <0.001 |
| Haemoglobin (g/dL)[3] | 0.58 (0.0059) | <0.001 | 0.53 (0.0056) | <0.001 |
| Mean Corpuscular Haemoglobin (g/dL)[3] | 0.65 (0.0071) | <0.001 | 0.63 (0.0067) | <0.001 |
| Mean Corpuscular Volume (fL)[3] | 0.71 (0.0091) | <0.001 | 0.69 (0.0081) | <0.001 |
| Red Blood Cell Count ($10^{12}$/L)[3] | 0.85 (0.012) | <0.001 | 0.85 (0.11) | <0.001 |
| White Blood Cell Count ($10^9$/L)[3] | 0.95 (0.013) | 0.001 | 0.95 (0.013) | <0.001 |
| Platelet Count ($10^9$/L)[3] | 1.09 (0.014) | <0.001 | 1.07 (0.013) | <0.001 |

[3] Per standard deviation increase. SDs reported in **Table 3.1** and **Table 3.2**

Variable selection amongst men resulted in five main effects; non-O blood group, baseline haemoglobin and ferritin, and the number of donations and low haemoglobin deferrals in the past two years, their corresponding interaction effects with inter-donation interval, alongside main effects of age, inter-donation interval, weight, donor status, and donor centre and the interaction effect of age with inter-donation interval. The significant associations with higher low haemoglobin deferrals were (in order of magnitude) higher number of low haemoglobin deferrals in the past two years, the inter-donation interval, and age. Conversely, higher baseline ferritin and haemoglobin levels, donations in the previous two years, and weight were significantly associated with fewer low haemoglobin deferrals (**Table 4.13**)

**Table 4.13**: Relative risks from the final selected model for number of low hb deferrals in the trial for men (N=20,945)

| **Main Effects** | | |
|---|---|---|
| **Variable** | **Coefficient** | **P Value** |
| Inter-donation interval (1 week decrease) | 1.42 (1.38, 1.46) | <0.001 |
| 2-Year Low Hb Deferrals (n, 1 SD higher) | 1.54 (1.36, 1.72) | <0.001 |
| Age (years, 1 SD higher) | 1.37 (1.28, 1.46) | <0.001 |
| 2-Year Donations (n, 1 SD higher) | 0.79 (0.73, 0.85) | <0.001 |
| Weight (kg, 1 SD higher) | 0.83 (0.79, 0.85) | <0.001 |
| Donor Status (New vs Returning) | 0.84 (0.69, 1.01) | 0.063 |
| Blood Group (Non-O vs O) | 0.93 (0.83, 1.05) | 0.242 |
| Log Ferritin (µg/L, 1 SD higher) | 0.53 (0.50, 0.57) | <0.001 |
| Haemoglobin (g/dL, 1 SD higher) | 0.54 (0.49, 0.59) | <0.001 |
| **Interaction with Inter-donation interval** | | |
| Haemoglobin (g/dL, 1 SD higher) | 1.09 (1.06, 1.12) | <0.001 |
| 2-Year Low Hb Deferrals (n, 1 SD higher) | 0.94 (0.91, 0.98) | 0.007 |
| Blood Group (Non-O vs O) | 1.04 (1.01, 1.08) | 0.023 |
| 2-Year Donations (n, 1 SD higher) | 1.03 (1.00, 1.05) | 0.020 |
| Age (years, 1 SD higher) | 0.98 (0.96, 0.99) | 0.013 |
| Log Ferritin (µg/L, 1 SD higher) | 1.02 (1.00, 1.05) | 0.029 |

Variable selection amongst women resulted in three main and corresponding interaction effects: non-O blood group, and the number of donations and low haemoglobin deferrals in the previous two years. The significant associations with higher frequency of low haemoglobin deferrals were (in order of magnitude) higher number of low haemoglobin deferrals in the past two years, age and inter-donation

interval. Conversely, significant associations with fewer low haemoglobin deferrals were higher age, weight, and number of donations in the previous two years (**Table 4.14**).

**Table 4.14**: Relative risks from the final selected model for number of low hb deferrals in the trial for women (N=22,672)

| **Main Effects** | | |
|---|---|---|
| **Variable** | **Coefficient** | **P Value** |
| Inter-donation interval (1 week decrease) | 1.17 (1.14, 1.20) | <0.001 |
| Age (years, 1 SD higher) | 0.97 (0.94, 1.00) | 0.04 |
| Blood Group (Non-O vs O) | 0.98 (0.89, 1.08) | 0.68 |
| Weight (kg, 1 SD higher) | 0.83 (0.80, 0.86) | <0.001 |
| Donor Status (New vs Returning) | 0.90 (0.81, 1.00) | 0.051 |
| 2-Year Low Hb Deferrals (n, 1 SD higher) | 2.05 (1.93, 2.20) | <0.001 |
| 2-Year Donations (n, 1 SD higher) | 0.90 (0.85, 0.94) | <0.001 |
| **Interaction with Inter-donation interval** | | |
| Blood Group (Non-O vs O) | 1.04 (0.99, 1.08) | 0.015 |
| 2-Year Low Hb Deferrals (n, 1 SD higher) | 0.97 (0.95, 0.99) | 0.01 |
| 2-Year Donations (n, 1 SD higher) | 1.03 (1.01, 1.04) | 0.003 |

#### 4.3.4.2 Effect Modification by Randomised Group

In men, a higher number of donations in the past two years, and higher baseline haemoglobin and ferritin were associated with a greater risk of low haemoglobin deferrals than would have been expected on shorter inter-donation intervals. In addition, non-O blood group was associated with a lesser risk of low haemoglobin deferrals on shorter inter-donation intervals (**Table 4.13**). However, at the highest levels of baseline ferritin there was little difference in the risk of low haemoglobin deferrals (**Figure 4.11**).

In women, a higher number of donations in the previous two years was associated with less risk of low haemoglobin deferrals on shorter intervals. Conversely, non-O blood group and a higher number of low haemoglobin deferrals in the previous two years were associated with less of an increased risk of low haemoglobin deferrals on shorter intervals (**Table 4.14**, **Figure 4.12**).

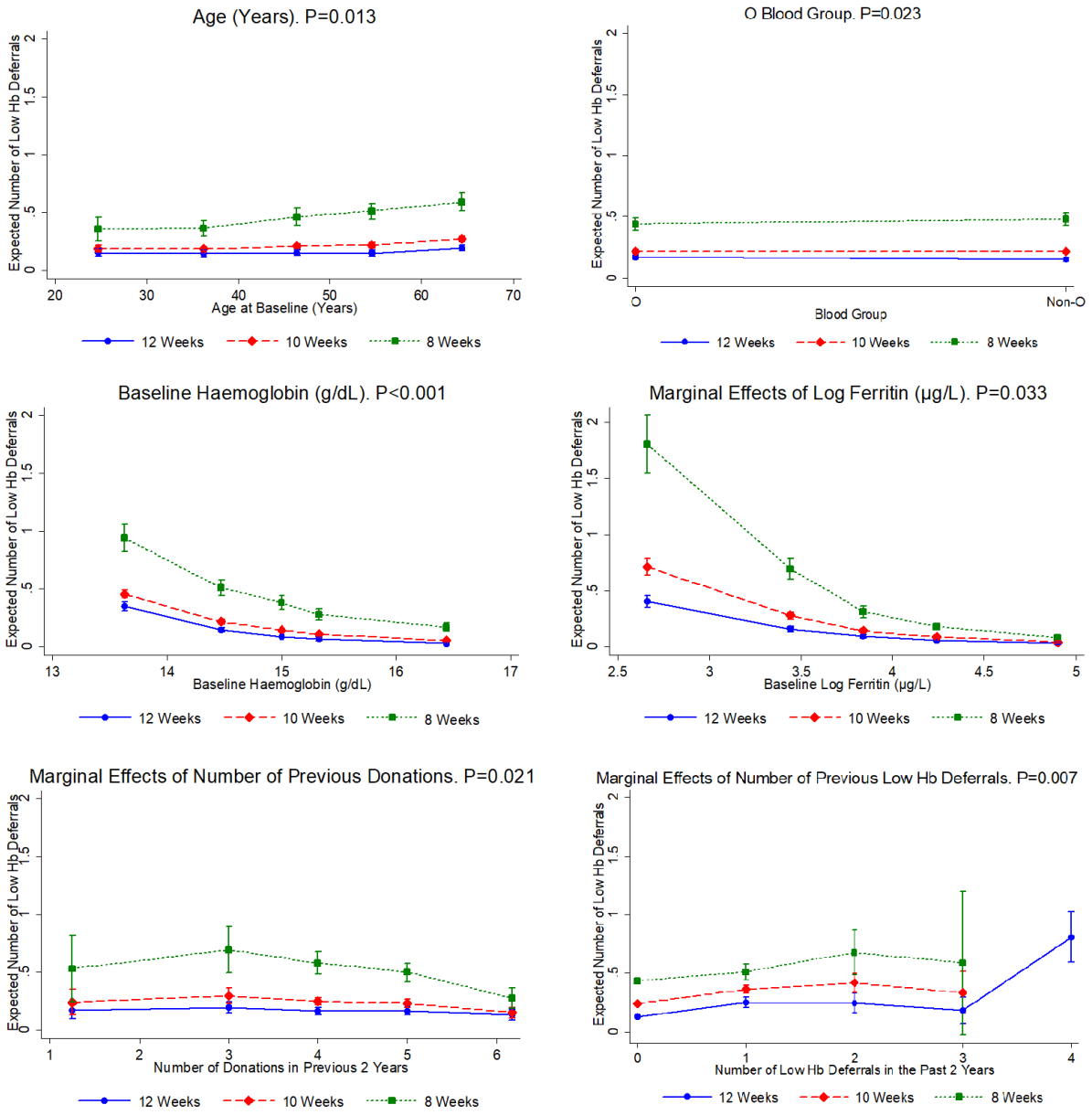



**Figure 4.11**: Marginal effects of age, blood group, baseline haemoglobin and log ferritin, and number of donations and low haemoglobin deferrals in the past two years on number of low haemoglobin deferrals in the trial for men. Green lines indicate the eight week group, red the 10 week group, and blue the 12 week group.

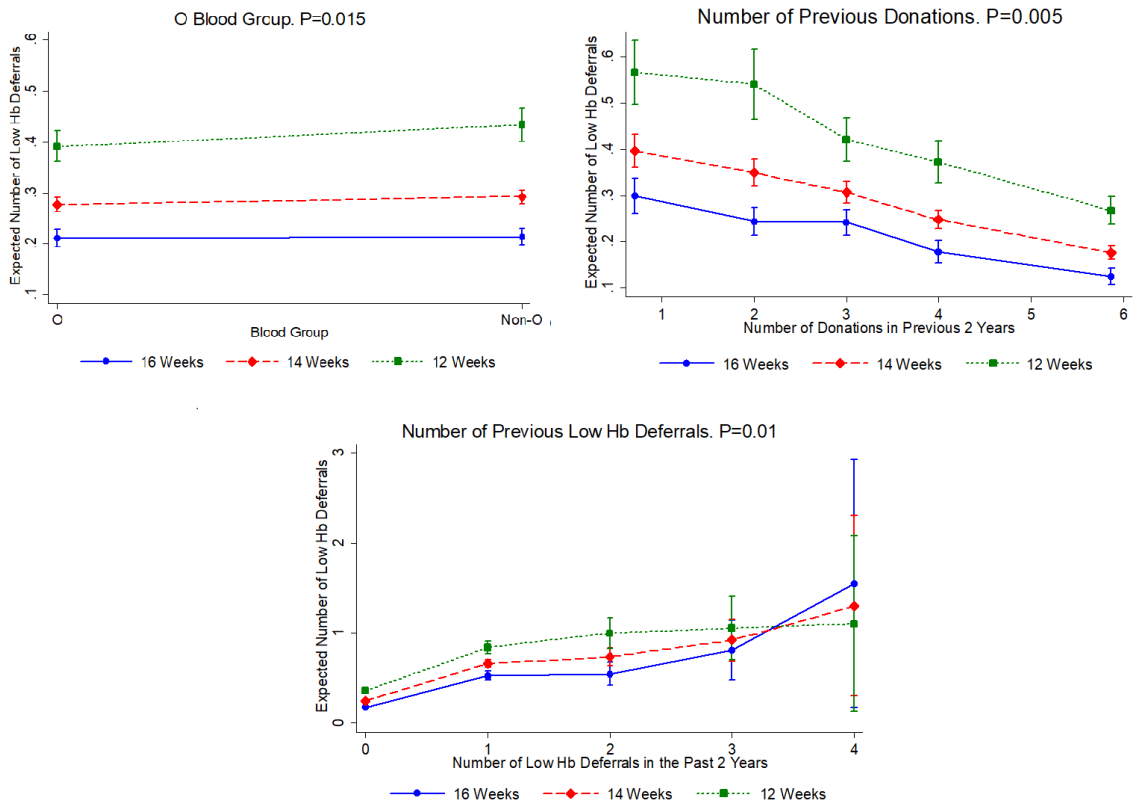



**Figure 4.12**: Marginal effects of blood group, number of donations and low haemoglobin deferrals in the past two years on number of low haemoglobin deferrals in the trial for women. Green lines indicate the 12 week group, red the 14 week group, and blue the 16 week group.

### 4.3.4.3 Performance of Models for Number of Low Haemoglobin Deferrals

Considering the performance of the models, the proportion of variance explained, as measured by the pseudo-$R^2$ values, increased marginally (<1%) when interaction effects were added to the model containing selected main effects with biomarkers, implying that the addition of interactions does little to explain variation in the number of low haemoglobin deferrals experienced by donors over two years. Comparing the models with and without biomarkers, in men adding biomarkers contributed an extra 6% to $R^2$. In women, no biomarkers were selected. The $R^2$ difference between the saturated models containing biomarkers and not containing biomarkers was larger for women (12%) than for men (6%) (**Table 4.15**).

**Table 4.15**: Pseudo-$R^2$ values for models of number of low haemoglobin deferrals in the trial for women

| | Men (N=16004) | | Women (N=14605) | |
|---|---|---|---|---|
| **No Biomarkers** | **$R^2$** | **$R^2$ Difference** | **$R^2$** | **$R^2$ Difference** |
| Saturated Model | 12.33% | - | 6.86% | - |
| Selected Main Effects & Interactions | 11.68% | 0.65% | 5.84% | 1.02% |
| Selected Main Effects Only | 11.5% | 0.18% | 5.74% | 0.1% |
| **Add Biomarkers** | **$R^2$** | **$R^2$ Difference** | **$R^2$** | **$R^2$ Difference** |
| Saturated Model | 18.97% | - | 18.70% | - |
| Selected Main Effects & Interactions | 17.01% | 1.96% | 5.84% | 12.86% |
| Selected Main Effects Only | 16.09% | 0.93% | 5.74% | 0.1% |

## 4.4 Discussion

In this chapter, I have conducted a comprehensive analysis of characteristics associated with four donor-health related outcomes, including identifying moderators of the effect of randomised inter-donation interval. Specifically, the findings suggest characteristics associated with a donor's ability to safely donate over the two year study period, risk of receiving low haemoglobin deferrals, and final haemoglobin and ferritin values, and which of these are moderators of the individual's interval assignment. The findings can be used to develop personalised donation intervals in the future.

### 4.4.1 Number of Donations

In both sexes, randomisation to shorter inter-donation intervals did result in an increase in blood collected during the trial. Among men, a key finding is that those with higher ferritin were able to donate more during the trial on shorter donation intervals, as were men with higher haemoglobin levels and more previous blood donations. For women, these associations were present at a smaller magnitude. In addition, age was associated with the number of donations given, with older women able to donate more than younger women. This is in agreement with previous studies which found more donations from older donors [34, 183-185], those of white ethnicity compared to non-white [1, 4], and a decrease in donations from those with lower ferritin [5]. The main INTERVAL findings also concluded that higher ferritin and haemoglobin at baseline were associated with more donations during the trial [74]. In addition, a week reduction in time between donations resulted in an increase

of 0·23 units in the amount of blood collected per year in men and 0·14 units in women compared with the currently-used 12 and 16 week inter-donation intervals [186].

Examining variables that were moderators of inter-donation interval in men, the largest differences were evident among the most frequent previous donors as well as those with highest baseline ferritin levels and use of iron multivitamins. The former can likely be explained by the fact that if a donor has already donated frequently in the previous two years, they are likely to be committed to the trial. Those using iron multivitamins were able to donate more during the trial than those who did not. This could be because those on iron supplementation could represent the more committed donors to the trial who were taking an extra precaution to control the risk of iron depletion afforded by more frequent blood donation, and this will be further examined in **Chapter 5**. In addition, those in full time employment were able to donate more blood during the trial than those in part time work or out of work. This could perhaps be because full time employees are used to managing a schedule and thus can schedule donations more easily compared to the additional commitments that may hinder those in part time work or not in employment such as childcare. Those out of work may be out of work due to mobility issues or family circumstances which may affect their availability to give blood.

In women, there were similar associations as men with number of previous donations and inter-donation interval. The difference between the 16 week group and the other groups widened for more frequent donors, implying that this group may have been committed enough to take full advantage of the extra donation opportunities afforded by more frequent donation intervals. A similar association was seen according to baseline PCS score, with the highest scoring women donating relatively more in the 12 week group than the 14 week group, likely due to being in better physical health and so able to withstand more frequent donation. The most notable association with number of donations in women was age, with older women able to donate half an extra donation in two years with a one week shorter donation interval. This could be because, as identified in **Chapter 2**, older women who have stopped menstruating are less susceptible to low haemoglobin levels. More biomarkers were

moderators of inter-donation interval in men than in women. While there was little difference between donations given in the trial at the lowest baseline ferritin levels, the groups separated as baseline ferritin increased.

### 4.4.2 Two Year Haemoglobin Levels

For both sexes, the most relevant correlate of a donor's two year haemoglobin level was their haemoglobin level at baseline. For women there was a more pronounced effect of the number of previous donations on haemoglobin levels than in men, perhaps because women had a smaller maximum number of donations they could have given in the previous two years due to the longer minimum inter-donation interval. This effect of previous donation was also found in other studies [113, 187, 188]. However, a previous study found more associations with haemoglobin levels than these results, including associations with smoking status, iron supplementation, and BMI, but concluded that dietary variables did not have a significant effect on haemoglobin levels [189].

Examining moderators of inter-donation interval, in men baseline haemoglobin levels had the highest-magnitude of association, with those who had the highest baseline haemoglobin having the highest two-year haemoglobin, and those on the 12 week group having higher haemoglobin compared to other groups. Considering the aim to safely maximise the blood supply, men with higher baseline haemoglobin levels could be allocated shorter inter-donation intervals, however there is currently a lack of routine haemoglobin measurement in practice.

For women, the association between haemoglobin at the start and end of the trial was such that those women on the 12 week group had a lower two year haemoglobin than those on the 14 and 16 week groups at the highest levels. Women with fewer than four donations in the previous two years had similar haemoglobin levels regardless of inter-donation interval while larger differences were observed between randomised inter-donation intervals with more previous donations. This could perhaps be explained by the fact that women with fewer donations in the past have had more time to recover haemoglobin between donations.

### 4.4.3 Ferritin Levels

For both sexes, the associations with baseline variables and two-year ferritin levels were similar. Baseline ferritin had the largest association with two-year ferritin. Those with higher donations in the past had a lower two-year ferritin, particularly in the shortest inter-donation interval. For men only, returning donors had lower two-year ferritin than those who were first time donors at the commencement of the trial. This could be due to the removal of iron from the body as a consequence of blood donation, and is in agreement with previous studies [113, 188]. Other studies also found associations between ferritin levels and donation history [76, 113, 190-192], and also found a lack of major association with dietary variables [190].

The effect of inter-donation interval on ferritin levels was moderated by red blood cell count, MCH and baseline ferritin in men. Baseline ferritin had the largest association with two year ferritin. As baseline ferritin levels rose, those on the 8 week group had lower two year ferritin than the 10 and 12 week group members, and at the highest level the 12 week group had higher ferritin than those in the 10 week group as well as the 8 week group. There was also a greater separation between randomised groups at the higher levels of baseline red blood cell count and MCH. If assessment of ferritin levels is to be considered in the future, biomarker analysis which is not routine would be required.

As for men, baseline ferritin had the largest association with two-year ferritin levels, and had significant interaction with randomised inter-donation interval in women. Differences in two-year ferritin levels diverged between inter-donation intervals as baseline ferritin increased. The association between red blood cell count and two year ferritin in women was similar to men. There was also an association between two-year ferritin and the number of previous donations, showing women who had donated more in the past had lower ferritin, likely due to the above relationship between previous donations and donation during the trial, and so they would lose more ferritin during the trial by donating more. In addition to the biomarkers, the interaction between use of iron supplements and inter-donation interval was significant, with women who did not take iron supplements having lower

two-year ferritin levels. This may be because women who take iron supplements have better overall management of their iron stores, which would be reflected in their ferritin levels.

### 4.4.4 Low Haemoglobin Deferrals

There were more variables found to be moderators of the effect of inter-donation interval on low haemoglobin deferrals in men than in women. The strongest associations were with baseline ferritin and previous low haemoglobin deferrals. At the lowest baseline ferritin levels, there were larger differences in deferral rates, with the 8 week group having the highest and 12 week the lowest, compared with the highest levels where deferral rates were similar across the inter-donation groups. This is perhaps unsurprising as ferritin is a measure of iron stores in the body, and so those with low ferritin levels are likely to have lower haemoglobin levels.

Men with more low haemoglobin deferrals in the past received more haemoglobin deferrals during the trial, perhaps because they are more susceptible to having lower haemoglobin and thus also deferrals. In concordance with **Chapter 2**, older men experienced more low haemoglobin deferrals than their younger counterparts. There was also a difference by blood group, with non-O blood group associated with slightly higher low haemoglobin deferrals on shorter inter-donation intervals. Baseline haemoglobin levels were also associated with low haemoglobin deferrals. Men with the highest baseline haemoglobin levels had fewer low haemoglobin deferrals, likely because these donors were more likely to be able to recover their haemoglobin levels as they started at a higher level. Repeat donors experienced more of an increase in low haemoglobin deferrals in the 8 week group than the 10 and 12 week groups.

In women two moderators of inter-donation interval on low haemoglobin deferrals were found – number of previous donations and low haemoglobin deferrals. More committed donors received fewer low haemoglobin deferrals during the trial. This may be because donors who have donated more in the past are more used to managing their iron levels so that they are more likely to come back

to donate only when their haemoglobin levels are sufficient. Women with more low haemoglobin deferrals in the past also experienced more such deferrals during the trial.

### 4.4.5 Implications

The INTERVAL trial's primary aim was to assess the efficacy and safety of shorter inter-donation intervals as one way to maximise the number of donations that can be given by the blood donor population in England. However, avoidance of low haemoglobin deferrals is a key issue, and this can be aided by knowing donors' haemoglobin and ferritin levels pre-donation. The variables found to have the strongest associations with donations in the trial were also moderators of the effect of inter-donation interval and could be conveniently collected from donors using existing records or questionnaires, such as number of previous donations, occupation status, iron multivitamin use, age, and PCS and MCS scores. The same was true of low haemoglobin deferrals, with a donor's donation and deferral history being key moderators of inter-donation interval for both sexes, as well as age and blood group for men.

However, haemoglobin levels, and particularly ferritin levels, often had a higher magnitude of association than these more easily available variables. While haemoglobin levels per visit can sometimes be recorded when haemoglobin is measured before donation, this is not current practice in the UK. In addition, ferritin levels are not routinely measured, and neither are other blood based biomarkers, some of which could also be useful in assigning personalised donation intervals alongside history of donations and deferrals. In addition, the cost of measuring ferritin is greater than if a haemotology analyser (e.g. Sysmex XN in the INTERVAL trial) were routinely used, the haemoglobin and other biomarker information could be readily available, although this comes at an additional cost. These results could also be used to predict donors' ferritin levels, which requires different assay for measurement. This information could be a useful counterpart to low haemoglobin deferral considerations when assigning donation intervals.

### 4.4.6 Strengths and Limitations

The primary strengths of INTERVAL were its trial design and scale. Due to the large sample size of 45,000 participants and high completeness of follow-up including 99.5% completeness for the primary outcome could be achieved due to the blood service's database [74], statistical power to compare outcomes across three donation intervals for each sex was achieved. Analysing the inter-donation interval as a continuous variable also allowed for greater statistical power. The design of the trial helped reduce confounding compared with observational studies and represented all areas of England as it was facilitated by the regular blood service [74]. It is possible that the true effect of the inter-donation interval may not have been accurately captured in the continuous modelling, as the assigned inter-donation interval may not reflect the participant's actual time to return to donate. However, this does not prevent interpretation on the basis of the intention-to-treat analysis, and while the true inter-donation interval may have varied around the randomly assigned interval, approximately 75% of trial participants attended within one week of their assigned inter-donation interval (**Figure 4.13**) [74]. Conversely, the trial context may have encouraged greater adherence to the assigned inter-donation interval, potentially reducing external validity in the usual blood donation context. While there may be concerns that non-random attrition may bias estimation of the true effect of the randomised inter-donation interval on outcomes, such bias seems unlikely as the dropout rates during the trial were not differential between the randomised inter-donation interval groups [74]. Furthermore, completeness of data for all primary and secondary outcomes was comparable across all three sex-specific inter-donation groups (**Figure 3.1**) [74].

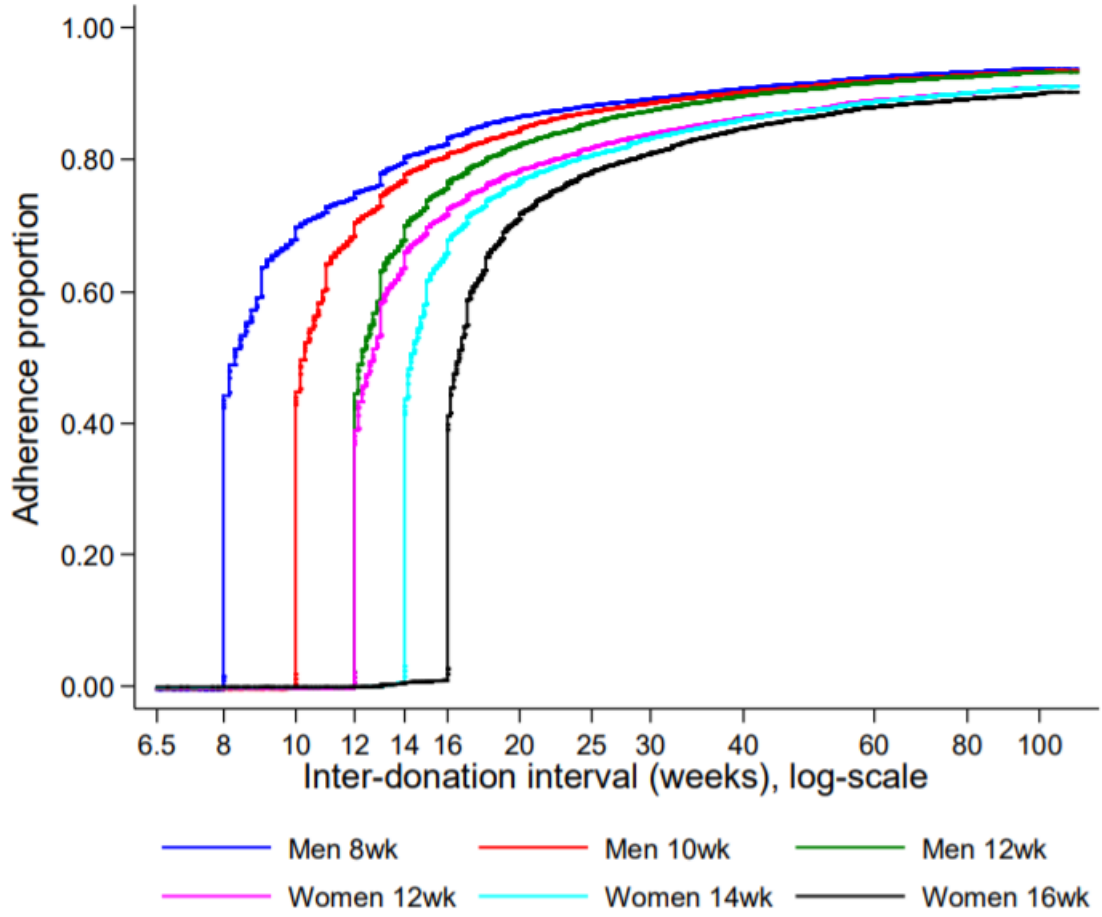



**Figure 4.13:** Adherence to assigned randomised inter-donation interval, either through making a donation or receiving a deferral. Approximately 75% of participants returned to donate within one week of their assigned interval, and adherence was not differential by trial arm [74].

Limitations of the study included the trial setting, which may overestimate the impact of the trial's primary outcome in particular if applied to the general UK blood donor population [73]. In addition, only around 45% of invited donors consented to participate in the trial. The trial also excluded donors without internet access, and so the results may not be generalisable to these donors [74]. Moreover, while few self-reported variables made it into the final models, several variables, particularly dietary variables, were self-reported, and so the true impact of these could be different than that which was observed. In addition, while variance inflation factors were low in the models, it is possible that there exists collinearity between biomarker variables such as MCV, MCH, and baseline haemoglobin, which could affect results. There are possible limitations in the statistical modelling used in these analyses. While the Poisson model assumptions may not have been strictly met by the deferral data, the

assumption of equality of mean and variance could be relaxed by using robust standard errors. In addition, there was a potential limitation in assuming that age adjustment also captured differences due to menopausal status in women. Menopausal status was self-reported at baseline (as Yes, No, or Unsure) but the exact age at menopause was not specifically ascertained. The mean age at baseline was 59.11 years for post-menopausal women and was 39.14 years for premenopausal women, a large 20 years difference that would partly explain the result that the menopausal status variable was not found to be an independent predictor in multivariable regression models adjusted for baseline age and other variables associated with menopausal status.

### 4.4.7 Conclusions

This chapter has identified key correlates and moderators of the effect of inter-donation interval on four key outcomes – the number of donations, low haemoglobin, and haemoglobin and ferritin levels. With the exception of ferritin, many of the variables that were key moderators of the effect of inter-donation interval on these outcomes are routinely collected by the blood service, or else can be collected easily using questionnaires. In general, maximising donations while minimising deferrals does not result in practical difficulties as these were often related to similar variables. When the same variable affected both, donations are maximised and deferrals minimised at the same levels of the variable. Consequently, personalising donation intervals is likely to be achievable in practice by the blood service. There are other considerations when assigning donors to shorter inter-donation intervals such as post-donation symptoms and well-being, which will be addressed in **Chapter 5** and **Chapter 6** respectively.

The primary question raised is whether ferritin testing should be used if the blood service intends to introduce personalised donation intervals. It was a moderator of the effect of inter-donation interval on most outcomes and had a high magnitude of association with number of donations for both sexes, and number of low haemoglobin deferrals for men. However, implementing ferritin testing would come at a cost to the blood service.

# Chapter 5 – Reported Occurrence of Symptoms in INTERVAL and the Role of Iron Supplementation as a Mediator of Symptoms

## Summary

Iron deficiency is a frequently reported adverse consequence of blood donation that can be exacerbated by more frequent donation as found in the INTERVAL trial. Iron supplementation has been suggested to relieve symptoms associated with iron deficiency. Mitigation of post-donation symptoms is important in donor management, as donors who experience symptoms are less likely to return to donate.

In the INTERVAL trial, participants were asked to self-report a range of symptoms every six months, including tiredness, breathlessness, fainting, and restless legs syndrome, as well as whether they were using iron supplementation. These data facilitate prospective analysis of risk factors for reported symptoms, and assessment of potential mediators of the effect of randomised inter-donation interval on symptoms, specifically iron supplementation.

In both sexes, randomised shorter inter-donation intervals significantly increased reported occurrence of symptoms, including tiredness, breathlessness, dizziness, fainting or feeling faint, restless legs syndrome and doctor diagnosed low iron. Additionally, in men only, reported occurrences of palpitations and chest pain were higher in shorter randomised inter-donation intervals. The reported use of doctor prescribed iron supplements gradually increased in the course of the trial from 2.2% at baseline to 4.4% at 2 years. While use of iron supplementation, whether through a supplement or prescribed by a doctor, was associated with symptoms in both sexes, it did not mediate the effect of inter-donation interval assignment on symptoms.

While there are other reasons why iron supplementation may be offered to blood donors, such as a desire to reduce low haemoglobin deferrals, the analyses herein suggest such strategy may not be sufficient to combat the effect of shorter inter-donation intervals on occurrence of symptoms.

## 5.1 Introduction

Iron deficiency affects 25-35% of blood donors and is a widely reported adverse consequence of blood donation [76, 84, 193]. A standard blood donation (about 470 ml of blood) removes around 240-260mg of iron [137, 194]. This can often be between one and two thirds of a woman's iron stores [195] and as much as 81% of iron stores in a menstruating woman (**Figure 5.1**). Post-menopausal women, while also at greater risk of iron deficiency than men, have much greater iron levels than pre-menopausal women, with average ferritin values increasing with age to an average of 86 ug/L post-menopause, compared to 32-53 ug/L in pre-menopausal women [193]. Physiological iron absorption is between 3 and 4 mg per day maximum [196, 197], and may not give sufficient time for a donor to recover iron levels if donating at short intervals.

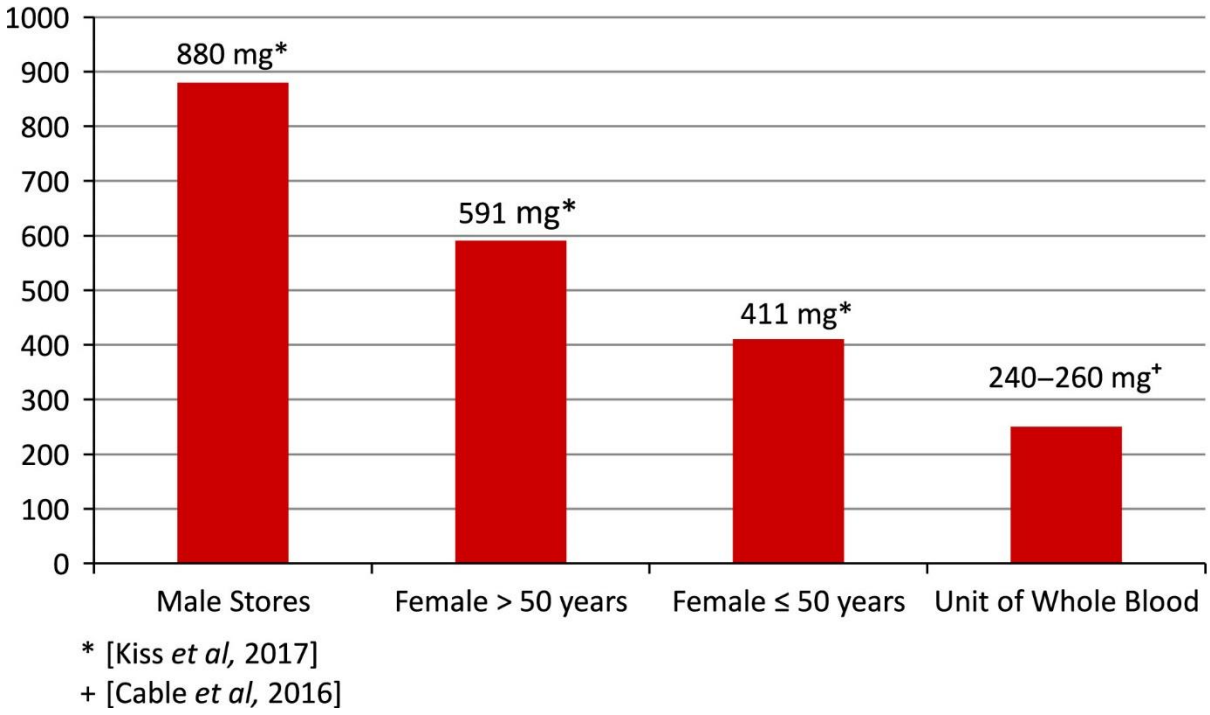



**Figure 5.1**: Iron stores in blood donors compared with iron lost in one donation [193].

Studies have shown that there is a higher prevalence of iron deficiency among younger women, and those who donate at higher intensity, whether through more donations or less time between donations [76, 84]. In the REDS -II study that assessed iron status in blood donors in the USA, donation intensity stood out as the most predictive factor of iron depletion [84]. It is possible that donors who

give blood will be subject to post-donation symptoms, such as vasovagal reactions (VVRs), due to iron deficiency following blood donation [198]. Subjective experience of mild VVRs was found to reduce donor return rates by 20% in first time donors and 33% for repeat donors, and male donors were found to be less likely to return following a VVR than females [199]. The statistic of repeat donors returning less after a VVR is of concern, as donations from repeat donors make up a large proportion of the blood supply, and so efforts to minimise symptoms from donation are crucial to maintaining a sufficient blood supply.

### 5.1.1 Possible Consequences of Iron Deficiency and Use of Iron Supplementation to Relieve Symptoms

#### 5.1.1.1 Restless Legs Syndrome

Previous studies have investigated the link between iron stores, iron supplementation and restless legs syndrome. Restless legs syndrome is characterised by paraesthesia in the legs, occurring at rest, which is relieved by movement. It frequently causes sleep disturbance, and has been linked to iron deficiency [200].

There have been inconclusive results from studies examining a relationship between restless legs syndrome and iron stores in blood donors. While two studies found that restless legs syndrome symptoms were improved when donors used iron supplementation [201, 202]. In a systematic review of studies with blood donor populations, six out of nine studies did not report an association between iron deficiency and restless legs syndrome [203].This may be because iron balance does not affect all causes of restless legs syndrome. Other conditions associated with restless legs syndrome include renal failure, pregnancy, and neuropathy [200, 204]. As such, restless legs syndrome may be inconsistently reversible with iron supplementation when associated with iron deficiency [193, 201, 202, 205]. Blood donors who develop such symptoms may not necessarily do so due to iron deficiency, and so it may not be appropriate to regard iron supplementation as a cure for restless legs syndrome.

#### 5.1.1.2 Pica

Pica is another documented side effect of iron deficiency and blood donation. The most common form of pica, the desire to eat non-nutritious substances, associated with iron deficiency is eating ice [201]. A study in a blood donor population in the USA found that pica and iron status were associated in female donors but not male donors, however incidence of pica was low [205].

Another study investigated the relationship between pica and haemoglobin levels and found that lower haemoglobin levels corresponded to a higher prevalence of pica. This suggests that, as with restless legs syndrome, there could be other mechanisms which cause pica in donors than simply iron deficiency [206].

#### 5.1.1.3 Fatigue

Fatigue is a widely reported symptom of iron deficiency [207, 208]. However, iron repletion has benefited patients complaining of chronic fatigue [193]. It is possible then that iron supplementation could help relieve fatigue if donors develop it as a consequence of more frequent donation.

A previous study found that the ability of iron supplementation to relieve fatigue was dependent on iron stores, with only those with ferritin values below 50 ug/L reporting a decrease in fatigue after one month of iron supplementation, and found an effect of iron supplementation in relieving fatigue in adolescent girls [209]. Another study found that the number of donors reporting fatigue more than halved following iron supplementation [210]. One meta-analysis of six randomised controlled trials and six cross-sectional studies in non-anaemic individuals and found that iron supplementation reduced fatigue. The meta-analysis was also robust to sensitivity analysis without evidence of publication bias [208].

#### 5.1.1.4 Physical Symptoms

Some studies have investigated the relationship between physical symptoms such as exercise capacity and iron supplementation in blood donors, as well as an effect on general quality of life.

A meta-analysis found that exercise capacity was reduced in donors two days following a blood donation [211]. While this could be due to decreased blood volume, it is possible that reduced iron levels could also play a role. Randomised double-blind controlled trials have found that iron supplementation can be associated with an increase in aerobic capacity [212-215] and a decrease in muscle fatigue [216].

The INTERVAL trial assessed several physical symptoms which have not been studied in the literature, including fainting, breathlessness and chest pain, and found that these were more prevalent in the shorter donation groups [74]. It is possible that, with such donors losing more iron during the trial, that this could be driven in part by iron stores.

#### 5.1.1.5 Cognitive Symptoms

Iron has an impact on the brain's development, and some have particularly pointed to risks associated with blood donation and the associated loss of iron in the development of adolescents.

Verbal learning and memory in adolescent girls has been shown to be improved with iron supplementation [217], and a systematic review of 14 studies on women and children's cognition found that iron supplementation improved attention, concentration and intelligence. There was no effect on non-anaemic participants [218]. It is possible therefore that iron supplementation could help reduce the risks of cognitive symptoms in blood donors due to iron deficiency.

#### 5.1.1.6 Side Effects of Iron Supplementation

Other symptoms have been studied in relation to iron supplementation, often by being reported as side effects by participants. These side effects include gastrointestinal symptoms, such as gastric discomfort, diarrhoea, and constipation [195, 210, 219].

There is also the question of whether donors who take iron supplementation are more susceptible to VVRs than those who do not. One study which randomised donors to take iron supplementation or not before donation found no significant difference between symptoms in the iron and placebo groups,

with the majority of adverse events reported by blood donors were related to venepuncture [220]. A meta-analysis of four studies found that the risk of adverse events was not different between the group of donors taking iron supplements and placebo group [221]. On the other hand, in another study, the iron group had significantly more people report any symptom (P=0.002) [195], and a meta-analysis of four studies (1748 participants) showed a significant increase in adverse events was associated with iron supplementation (Risk ratio 16 (1.23-2.07)) [155]. It is therefore unclear if iron supplementation affects adverse events.

### 5.1.2 Conclusions From Current Literature

Short-term iron supplementation in donors may help to reduce the risk of post-donation adverse symptoms, including restless legs syndrome, pica, and fatigue.

One limitation of the studies that have been carried out on iron supplementation and symptoms is that they often suffer from low sample sizes. It is possible that, due to its much larger sample size, INTERVAL data could address this limitation. Moreover, characteristics of study populations are variable and so conclusions from previous literature may not be generalisable to a healthy blood donor population. In addition, many symptoms recorded in INTERVAL such as feeling faint, breathlessness, and chest pain, had not been widely studied, or studied at all. However, iron supplementation was not included in INTERVAL's trial design, and symptoms was a secondary outcome of the trial.

### 5.1.3 Post-Donation Symptoms and Iron Supplementation in INTERVAL

Variation of the inter-donation interval as conducted in INTERVAL could present an increased challenge to the UK blood service from iron deficiency as donors lose iron more regularly due to more regular blood donation.

INTERVAL studied not just serious adverse events and VVRs such as heart attacks, but also less serious post-donation symptoms which a donor may attribute to frequent blood donation and may affect their willingness to return.

The symptoms studied in INTERVAL were tiredness, breathlessness, dizziness, chest pain, palpitations, fainting or feeling faint, and restless legs syndrome. Previous findings from the INTERVAL study have found that increasing the inter-donation interval did not cause an increase in fainting events, or harm cognitive function or physical ability of donors. However, there was a moderate increase in other symptoms, particularly amongst men [74]. In an extension study, there were no clear differences between frequency of reporting symptoms by randomised group [186]. This may be because those who experienced increased symptoms during the INTERVAL's two-year period may have been less likely to participate in the extension study.

### 5.1.4 Statistical Mediation

In clinical trials, it is possible that there is an event or change in other characteristics influenced by the treatment, which may explain the effect of the intervention observed on outcomes [222]. In INTERVAL, the randomised inter-donation interval may affect symptoms directly or via iron-related pathways, which would also be influenced by iron supplementation. In this case, iron supplementation would be a mediator of the effect of inter-donation interval. If this is not considered when performing analysis, it is possible that models will over or underestimate the effects of predictors, and some which appear significant may not be due to confounders which are not present in the model.

**Figure 5.2** gives a graphical interpretation of a mediator. Without the mediator (iron supplementation), it is assumed that the effect of the independent variable (randomised inter-donation interval) on the outcome variable (symptom) is from the direct effect (path c). It is possible instead that there could be the mediator variable the indirect effect of the independent variable on outcome (via path a and path b) may be not be ignorable.

A mediator meets the following conditions: that variations in the independent variable significantly account for variations in the mediator, and vice-versa, and that there is a significant change in the direct effect of the independent variable on the outcome (path c vs path $c_{adjusted}$) when the mediator is added to the model [223]. An extension to these criteria is the MacArthur method, which extends

this framework by requiring the inclusion of an interaction between the independent variable (i.e. inter-donation interval) and the proposed mediator [224]. The MacArthur method essentially accounts for the possibility that a statistically significant interaction may reflect statistical mediation [225], thereby decreasing the probabilities of both a type I error (rejecting the null hypothesis when it is true) and a type II error (failing to reject the null hypothesis when it is false).

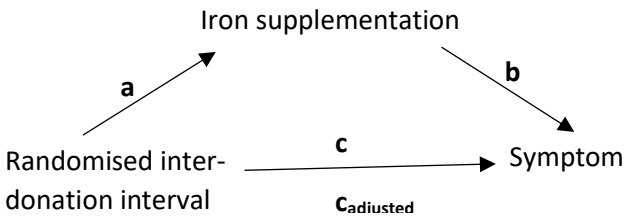



**Figure 5.2**: Diagram of the effect paths when a mediator is present. When no mediator is present, only effect c is recorded by the model. When the mediator is added, c becomes $c_{adjusted}$.

### 5.1.5 Chapter Aims

To enhance the evidence base and overcome the limitations of previous studies on the occurrence of donor symptoms including fatigue, fainting, and restless legs syndrome, in this chapter I investigated the relationship between randomised inter-donation interval and symptoms thought to be related to iron deficiency. I also investigated iron supplementation as potential mediator of the effect of randomised inter-donation interval on symptoms.

## 5.2 Methods

### 5.2.1 Measurement of Symptoms in INTERVAL

All symptoms were assessed in the six-monthly questionnaires that asked participants if they had experienced a list of symptoms in the past six months. Clinical Restless legs syndrome was also assessed via the Cambridge-Hopkins questionnaire at the 18$^{th}$ and 24$^{th}$ months of follow [7, 169]. Pica was assessed by questions which asked participants if they had craved and regularly eaten or chewed ice, clay, dirt, raw pasta, chalk, or coal. The same questions were asked at all time points.

### 5.2.2 Iron Supplementation in the INTERVAL trial

Iron supplementation was not randomised in the INTERVAL trial, however its usage was enquired in the questionnaires completed by participants. At baseline and after two years participants were asked if they took iron supplements prescribed by a doctor, or contained within a multivitamin, or iron only supplements. Every six months, participants were asked if they had seen a doctor who had diagnosed them with low iron or prescribed them iron supplements.

### 5.2.3 Statistical Analysis

Logistic regression models fitted using the generalised estimating equation (GEE) modelling framework [226, 227] were used to assess the effect of inter-donation interval on symptoms and assess role of iron supplementation use as a potential mediator. The GEE modelling framework is primarily designed to assess predictors of the mean of a transformed response (e.g. log odds) while accounting for relatedness of observations within clusters based on an assumed working correlation matrix. This framework was used in order to take into account to account correlation of repeated observations on the same individual [228, 229]. Each symptom was assessed up to four times during the study [7] and so data rows were not independent and identically distributed [230, 231]. An exchangeable correlation structure was assumed for the repeated measures within individuals, meaning equally correlated across time points [232], with individuals considered independent. However, for unbiased inferences, the GEE model requires that data be missing completely at random (MCAR), i.e. that the probability distribution of missing measurements is independent of both the unobserved and observed measurements. Because this may be a rather strong assumption, sensitivity analyses were conducted based on mixed effects logistic regression (MELOGIT) which provides unbiased inferences under the less strict, but more plausible, missing at random (MAR) assumption, i.e. that the probability distribution of missing measurements is independent of the unobserved measurements given the observed measurements.

Analyses were sex-specific and the effect of randomised inter-donation interval was modelled as a linear trend. Covariate adjustments were introduced in three progressive models to assess changes in the effect of randomised inter-donation interval with adjustments. Model 1 adjusted for age, weight, new donor status, and donation centre. Model 2 further adjusted for the participant's use of iron supplementation at baseline. Model 3 further adjusted for the participant's use of iron supplementation during the trial as assessed by the six-month questionnaires. All associations were presented with a 95% confidence interval. Statistical significance was based on $p < 0.05$ throughout.

## 5.3 Results

### 5.3.1 Frequency of Symptoms

The three most frequently reported symptoms were tiredness, restless legs, and dizziness. Comparing the results of the 12-week group, the frequency of symptoms reporting was generally higher in women than men (**Table 5.1)**.

### 5.3.2 Completion of Questionnaire

For all symptoms in all randomised groups, the proportion of INTERVAL participants completing the questionnaire reduced over the 2-year trial period. (**Table 5.2**). However, dropout during follow up was not differential across the randomised inter-donation intervals, and therefore inferences should remain unbiased. Furthermore, the GEE modelling framework used for analyses allowed the inclusion of information from all available questionnaires completed during follow up, as opposed to requiring restriction to participants with all questionnaires completed.

**Table 5.1**: Number of donors (%) who reported symptoms or iron prescription at any point during the trial by randomised group

| | Men | | | Women | | |
|---|---|---|---|---|---|---|
| | **8 weeks (N = 7456)** | **10 weeks (N = 7446)** | **12 weeks (N = 7452)** | **12 weeks (N = 7567)** | **14 weeks (N = 7565)** | **16 weeks (N = 7548)** |
| **Any Symptom** | | | | | | |
| Yes | 3458 (46.4) | 3244 (43.6) | 3090 (41.5) | 4210 (55.6) | 4060 (53.7) | 3967 (52.6) |
| **Tiredness** | | | | | | |
| Yes | 2034 (27.3) | 1774 (23.8) | 1720 (23.1) | 2561 (33.8) | 2343 (31.0) | 2277 (30.2) |
| **Breathlessness** | | | | | | |
| Yes | 942 (12.6) | 783 (10.5) | 710 (9.5) | 1083 (14.3) | 963 (12.7) | 967 (12.8) |
| **Palpitations** | | | | | | |
| Yes | 698 (9.4) | 633 (8.5) | 585 (7.9) | 1234 (16.3) | 1116 (14.8) | 1150 (15.2) |
| **Dizziness** | | | | | | |
| Yes | 1039 (13.9) | 947 (12.7) | 886 (11.9) | 1706 (22.5) | 1618 (21.4) | 1525 (20.2) |
| **Feeling Faint** | | | | | | |
| Yes | 774 (10.4) | 660 (8.9) | 598 (8.0) | 1257 (16.6) | 1162 (15.4) | 1119 (14.8) |
| **Fainting** | | | | | | |
| Yes | 125 (1.7) | 96 (1.3) | 82 (1.1) | 249 (3.3) | 225 (3.0) | 211 (2.8) |
| **Chest Pain** | | | | | | |
| Yes | 455 (6.1) | 405 (5.4) | 384 (5.2) | 415 (5.5) | 367 (4.9) | 409 (5.4) |
| **RLS** | | | | | | |
| Yes | 1442 (19.3) | 1334 (17.9) | 1245 (16.7) | 1841 (24.3) | 1764 (23.3) | 1741 (23.1) |
| **Iron Prescription** | | | | | | |
| Yes | 460 (6.2) | 343 (4.6) | 260 (3.5) | 691 (9.1) | 591 (7.8) | 538 (7.1) |

**Table 5.2**: Number (%) of INTERVAL participants who completed the questions for reporting symptoms at each time point in the trial by randomised group

| Symptom | Men | | | Women | | |
|---|---|---|---|---|---|---|
| | 8 weeks (N = 7456) | 10 weeks (N = 7446) | 12 weeks (N = 7452) | 12 weeks (N = 7567) | 14 weeks (N = 7565) | 16 weeks (N = 7548) |
| **Tiredness** | | | | | | |
| 6 months | 5927 (79%) | 5869 (79%) | 5733 (77%) | 5889 (78%) | 5953 (79%) | 5875 (78%) |
| 12 months | 5396 (72%) | 5395 (72%) | 5369 (72%) | 5330 (70%) | 5479 (72%) | 5301 (70%) |
| 18 months | 4789 (64%) | 4814 (65%) | 4831 (65%) | 4679 (62%) | 4737 (63%) | 4694 (62%) |
| 24 months | 4908 (66%) | 4922 (66%) | 4937 (66%) | 4715 (62%) | 4714 (62%) | 4651 (62%) |
| **Breathlessness** | | | | | | |
| 6 months | 5921 (79%) | 5862 (79%) | 5725 (77%) | 5875 (78%) | 5943 (79%) | 5866 (78%) |
| 12 months | 5387 (72%) | 5385 (72%) | 5364 (72%) | 5322 (70%) | 5372 (71%) | 5298 (70%) |
| 18 months | 4787 (64%) | 4812 (65%) | 4827 (65%) | 4675 (62%) | 4730 (63%) | 4690 (62%) |
| 24 months | 4886 (66%) | 4896 (66%) | 4926 (66%) | 4695 (62%) | 4701 (62%) | 4633 (61%) |
| **Palpitations** | | | | | | |
| 6 months | 5913 (79%) | 5855 79%) | 5721 (77%) | 5869 (78%) | 5946 (79%) | 5869 (78%) |
| 12 months | 5385 (72%) | 5379 (72%) | 5362 (72%) | 5311 (70%) | 5371 (71%) | 5282 (70%) |
| 18 months | 4780 (64%) | 4816 (65%) | 4815 (65%) | 4665 (62%) | 4762 (63%) | 4683 (62%) |
| 24 months | 4895 (66%) | 4914 (66%) | 4926 (66%) | 4702 (62%) | 4707 (62%) | 4641 (61%) |
| **Dizziness** | | | | | | |
| 6 months | 5917 (79%) | 5853 (79%) | 5723 (77%) | 5868 (78%) | 5938 (78%) | 5867 (78%) |
| 12 months | 5387 (72%) | 5380 (72%) | 5360 (72%) | 5312 (70%) | 5365 71%) | 5291 (70%) |
| 18 months | 4777 (64%) | 4810 (65%) | 4820 (65%) | 4671 (62%) | 4731 (63%) | 4680 (62%) |
| 24 months | 4908 (66%) | 4916 (66%) | 4938 (66%) | 4715 (62%) | 4714 (62%) | 4651 (62%) |
| **Feeling Faint** | | | | | | |
| 6 months | 5912 (79%) | 5846 (79%) | 5715 (77%) | 5866 (78%) | 5933 (78%) | 5865 (78%) |
| 12 months | 5377 (72%) | 5381 (72%) | 5353 (72%) | 5312 (70%) | 5366 (71%) | 5292 (70%) |
| 18 months | 4784 (64%) | 4804 (65%) | 4823 (65%) | 4666 (62%) | 4716 (62%) | 4681 (62%) |
| 24 months | 4908 (66%) | 4914 (66%) | 4938 (66%) | 4715 (62%) | 4714 (62%) | 4651 (62%) |
| **Fainting** | | | | | | |
| 6 months | 5904 (79%) | 5844 (79%) | 5707 (77%) | 5849 (77%) | 5925 (78%) | 5837 (77%) |
| 12 months | 5367 (72%) | 5376 (72%) | 5345 (72%) | 5300 (70%) | 5352 (71%) | 5270 (70%) |
| 18 months | 4763 (64%) | 4794 (64%) | 4817 (65%) | 4653 (61%) | 4704 (62%) | 4661 (62%) |
| 24 months | 4907 (66%) | 4917 (66%) | 4935 (66%) | 4709 (62%) | 4711 (62%) | 4647 (62%) |
| **Chest Pain** | | | | | | |
| 6 months | 5905 (79%) | 5842 (79%) | 5718 (77%) | 5857 (77%) | 5919 (78%) | 5838 (77%) |
| 12 months | 5362 (72%) | 5377 (72%) | 5339 (72%) | 5299 (70%) | 5341 (71%) | 5267 (70%) |
| 18 months | 4767 (64%) | 4799 (64%) | 4814 (65%) | 4663 (62%) | 4712 (62%) | 4669 (72%) |
| 24 months | 4886 (66%) | 4905 (66%) | 4909 (66%) | 4689 (62%) | 4692 (62%) | 4624 (61%) |
| **RLS** | | | | | | |
| 6 months | 5896 (79%) | 5835 (79%) | 5700 (77%) | 5853 (77%) | 5918 (78%) | 5839 (77%) |
| 12 months | 5387 (72%) | 5387 (72%) | 5362 (72%) | 5318 (70%) | 5370 (71%) | 5283 (70%) |
| 18 months | 4795 (64%) | 4814 (65%) | 4834 (65%) | 4686 (62%) | 4747 (63%) | 4704 (62%) |
| 24 months | 4851 (65%) | 4863 (65%) | 4894 (66%) | 4664 (62%) | 4668 (62%) | 4601 (61%) |
| **Iron Prescription** | | | | | | |
| 6 months | 5900 (79%) | 5835 (78%) | 5694 (76%) | 5871 (78%) | 5935 (78%) | 5856 (78%) |
| 12 months | 5370 (72%) | 5364 (72%) | 5267 (71%) | 5308 (70%) | 5369 (71%) | 5268 (70%) |
| 18 months | 4687 (63%) | 4733 (64%) | 4656 (62%) | 4594 (61%) | 4662 (62%) | 4620 (61%) |
| 24 months | 4923 (66%) | 4940 (66%) | 4957 (67%) | 4739 (63%) | 4737 (63%) | 4672 (62%) |

### 5.3.3 Associations with Baseline Variables and Symptoms

Multivariable adjusted associations of randomised inter-donation interval and other baseline variables and reported symptoms are summarised in **Table 5.3**. In both men and women, shorter randomised inter-donation interval significantly increased the odds of all symptoms assessed, other than pica (in both sexes) and chest pain (in women).

As regards other baseline variables, for men, older age was associated with lower odds of reporting fainting or feeling faint, palpitations, chest pain and pica. Higher weight was associated with lower odds of reporting fainting or feeling faint and doctor diagnosed low iron. Higher baseline haemoglobin and baseline ferritin were associated with lower odds of reporting all symptoms, and a higher number of donations in the past two years was associated with lower odds of reporting all symptoms except pica. A higher number of low haemoglobin deferrals in the past was associated with lower odds of reporting fainting or feeling faint. On the other hand, older age was associated with higher odds of reporting breathlessness, restless legs syndrome, and doctor diagnosed low iron, and higher weight was associated with higher odds of reporting breathlessness and pica (**Table 5.3**).

For women, older age was associated with lower odds of reporting fainting or feeling faint, tiredness, dizziness, chest pain, and pica. Higher weight was associated with lower odds of reporting fainting or feeling faint. First time donor status was associated with lower odds of reporting dizziness. A higher number of low haemoglobin deferrals was associated with lower odds of reporting tiredness and breathlessness. Higher baseline ferritin was associated with lower odds of reporting all symptoms except for chest pain and pica. As in men, higher haemoglobin levels were associated with lower odds of reporting all symptoms, a higher number of donations in the past two years was associated with lower odds of reporting all symptoms except pica. On the other hand, older age was associated with higher odds of reporting restless legs syndrome; higher weight was associated with higher odds of reporting tiredness and breathlessness, and higher low haemoglobin deferrals in the previous two years was associated with higher odds of reporting doctor diagnosed low iron (**Table 5.3**).

**Table 5.3**: Odds ratios of associations between baseline variables and reported symptoms over the two year trial period

| Variable | Fainting or Feeling Faint | Tiredness | Breathlessness | Palpitations | Dizziness | Chest Pain | RLS | Doctor Diagnosed Low Iron | Pica |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **Men** | | | | | |
| Inter-Donation Interval[4] | 1.07 (1.04, 1.11) | 1.06 (1.04, 1.08) | 1.09 (1.06, 1.12) | 1.05 (1.02, 1.08) | 1.06 (1.04, 1.09) | 1.05 (1.01, 1.10) | 1.03 (1.01, 1.05) | 1.20 (1.16, 1.24) | 1.05 (0.93, 1.19) |
| Age (years)[5] | 0.83 (0.79, 0.88) | 0.96 (0.93, 1.00) | 1.11 (1.06, 1.17) | 0.85 (0.80, 0.90) | 0.98 (0.94, 1.03) | 0.86 (0.80, 0.93) | 1.20 (1.16, 1.24) | 1.46 (1.37, 1.55) | 0.42 (0.33, 0.54) |
| Weight (kg)[5] | 0.87 (0.82, 0.92) | 1.02 (0.98, 1.05) | 1.17 (1.11, 1.23) | 1.03 (0.97, 1.09) | 0.96 (0.92, 1.01) | 1.04 (0.97, 1.12) | 1.03 (0.99, 1.07) | 0.83 (0.78, 0.89) | 1.48 (1.24, 1.77) |
| New Donor (Y/N) | 0.92 (0.75, 1.13) | 0.96 (0.84, 1.09) | 0.87 (0.71, 1.08) | 0.84 (0.67, 1.06) | 0.97 (0.80, 1.17) | 0.83 (0.62, 1.11) | 1.06 (0.92, 1.22) | 0.87 (0.66, 1.18) | 0.84 (0.38, 1.84) |
| Haemoglobin (g/dL)[5] | 0.81 (0.77, 0.86) | 0.92 (0.89, 0.95) | 0.99 (0.94, 1.04) | 0.92 (0.87, 0.98) | 0.84 (0.80, 0.89) | 0.93 (0.85, 1.00) | 0.92 (0.89, 0.96) | 0.71 (0.66, 0.75) | 0.48 (0.38, 0.61) |
| Ferritin (µg/L)[5] | 0.88 (0.84, 0.93) | 0.92 (0.89, 0.95) | 0.84 (0.80, 0.89) | 0.92 (0.87, 0.97) | 0.86 (0.82, 0.90) | 0.89 (0.83, 0.96) | 0.85 (0.82, 0.88) | 0.64 (0.61, 0.75) | 0.70 (0.56, 0.86) |
| 2-Year Donations (n)[5] | 0.84 (0.80, 0.89) | 0.88 (0.85, 0.91) | 0.84 (0.80, 0.89) | 0.84 (0.79, 0.90) | 0.87 (0.83, 0.91) | 0.90 (0.83, 0.97) | 0.88 (0.85, 0.92) | 0.86 (0.81, 0.92) | 0.82 (0.64, 1.04) |
| 2-Year Low Hb Deferrals (n)[5] | 0.91 (0.84, 0.98) | 0.99 (0.96, 1.03) | 1.02 (0.96, 1.08) | 0.96 (0.89, 1.04) | 0.96 (0.90, 1.01) | 1.00 (0.92, 1.09) | 0.96 (0.92, 1.00) | 1.10 (1.05, 1.16) | 0.85 (0.60, 1.03) |
| | | | | **Women** | | | | | |
| Inter-Donation Interval[4] | 1.04 (1.01, 1.06) | 1.04 (1.02, 1.06) | 1.04 (1.01, 1.06) | 1.03 (1.00, 1.05) | 1.04 (1.02, 1.06) | 1.00 (0.96, 1.04) | 1.01 (0.99, 1.03) | 1.09 (1.06, 1.12) | 1.00 (0.92, 1.09) |
| Age (years)[5] | 0.62 (0.60, 0.65) | 0.84 (0.81, 0.86) | 0.97 (0.92, 1.02) | 0.99 (0.94, 1.03) | 0.83 (0.80, 0.86) | 0.72 (0.67, 0.78) | 1.30 (1.26, 1.34) | 0.95 (0.91, 1.00) | 0.94 (0.93, 0.96) |
| Weight (kg)[5] | 0.90 (0.86, 0.94) | 1.07 (1.04, 1.10) | 1.13 (1.08, 1.18) | 0.97 (0.93, 1.02) | 1.01 (0.98, 1.05) | 1.03 (0.96, 1.11) | 1.03 (1.00, 1.07) | 0.93 (0.88, 0.98) | 1.01 (1.00, 1.02) |
| New Donor (Y/N) | 0.98 (0.86, 1.12) | 0.92 (0.83, 1.01) | 0.89 (0.76, 1.04) | 0.86 (0.74, 1.00) | 0.87 (0.77, 0.99) | 0.86 (0.67, 1.09) | 0.93 (0.83, 1.03) | 0.92 (0.78, 1.09) | 0.80 (0.48, 1.36) |
| Haemoglobin (g/dL)[5] | 0.87 (0.83, 0.92) | 0.91 (0.88, 0.94) | 0.91 (0.86, 0.96) | 0.95 (0.90, 0.99) | 0.87 (0.83, 0.91) | 0.92 (0.84, 1.00) | 0.94 (0.91, 0.97) | 0.69 (0.65, 0.73) | 0.49 (0.43, 0.56) |
| Ferritin (µg/L)[5] | 0.91 (0.88, 0.95) | 0.96 (0.94, 0.99) | 0.88 (0.84, 0.92) | 0.95 (0.91, 0.99) | 0.92 (0.89, 0.95) | 0.95 (0.89, 1.02) | 0.91 (0.89, 0.94) | 0.67 (0.64, 0.70) | 0.99 (0.98, 1.00) |
| 2-Year Donations (n)[5] | 0.82 (0.78, 0.87) | 0.81 (0.78, 0.84) | 0.77 (0.73, 0.82) | 0.83 (0.79, 0.88) | 0.81 (0.77, 0.84) | 0.83 (0.76, 0.91) | 0.86 (0.83, 0.89) | 0.74 (0.69, 0.78) | 0.94 (0.85, 1.04) |
| 2-Year Low Hb Deferrals (n)[5] | 0.98 (0.95, 1.01) | 0.96 (0.94, 0.99) | 0.95 (0.92, 0.98) | 0.99 (0.95, 1.02) | 0.98 (0.96, 1.01) | 0.98 (0.92, 1.03) | 0.98 (0.96, 1.01) | 1.07 (1.04, 1.10) | 0.92 (0.67, 1.28) |

[4] Per one week decrease

[5] Per standard deviation increase. SDs reported in **Table 3.1** and **Table 3.2**

### 5.3.4 Trends of Symptoms over Trial Period

The proportion of donors reporting symptoms generally increased over the 2-year trial period and was generally higher in women than men (**Figure 5.3**). Approximately 15-20% of men and 20-28% of women reported feeling tired during at least one six month period of the trial and between 5-10% of men and women reported feeling breathless. The proportion of donors reporting palpitations steadily increased from ~4% to ~6% for male donors and from ~7% to ~13% for women. The proportion of donors experiencing dizziness rose from ~4-6% in men and ~9-11% in women after six months on the trial to ~5-7% in men and ~11-13% in women after 12 months. This further rose to ~9-11% in men and ~15-17% in women, with the shortest donation group reporting most symptoms. The proportion of donors who reported fainting or feeling faint steadily rose from ~4% to ~6-8% for men during the study period, and ~7-8% to ~10-11% in women. The proportion of participants reporting chest pain did not vary by sex or randomised group, rising from ~2% to ~4% during the trial. Restless legs syndrome also steadily rose during the trial, with men on the eight week group experiencing it more often. RLS levels rose from ~9-10% to ~14-16% by the end of the trial. There was less of a difference by randomised group for women, with RLS prevalence rising from ~14-15% at the start of the trial to ~22% irrespective of randomised group by the end. Prevalence of pica, which was only measured at the end of the trial, was low for both sexes and did not vary significantly by randomised group. Approximately 1% of men and ~2% of women reported it at the end of two years.

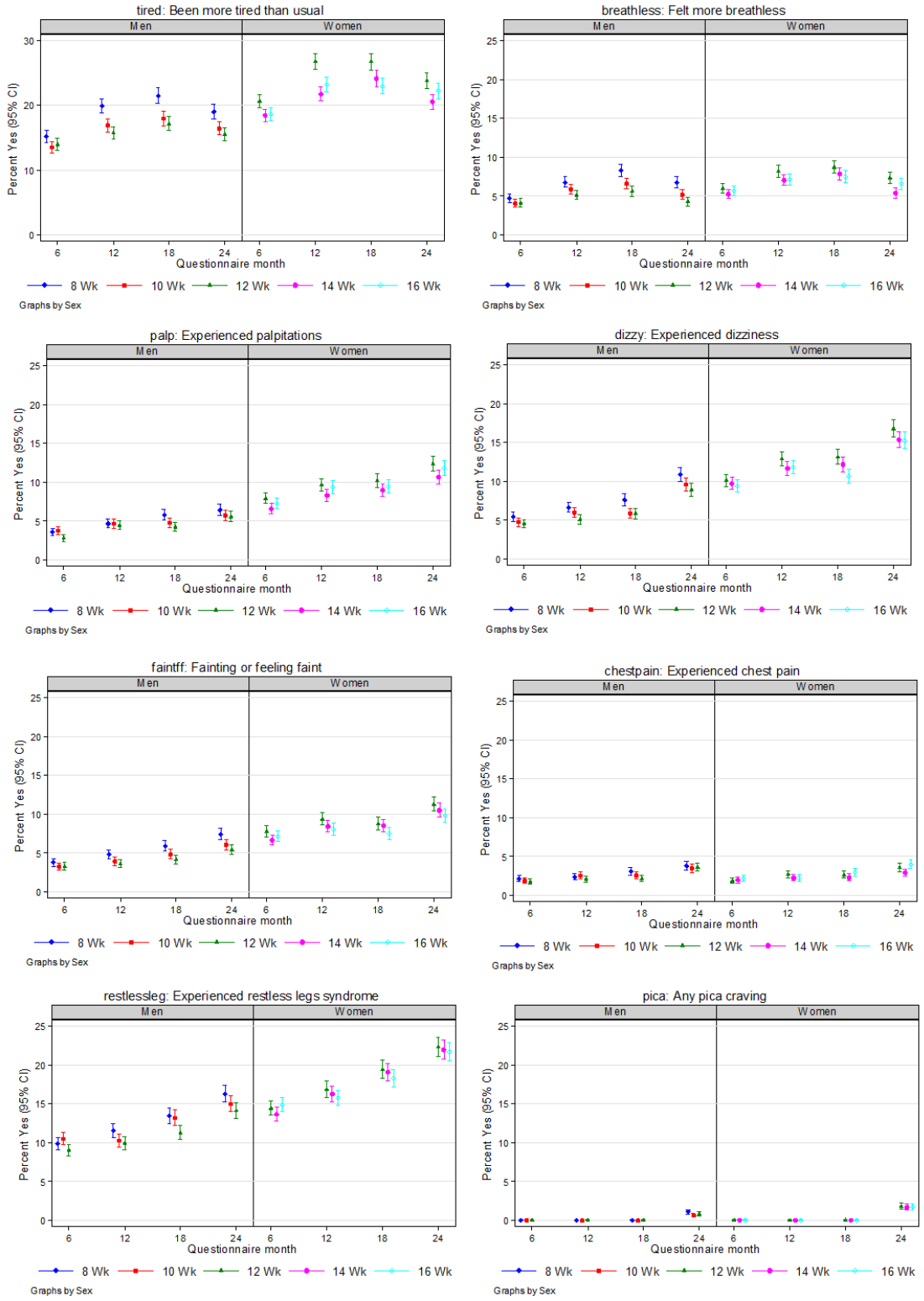



**Figure 5.3**: Frequency of tiredness, breathlessness, palpitations, dizziness, fainting or feeling faint, chest pain, restless legs syndrome, and pica over the two-year trial period by sex and randomised group, denoted by the percentage who indicated each symptom on the questionnaire.

### 5.3.5 Trends of Symptoms over Donation Period by Iron Supplementation

The proportion of donors using doctor prescribed iron supplements rose during the trial in both sexes. In men, the eight week group had the highest usage of iron supplements, and differences between the randomised groups significantly diverged as the trial progressed. More women than men used iron supplements at all time points during the trial. The 12 week group usually had the highest proportion of iron supplement users in women, but there was less clear divergence over time in women as compared to men (**Figure 5.4**)

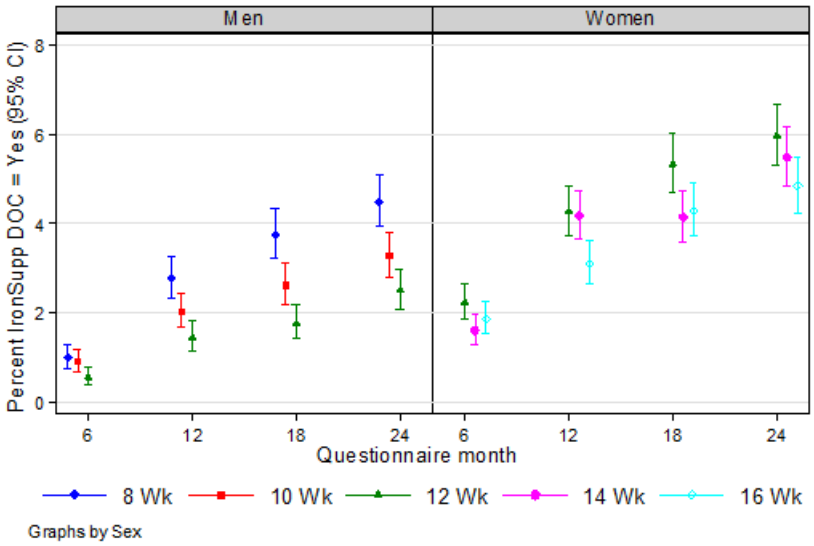



**Figure 5.4**: Proportion of donors using doctor prescribed iron supplements during INTERVAL, denoted by the percentage who indicated yes on the questionnaire. Higher numbers of women than men used iron supplementation.

The proportion of donors reporting symptoms was higher among donors who also reported iron-supplementation use over the 2-year trial period than those who did not (**Figure 5.5**). Moreover, the proportion of iron-supplementation users reporting symptoms appeared stable over the 2-year donation period, in contrast to an apparent rise in reporting of symptoms for donors not using iron-supplementation. The only exceptions were palpitations in men and chest pain in both sexes, which consistently had a similar proportion of donors reporting these symptoms regardless of whether they were taking iron supplementation.

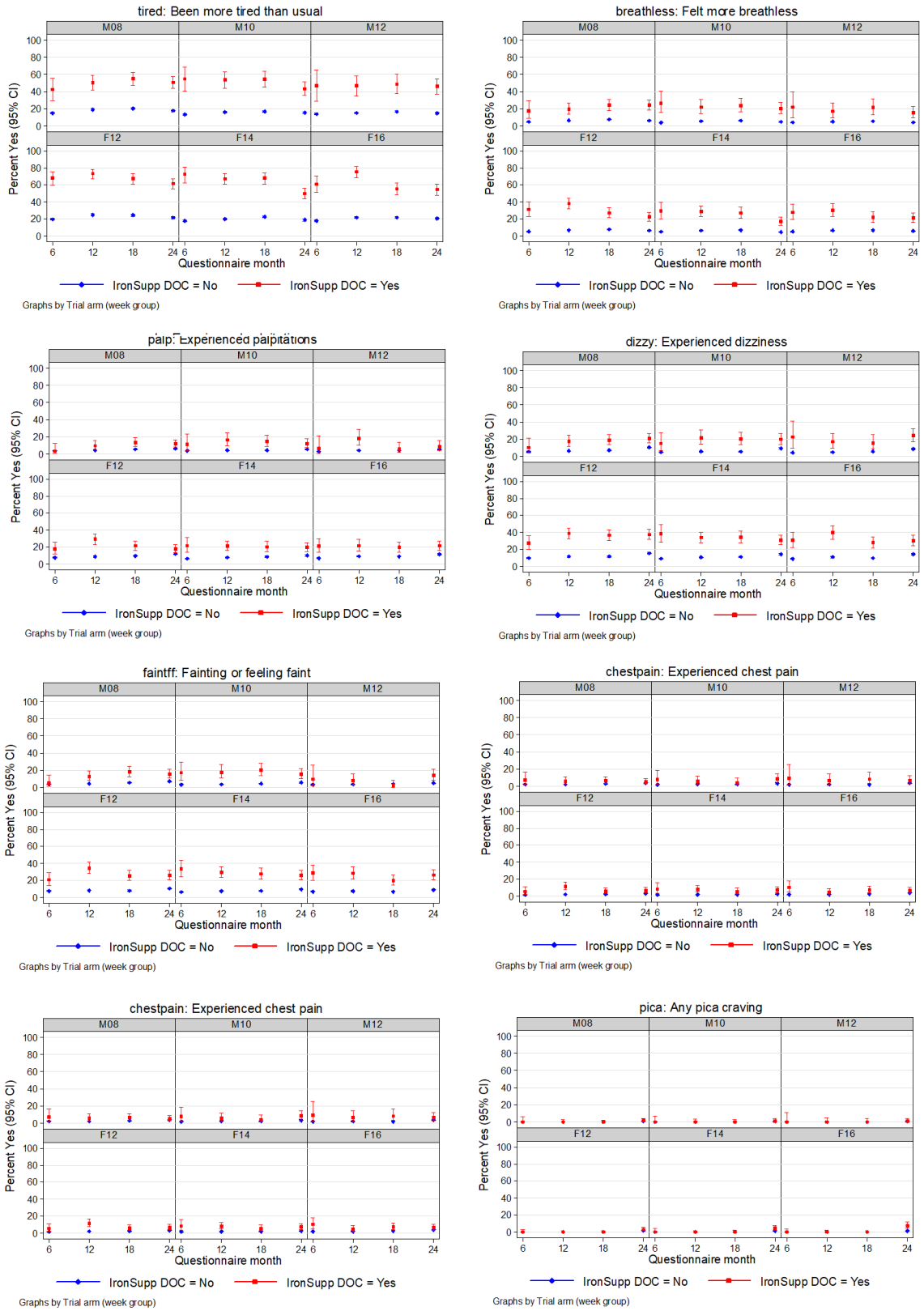



**Figure 5.5**: Frequency of tiredness, breathlessness, palpitations, dizziness, fainting or feeling faint, chest pain, restless legs syndrome, and pica over time by randomised group and iron supplement use. Panels indicate each inter-donation interval group, blue lines those who did not use iron supplementation, and red lines those who did.

### 5.3.6 Assessing mediation with Iron Supplementation

The proportion of donors reporting symptoms was generally higher in the shorter inter-donation intervals. The odds of reporting feeling faint or breathless, increased by approximately 10%, whereas the odds of reporting feeling tired, experiencing papulations, dizziness, and chest pain increased by approximately 4-5% per week shorter inter-donation interval. Odds of RLS increased by 3%. There was no significant increase in the odds of reporting pica (**Table 5.4**). For women, the odds of reporting fainting or feeling faint, tiredness, breathlessness and dizziness increased by 3-4% for each week shorter donation interval. The odds of reporting palpitations rose by 2%. There was no significant difference in the odds of reporting chest pain, restless legs syndrome, or pica (**Table 5.5**).

Use of iron supplementation, whether through a supplement or prescribed by a doctor, was significantly associated with symptoms in both men (**Table 5.4**) and women (**Table 5.5**). However, the effect of randomised inter-donation interval on symptoms did not change with the progressive addition of iron supplementation variables in the models, suggesting it did not mediate the effect of inter-donation interval assignment on symptoms.

As regards the prospective observational associations of iron supplementation and symptoms, in men baseline iron supplementation was most strongly associated with reported palpitations, dizziness, tiredness, and fainting or feeling faint, with 15-30% increase in odds found (**Table 5.4**). In women, baseline iron supplementation was most strongly associated with reported tiredness, breathlessness, palpitations, and fainting or feeling faint, with 14-21% increase in odds found (**Table 5.5**).

Iron supplementation during the trial was a notably stronger correlate of symptoms than baseline iron supplement use, and was statistically significantly associated with all symptoms studied in both men (**Table 5.4**) and women (**Table 5.4**). However, the further adjustment for iron supplementation during the trial did not importantly change the magnitude of association between baseline iron supplementation and symptoms nor the effect of randomised inter-donation interval on the symptoms. Application of the MacArthur method did not significantly alter the effect of randomised inter-donation interval on symptoms; there was no statistically significant interaction between

randomised inter-donation interval and iron supplementation for any symptom in men or women (all p>0.05, range 0.13 – 0.91).

**Table 5.4**: Odds ratios (95% CI) of the effect of inter-donation interval on symptoms from the models assessing symptoms by randomised group and iron supplementation in men

| Symptom/Variable | Model 1 | Model 2 | Model 3 |
|---|---|---|---|
| **Tiredness (N=61979)** | | | |
| Randomised Group | 1.06 (1.04, 1.08) | 1.06 (1.04, 1.08) | 1.06 (1.04, 1.07) |
| Baseline Iron Supplementation | | 1.15 (1.06, 1.25) | 1.15 (1.06, 1.25) |
| Iron Supplementation during Trial | | | 3.84 (3.42, 4.32) |
| **Breathlessness (N=61875)** | | | |
| Randomised Group | 1.09 (1.06, 1.12) | 1.09 (1.06, 1.12) | 1.08 (1.05, 1.11) |
| Baseline Iron Supplementation | | 1.11 (0.97, 1.27) | 1.11 (0.97, 1.26) |
| Iron Supplementation during Trial | | | 3.66 (3.13, 4.28) |
| **Palpitations (N=61861)** | | | |
| Randomised Group | 1.05 (1.02, 1.08) | 1.05 (1.02, 1.08) | 1.05 (1.01, 1.08) |
| Baseline Iron Supplementation | | 1.32 (1.14, 1.52) | 1.32 (1.14, 1.52) |
| Iron Supplementation during Trial | | | 2.28 (1.87, 2.77) |
| **Dizziness (N=61892)** | | | |
| Randomised Group | 1.06 (1.04, 1.09) | 1.06 (1.04, 1.09) | 1.06 (1.03, 1.09) |
| Baseline Iron Supplementation | | 1.28 (1.14, 1.44) | 1.28 (1.14, 1.44) |
| Iron Supplementation during Trial | | | 2.65 (2.26, 3.10) |
| **Fainting or Feeling Faint (N=61931)** | | | |
| Randomised Group | 1.07 (1.04, 1.11) | 1.07 (1.04, 1.11) | 1.07 (1.04, 1.10) |
| Baseline Iron Supplementation | | 1.20 (1.05, 1.38) | 1.20 (1.05, 1.38) |
| Iron Supplementation during Trial | | | 3.12 (2.61, 3.74) |
| **Chest Pain (N=61724)** | | | |
| Randomised Group | 1.05 (1.01, 1.10) | 1.05 (1.01, 1.10) | 1.05 (1.01, 1.09) |
| Baseline Iron Supplementation | | 1.13 (0.95, 1.36) | 1.13 (0.95, 1.36) |
| Iron Supplementation during Trial | | | 2.28 (1.75, 2.97) |
| **RLS (N=83115)** | | | |
| Randomised Group | 1.03 (1.01, 1.05) | 1.03 (1.01, 1.05) | 1.03 (1.01, 1.05) |
| Baseline Iron Supplementation | | 1.10 (0.99, 1.21) | 1.10 (1.00, 1.21) |
| Iron Supplementation during Trial | | | 1.40 (1.23, 1.59) |
| **Pica (N=80425)** | | | |
| Randomised Group | 1.05 (0.93, 1.19) | 1.05 (0.93, 1.19) | 1.05 (0.93, 1.19) |
| Baseline Iron Supplementation | | 1.56 (0.92, 2.65) | 1.55 (0.92, 2.64) |
| Iron Supplementation during Trial | | | 2.79 (1.07, 7.28) |
| **Doctor Diagnosed Low Iron (N=62092)** | | | |
| Randomised Group | 1.20 (1.16, 1.24) | 1.20 (1.16, 1.24) | 1.23 (1.17, 1.28) |
| Baseline Iron Supplementation | | 0.94 (0.80, 1.10) | 0.78 (0.63, 0.97) |
| Iron Supplementation during Trial | | | NA |

**Table 5.5**: Coefficients (95% CI) of the effect of inter-donation interval on symptoms from the models assessing symptoms by randomised group and iron supplementation in women

| Symptom/Variable | Model 1 | Model 2 | Model 3 |
|---|---|---|---|
| **Tiredness (N=61025)** | | | |
| Randomised Group | 1.04 (1.02, 1.06) | 1.04 (1.02, 1.06) | 1.04 (1.02, 1.05) |
| Baseline Iron Supplementation | | 1.22 (1.15, 1.30) | 1.23 (1.15, 1.31) |
| Iron Supplementation during Trial | | | 5.45 (4.97, 5.98) |
| **Breathlessness (N=60913)** | | | |
| Randomised Group | 1.04 (1.01, 1.06) | 1.04 (1.01, 1.06) | 1.03 (1.00, 1.05) |
| Baseline Iron Supplementation | | 1.20 (1.09, 1.32) | 1.20 (1.08, 1.32) |
| Iron Supplementation during Trial | | | 4.54 (4.06, 5.09) |
| **Palpitations(N=60886)** | | | |
| Randomised Group | 1.03 (1.00, 1.05) | 1.02 (1.00, 1.05) | 1.02 (1.00, 1.05) |
| Baseline Iron Supplementation | | 1.17 (1.06, 1.28) | 1.16 (1.06, 1.28) |
| Iron Supplementation during Trial | | | 2.45 (2.19, 2.74) |
| **Dizziness (N=60910)** | | | |
| Randomised Group | 1.04 (1.02, 1.06) | 1.04 (1.02, 1.06) | 1.03 (1.01, 1.05) |
| Baseline Iron Supplementation | | 1.08 (0.99, 1.17) | 1.07 (0.99, 1.17) |
| Iron Supplementation during Trial | | | 3.14 (2.85, 3.47) |
| **Fainting or Feeling Faint (N=60960)** | | | |
| Randomised Group | 1.04 (1.01, 1.06) | 1.04 (1.01, 1.06) | 1.03 (1.01, 1.05) |
| Baseline Iron Supplementation | | 1.14 (1.05, 1.25) | 1.14 (1.04, 1.24) |
| Iron Supplementation during Trial | | | 3.64 (3.26, 4.07) |
| **Chest Pain (N=60690)** | | | |
| Randomised Group | 1.00 (0.96, 1.04) | 1.00 (0.96, 1.04) | 0.99 (0.95, 1.03) |
| Baseline Iron Supplementation | | 0.99 (0.84, 1.17) | 0.99 (0.84, 1.17) |
| Iron Supplementation during Trial | | | 2.84 (2.35, 3.43) |
| **RLS (N=82131)** | | | |
| Randomised Group | 1.01 (0.99, 1.03) | 1.01 (0.99, 1.03) | 1.01 (0.99, 1.03) |
| Baseline Iron Supplementation | | 1.08 (1.01, 1.16) | 1.08 (1.01, 1.16) |
| Iron Supplementation during Trial | | | 1.23 (1.13, 1.34) |
| **Pica (N=83085)** | | | |
| Randomised Group | 1.01 (0.92, 1.10) | 1.01 (0.92, 1.10) | 1.00 (0.92, 1.09) |
| Baseline Iron Supplementation | | 0.96 (0.67, 1.37) | 0.95 (0.66, 1.36) |
| Iron Supplementation during Trial | | | 2.82 (1.79, 4.43) |
| **Doctor Diagnosed Low Iron (N=61194)** | | | |
| Randomised Group | 1.09 (1.06, 1.12) | 1.09 (1.06, 1.12) | 1.09 (1.05, 1.13) |
| Baseline Iron Supplementation | | 0.99 (0.89, 1.11) | 0.88 (0.75, 1.04) |
| Iron Supplementation during Trial | | | NA |

### 5.3.7 Sensitivity analyses

Results of sensitivity analyses comparing the effect of inter-donation interval on symptoms estimated from logistic regression models fitted under the generalised estimating equations framework (GEE-

LOGIT) versus mixed effects framework (ME-LOGIT) were similar (**Figure 5.5**), suggesting results were fairly robust to assumptions of missing data mechanisms.

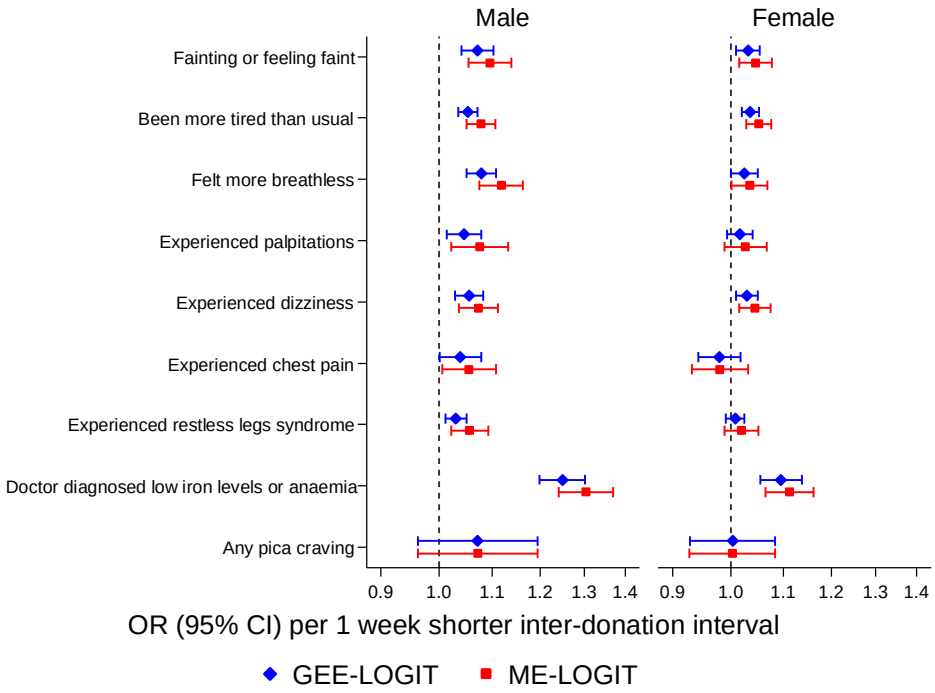



**Figure 5.6**: Comparison of effect of randomised inter-donation interval on symptoms estimated from logistic regression models fitted under the generalised estimating equations framework (GEE-LOGIT) versus mixed effects framework (ME-LOGIT).

## 5.4 Discussion

In this chapter, I have investigated the symptoms that donors reported during the INTERVAL trial. Eight symptoms were recorded during the trial: tiredness, breathlessness, palpitations, dizziness, fainting or feeling faint, chest pain, restless legs syndrome, and pica. These symptoms were reported every six months during the trial by self-reported questionnaires. Minimising post-donation symptoms is a key consideration for donor retention [199]. The three most commonly reported symptoms were tiredness, dizziness, and restless legs syndrome. Participants who took iron supplements, whether before or during the trial period, reported symptoms more often than those who did not.

### 5.4.1 Symptom Levels in INTERVAL

In both men and women, the most frequently reported symptom during the trial was feeling more tired than usual. This was reported by up to 25% of men and 28% of women, with a higher percentage of women than men reporting this symptom at all time points during the trial. This is likely because tiredness is a known symptom of iron deficiency, and women, particularly premenopausal women, lose a higher percentage of their body's iron stores in a blood donation than men.

Restless legs syndrome was the next most commonly reported. Other symptoms had lower frequency of reporting, with pica in particular reported by a very low proportion of the trial participants despite being a documented side effect of blood donation and iron deficiency [198]. It is possible, however, that some of those who experienced symptoms dropped out of the trial as a consequence, and so the true levels of symptoms in INTERVAL could have been higher.

### 5.4.2 Mediation of Effect of Randomised Inter-donation interval by Iron Supplementation

Examining the effect of randomised group on the odds of developing symptom quantitatively, I found that in men, the only symptoms that did not significantly increase with shorter inter-donation intervals were chest pain and pica. This may partly be due to low statistical power as few men experienced

these symptoms during the trial, especially pica. In women, fewer symptoms were increased by more frequent donation. In addition to chest pain and pica, prevalence of palpitations and restless legs syndrome did not significantly increase with shorter inter-donation intervals.

It was hypothesised that iron supplementation may mediate the effect of randomised group. This would mean that some of the changes in symptoms observed were actually attributable to iron supplementation during the trial rather than more frequent donation. However for both sexes, and all symptoms, the effect of randomised group was not significantly different when iron supplementation at baseline, and during the trial, were accounted for.

### 5.4.3 Implications

One proposed method of reducing post-donation symptoms in blood donors is by recommending iron supplementation [193]. However, the INTERVAL trial results presented in this chapter suggested that iron supplementation did not mediate the effect of randomised group on development of symptoms. This means that iron supplementation in the INTERVAL trial setting did not offset the risk of donors reporting symptoms due to donating blood more frequently than usual. While there are other reasons why iron supplementation would be offered to blood donors such as a desire to reduce low haemoglobin deferrals, it may not be sufficient to combat the effect of shorter inter-donation intervals on occurrence of symptoms.

### 5.4.4 Strengths and Limitations

The primary strength in this analysis is the use of the longitudinal data from INTERVAL trial, which were collected routinely every six months from a very large sample. In addition, the modelling framework adopted allowed the use of all information from all available time points to minimise selection biases and increase statistical power.

The primary limitation of this analysis is the method of reporting symptoms. This was performed using self-reported questionnaires. These contained questions asking donors if they had been more tired, experienced chest pain, fainted etc during the past six months. While some of these events, such as

fainting, are unlikely to be forgotten and are objective, other questions, such as “whether you have been more tired than usual” have a subjective interpretation. This could affect results: one donor’s perception of becoming sufficiently more tired, dizzy, or breathless to report that they had experienced this symptom on the questionnaire could be quite different from that of another donor. In addition, it is possible that, due to the non-blinded nature of the trial, those on shorter randomised inter-donation intervals were more aware of symptoms, thus more likely to attribute these to their increased donation frequency and report these on their questionnaires.

A specific limitation to the mediation analysis of the models investigating doctor prescribed low iron levels during the trial is illustrated in **Figure 5.6**. It is perhaps unsurprising that almost all those who had doctor prescribed iron supplements in a six month period also took iron supplements in the same period, as this would likely be what has been prescribed to them. This interpretation would present iron supplementation as a predictor of doctor diagnosed iron levels, rather than a consequence of it. It is possible that this relationship may apply to other symptoms.

Dropout levels in the trials could also influence the results from these analyses. It is possible that donors who experienced more severe or more frequent symptoms dropped out of the trial as a consequence. The true symptom levels of donors in the trial are therefore unknown, and it is possible that only healthier donors completed the trial, which would bias these results. However, as dropout was not differential by randomised inter-donation interval, the assessment of the effect of randomised inter-donation interval should remain unbiased.

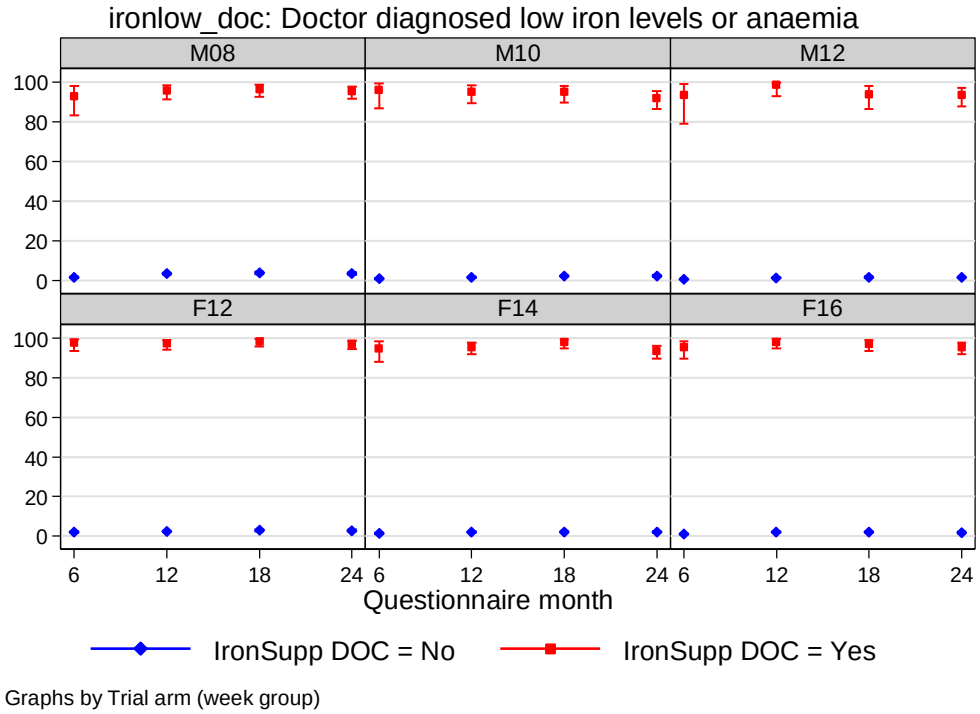



**Figure 5.7**: Comparison of doctor diagnosed low iron levels by randomised group and iron supplement use, denoted by the percentage who indicated yes on the questionnaire. Almost all those who indicated they were using iron supplementation on the questionnaire had these prescribed by a doctor in the prior six months.

### 5.4.5 Conclusions

Development of post-donation symptoms is a risk of blood donation. These range in severity from feeling more tired, to fainting, or developing complications such as RLS and pica. Decreasing the inter-donation interval will slightly increase the risk of experiencing any such symptom, although for some of these such as pica and chest pain, the risk is low.

Iron supplementation, while often proposed as a solution to this problem, does not offset the increased risk of developing symptoms with more frequent donation, and so alternative strategies for minimising the risk of post-donation symptoms should be studied. It is possible that there are dynamics unaccounted for in these results, as donors who were experiencing symptoms post-donation may have subsequently taken iron supplements. In addition, they may have stopped doing this if symptoms subsided while others who, by this point, had donated for a longer period of time

without taking iron supplementation started doing so upon developing symptoms. So, it is possible that iron supplementation may be a consequence, rather than a predictor, of a donor developing symptoms.

# Chapter 6 – Effect of Shorter Inter-donation Interval on Blood Donor Well-being.


## Summary

Shortening the length of time between donations could lead to consequences such as more low haemoglobin deferrals, lower haemoglobin and ferritin levels, and some post-donation symptoms for some donors. These could have a detrimental effect on well-being and overall quality of life.

In INTERVAL, donor well-being was assessed using the Sf-36 questionnaire at baseline and after two years, and the shorter Sf-12 questionnaire every six months. These questionnaires allow calculation of well-being scores in eight domains, which are then used to form two summary scores, the Physical Component Score (PCS) and Mental Component Score (MCS), assessing physical and mental wellbeing respectively. These well-being measures have seldom been studied in blood donor populations.

In both sexes, shorter randomised inter-donation interval did not have a significant effect on the PCS, MCS, and their associated sub-components, with the exception of physical role functioning in men which decreased with shorter inter-donation interval (coefficient: -0.026 95%CI (-0.052, -0.005), p=0.016). PCS and MCS scores during the trial were modestly associated with other baseline characteristics, including age, weight, and iron supplementation use.

While some changes in PCS, MCS and their sub-components over time were observed, they were not of clinical significance. The same was found of associations with baseline variables. Consequently, it is unlikely that there are major risks of increased frequency of blood donation to donors' physical and mental wellbeing.


## 6.1 Introduction

It is possible that shortening inter-donation intervals could lead to additional safety concerns as donors attempt to donate more frequently than they should. Some donors may find that more

frequent donation and its effects such as losing more iron more regularly affects their capacity to carry out physical activities. It is not clear how changes to the inter-donation interval, such as those trialled in INTERVAL, may affect physical and mental wellbeing. This is a largely unaddressed question in the literature on blood donors and trials containing blood donor participants. Just one previous study, the Danish Blood Donor Study, has considered quality of life scores as an outcome in a blood donor population, and it found no relationship between donation frequency and quality of life [233].

### 6.1.1 Well-being Assessment using the Sf-36 Questionnaire

The Sf-36 questionnaire is a widely used questionnaire that captures quality of life in populations. It is commonly used in the UK in order to help with planning of services, and to measure the effect of clinical and social interventions [234-237]. It has been effective at comparing general populations and specific subgroups within these populations, such as populations stratified by sex or socio-economic status [238-241]. While it has proven suitable for use on populations, its effectiveness at the individual level is uncertain [242].

### 6.1.2 Construction of the PCS and MCS Summary Scores

The Sf-36 questionnaire comprises 36 questions that are grouped into eight domains (**Figure 6.1**): limitations to all physical activities (Physical Functioning); problems with work and daily activities due to physical health (Role limitation due to Physical health problems); pain and limitations due to pain (Bodily Pain); problems with work and daily activities due to emotional problems (Role Limitations due to Emotional problems); assessment of health and how it may improve (General Health Perception); interference with social activities due to physical or emotional problems (Social Functioning); tiredness and energy levels (Vitality); and feelings of nervousness, depression, happiness and calm (Mental Health) [243-245]. These domains are then used to form two overall summary scores, the Physical Component Score (PCS) and the Mental Component Score (MCS), measuring a physical and mental health well-being respectively. All eight domains contribute to calculation of PCS and MCS with different weights [176, 246-248]. The exact weights of the sub-components vary by country. In most

countries, a sub-component that contributes positively to one score will contribute negatively to the other aside from vitality, which contributes positively to both. This causes the scores to be orthogonal and so uncorrelated [249]. In the UK, general health contributed positively to both scores [176] (**Table 6.1**).

**Table 6.1**: Coefficients (weights) used to derive the PCS and MCS for the Sf-36v2 in the UK [176]

| Sub-component | PCS coefficient | MCS coefficient |
|---|---|---|
| Physical functioning | 0.456 | -0.227 |
| Role physical | 0.362 | -0.102 |
| Bodily pain | 0.367 | -0.130 |
| General health | 0.199 | 0.036 |
| Vitality | -0.050 | 0.278 |
| Social functioning | -0.028 | 0.272 |
| Role emotional | -0.110 | 0.329 |
| Mental Health | -0.256 | 0.460 |

The PCS and MCS scores can be standardised using population norms, transforming them to have a mean of 50 and standard deviation of 10 [250]. This allows scores to have a recognisable interpretation, with a value of above 50 indicating above-average health and below 50 indicating below-average health. In addition, norm-based scoring has an advantage for the clinicians and researchers administering the questionnaire as they do not need to remember or refer to norms for the summary scores and their subscales [251]. It also allows comparison across populations and sub-populations [246]. The population norms used in the UK are taken from the ONS survey of Britain in 1992, the Health Survey for England, the Oxford Healthy Life Survey General Household Survey conducted in 1992 and an additional survey conducted in Surrey in 1992 [242, 252, 253].

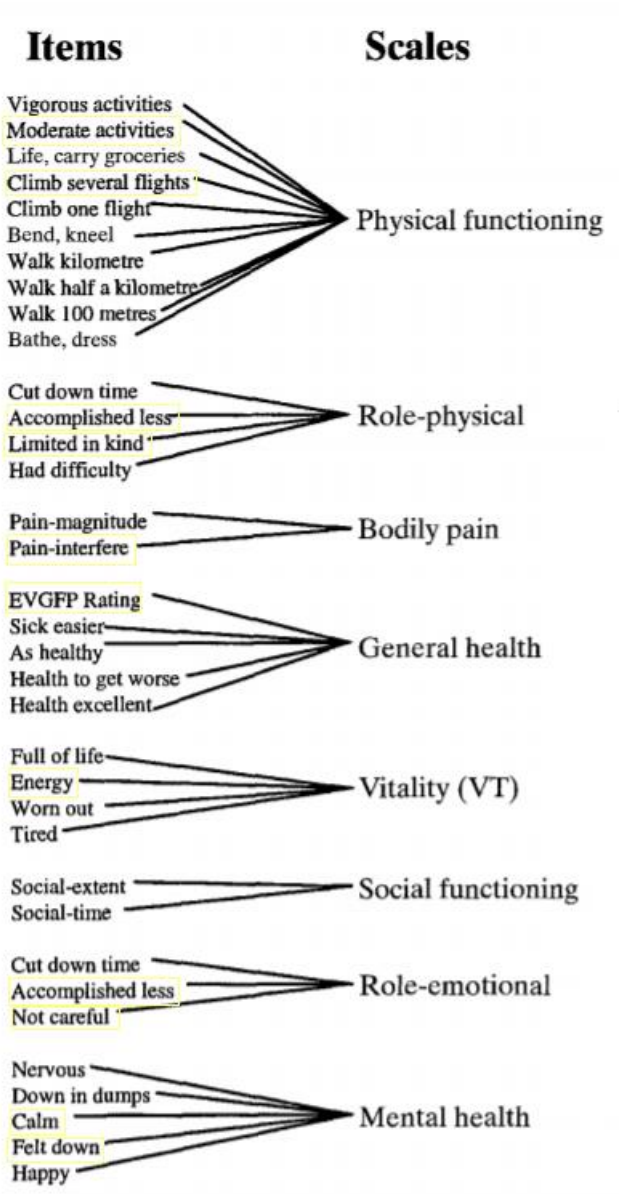



**Figure 6.1**: Diagram showing which questions in the Sf-36 survey correspond to which components of the PCS and MCS [254]. Highlighted questions comprise the Sf-12 survey.

### 6.1.3 Performance and Validity of the Sf-36

In a UK based population, the mean PCS was calculated as 50.8 and the mean MCS was 52.2 [255]. However, there have been recorded gender differences in scores. Women reported lower scores for all sub-sections of the Sf-36 than men except general health [242]. The Sf-36 was also found to have good internal validity [242] and was able to detect health differences expected from those in manual and non-manual jobs, and those who live with a chronic illness [176]. One UK based longitudinal study found that unadjusted mean scores were lower than baseline after three years, and that men and

women reported declines in different subscales by age [256]. Younger men had a greater decline in mental health, emotional role limitation, vitality and social role functioning than older men. In women, this relationship was seen in the vitality and general health sub-components [256].

### 6.1.4 The Sf-12 Questionnaire

The Sf-12 questionnaire is a shorter version of the Sf-36 containing the 12 questions that contribute the most to the Sf-36 components [257]. The questions selected for the Sf-12 questionnaire are two from the physical functioning and mental health scales, as these best predict physical and mental health overall, two items from the physical and emotional role domains, and one item from the remaining four domains [255]. This has the advantage of lessening the burden on those taking the questionnaire by reducing the length of time taken to complete the questionnaire to five minutes.

The initial validation study of the Sf-12 questionnaire found that correlations between the Sf-36 and Sf-12 PCS was 0.95, and for MCS this was 0.97 [257]. Other studies have similarly found that scores from the Sf-36 and Sf-12 are highly correlated [255, 257, 258].

### 6.1.5 Chapter Aims

The key secondary outcome in the INTERVAL trial was self-reported well-being [7] as measured by the Sf-36 questionnaire [245, 259, 260] at baseline and after two years of follow-up, and the Sf-12 every six months [257]. Findings from INTERVAL showed no significant effect of shorter inter-donation interval on physical and mental health well-being scores [74]. However, there was no analysis of the sub-components. It is possible that increased donation frequency could affect some of these subcomponents more than others, and this variation may not be captured by the PCS and MCS summary scores. The aim of this chapter was to assess the effect randomised inter-donation intervals on the Sf-36 subscales not analysed in previous INTERVAL results.

## 6.2 Methods

### 6.2.1 Sf-36 and Sf-12 Data Collection in INTERVAL

At baseline and after two years of follow-up, participants completed the full Sf-36 questionnaire, and every six months, the Sf-12 questionnaire was asked [7]. No question was compulsory and it was possible for participants not to answer all questions. The exact questions and their ordering are provided in **Appendix C**.

### 6.2.2 Statistical Analysis

To assess characteristics associated with well-being scores irrespective of inter-donation interval assignment, cross-sectional correlates of baseline PCS and MCS were assessed using a linear model adjusted for age, sex, and centre (**Chapter 4**). The difference in $R^2$ when each cross-sectional correlate was added to the model was examined to assess its added value.

Longitudinal analyses were conducted using the GEE modelling framework (see **Chapter 5**). This was chosen as the GEE model can take into account correlation of repeated observations on the same individual, and its estimation of fixed effect coefficients is generally more robust to assumed structure of working correlation matrix [228, 229]. There was a maximum of five observations per individual for each of PCS, MCS and their sub-components during the study [7] and so data rows were not independent and identically distributed [230, 231]. An exchangeable correlation structure was assumed for the repeated measures within individuals, meaning equally correlated across time points [232], with individuals considered independent.

Three models were constructed to assess the longitudinal associations with PCS and MCS scores and their sub-components. The first model contained inter-donation interval, the second added donor centre, age, weight, and new donor status, and the third model added iron supplementation and donation history data, specifically: whether a participant had been previously diagnosed with anaemia or was using iron prescriptions, the number of donations in the previous two years, and the number

of low haemoglobin deferrals the participant had experienced in the previous two years. Statistical significance was based on $p < 0.05$ throughout.

## 6.3 Results

### 6.3.1 Response Rates of the Sf-36 and Sf-12 Questionnaires

For both the PCS and MCS in all randomised groups, the proportion of INTERVAL participants completing the questionnaire generally reduced over the trial period and this was similar across randomised group. Slightly more participants completed the two-year follow-up questionnaire than the 18 month questionnaire. At two-year follow-up, slightly lower proportions of women than men completed the Sf-36 questionnaire (**Table 6.2**). However, dropout during follow up was not differential across the randomised inter-donation intervals, and therefore inferences should remain unbiased. Furthermore, the GEE modelling framework used for analyses allowed the inclusion of information from all available questionnaires completed during follow up, as opposed to requiring restriction to participants with all questionnaires completed.

**Table 6.2**: Number (%) of INTERVAL participants completing the Sf-36 and Sf-12 questionnaire at each time point in the trial by randomised group

| | Men | | | Women | | |
|---|---|---|---|---|---|---|
| **Score** | **8 weeks (N = 7456)** | **10 weeks (N = 7446)** | **12 weeks (N = 7452)** | **12 weeks (N = 7567)** | **14 weeks (N = 7565)** | **16 weeks (N = 7548)** |
| **PCS** | | | | | | |
| Baseline | 7398 (99%) | 7395 (99%) | 7412 (99%) | 7507 (99%) | 7515 (99%) | 7495 (99%) |
| 6 months | 5910 (79%) | 5852 (79%) | 5724 (77%) | 5893 (78%) | 5950 (79%) | 5879 (79%) |
| 12 months | 5386 (72%) | 5377 (72%) | 5362 (72%) | 5329 (70%) | 5380 (71%) | 5308 (70%) |
| 18 months | 4785 (64%) | 4810 (65%) | 4823 (65%) | 4671 (62%) | 4747 (63%) | 4691 (62%) |
| 24 months | 4924 (66%) | 4942 (66%) | 4968 (67%) | 4745 (63%) | 4739 (63%) | 4683 (62%) |
| **MCS** | | | | | | |
| Baseline | 7398 (99%) | 7396 (99%) | 7415 (99%) | 7509 (99%) | 7516 (99%) | 7498 (99%) |
| 6 months | 5912 (79%) | 5853 (79%) | 5727 (77%) | 5894 (78%) | 5957 (79%) | 5880 (78%) |
| 12 months | 5393 (72%) | 5376 (72%) | 5372 (72%) | 5335 (71%) | 5381 (71%) | 5309 (70%) |
| 18 months | 4789 (64%) | 4812 (65%) | 4827 (65%) | 4676 (62%) | 4750 (63%) | 4695 (62%) |
| 24 months | 4925 (66%) | 4945 (66%) | 4969 (67%) | 4746 (63%) | 4741 (63%) | 4684 (62%) |

### 6.3.3 Characteristics of Respondents at Baseline and after Two Years

Characteristics of the INTERVAL participants who completed the baseline and two year Sf-36 questionnaires were broadly similar (**Table 6.3**). Comparing men and women, it was found that women had a lower height and weight than men on average and were younger. Women had substantially lower ferritin than men, and more low haemoglobin deferrals in the previous two years. In addition, a higher proportion of women had a previous diagnosis of anaemia than men (~20% difference) and more women than men were taking iron multivitamins and iron supplements on a regular basis. Comparing those who completed the survey after two years with participants at baseline, in men, those who completed the two-year questionnaire were slightly older (mean age 45.2 vs 47.7), and other characteristics were similar. Similarly, women who completed the two-year survey were slightly older (mean age 41.3 vs 44.4) and other characteristics were broadly similar (**Table 6.3**).

**Table 6.3**: Baseline characteristics of INTERVAL participants who completed the Sf-36 questionnaire at baseline and after two-years of follow-up

| | Men | | Women | |
|---|---|---|---|---|
| **Variable** | **Baseline (N=22202)** | **24 months (N=14836)** | **Baseline (N= 22516)** | **24 months (N=14168)** |
| Height (m) | 1.79 (0.08) | 1.79 (0.08) | 1.65 (0.07) | 1.65 (0.07) |
| Weight (kg) | 85.1 (14.5) | 84.9 (14.1) | 71.6 (14.8) | 71.2 (14.3) |
| Age (years) | 45.2 (14.2) | 47.7 (13.4) | 41.3 (14.0) | 44.4 (13.6) |
| **Ethnicity** | | | | |
| White | 18576 (95%) | 12602 (96%) | 19417 (96%) | 12458 (96%) |
| Mixed | 245 (1%) | 141 (1%) | 308 (2%) | 167 (1%) |
| Asian | 507 (3%) | 265 (2%) | 268 (1%) | 121 (1%) |
| Black | 169 (1%) | 99% (1%) | 173 (1%) | 112 (1%) |
| Chinese | 61 (<1%) | 36 (<1%) | 71 (<1%) | 44 (<1%) |
| Other | 83 (<1%) | 54 (<1%) | 58 (<1%) | 29 (<1%) |
| PCS (score) | 56.8 (4.56) | 56.9 (4.29) | 57.0 (4.67) | 57.0 (4.58) |
| MCS (score) | 54.5 (6.12) | 54.9 (5.70) | 53.5 (6.61) | 53.9 (6.27) |
| Blood Group – O | 10574 (47%) | 7082 (48%) | 10869 (48%) | 6790 (48%) |
| Previous Anaemia – Yes | 971 (4%) | 675 (5%) | 5411 (25%) | 3536 (26%) |
| Iron Prescription Use - Yes | 48 (<1%) | 32 (<1%) | 132 (1%) | 84 (1%) |
| Iron Multivitamin Use - Yes | 2809 (13%) | 1870 (13%) | 4050 (18%) | 2680 (19%) |
| Iron Supplement Use - Yes | 202 (1%) | 146 (1%) | 603 (3%) | 410 (3%) |
| 2-Year Donations (n) | 3.58 (1.86) | 3.86 (1.80) | 2.87 (1.66) | 3.15 (1.64) |
| 2-Year Low Hb Deferrals (n) | 0.04 (0.23) | 0.05 (0.25) | 0.12 (0.39) | 0.13 (0.39) |
| Haemoglobin (g/dL) | 15.0 (1.00) | 14.9 (0.98) | 13.4 (0.92) | 13.4 (0.88) |
| Ferritin (µg/L) | 62.2 (66.0) | 59.4 (63.3) | 34.4 (34.3) | 34.9 (35.2) |

### 6.3.4 Cross-Sectional Correlates of PCS and MCS

Baseline PCS and MCS were minimally correlated with other baseline variables as evidenced by the $R^2$ change when correlates were added to models adjusted for age, and sex (**Figures 6.2-6.5**). The majority of characteristics explained less than 1% of variation in baseline PCS and MCS. Higher weight was associated with slightly lower PCS (**Figure 6.2**), and more active leisure activity was associated with a slightly higher PCS and MCS (**Figure 6.3**). The baseline PCS and MCS explained the most variation in each other out of all baseline variables considered (**Figures 6.2-6.5**).

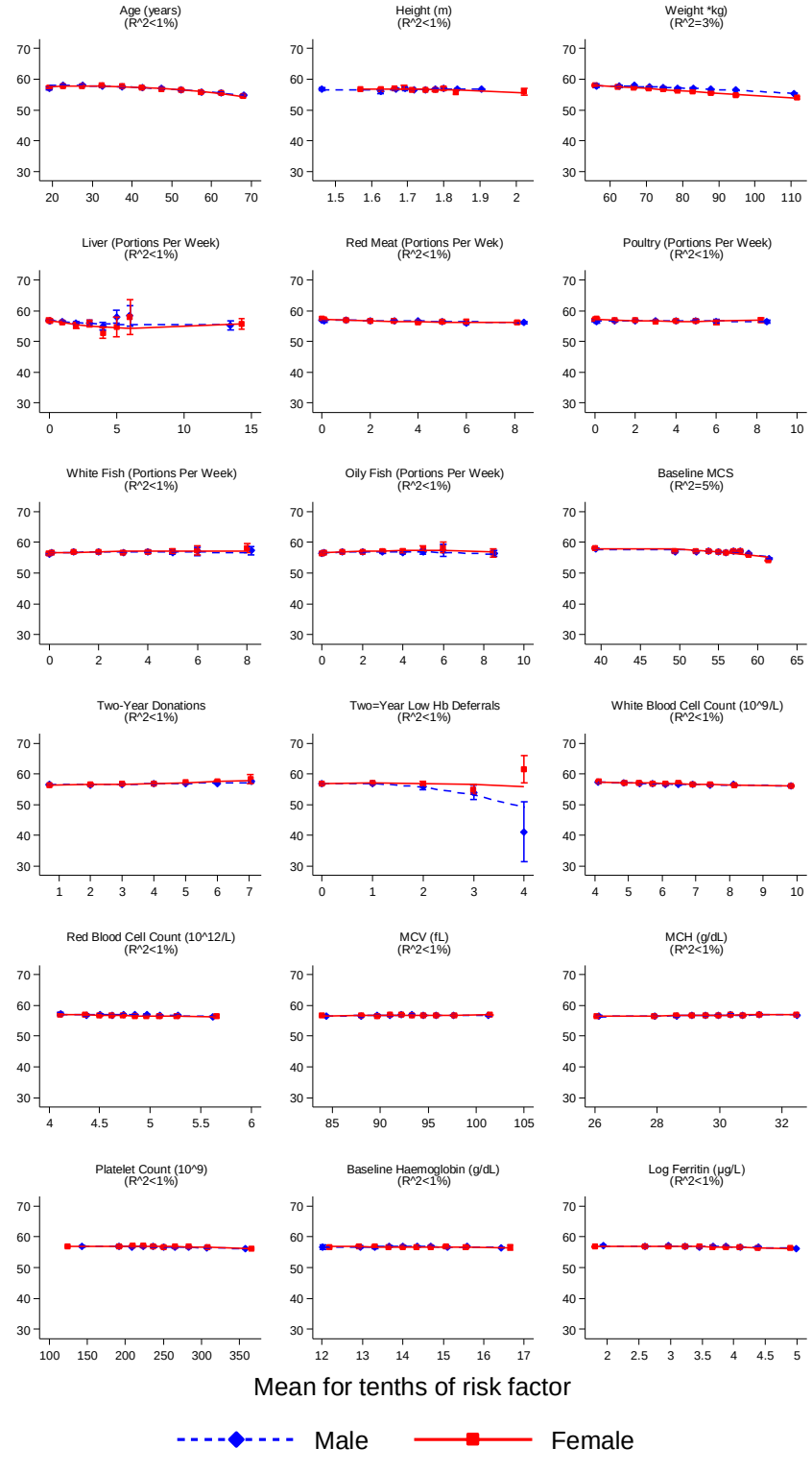



**Figure 6.2**: Cross-sectional correlates of baseline PCS and continuous variables ($R^2$ difference in brackets). Variables are plotted against PCS for men (blue) and women (red).

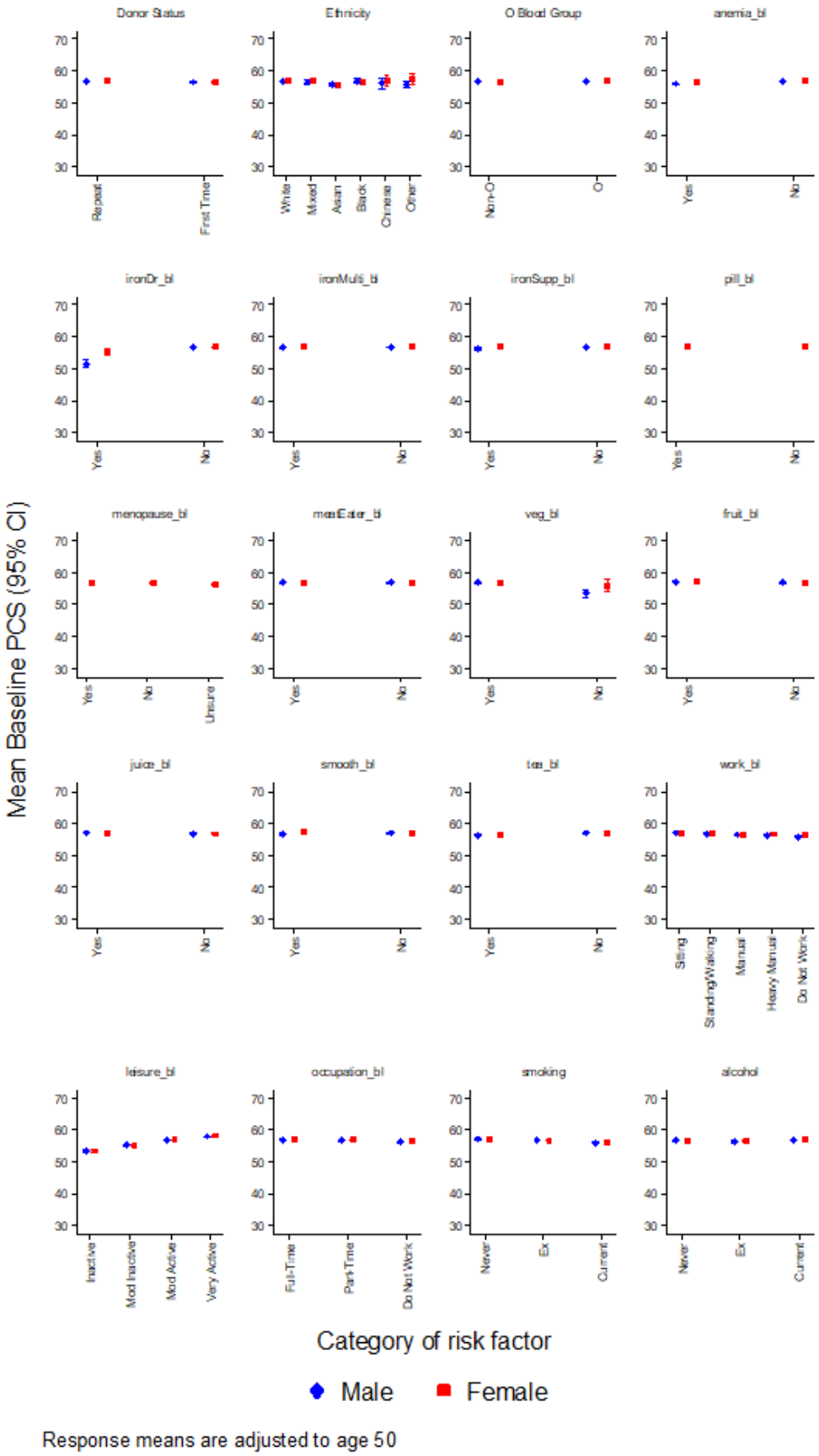
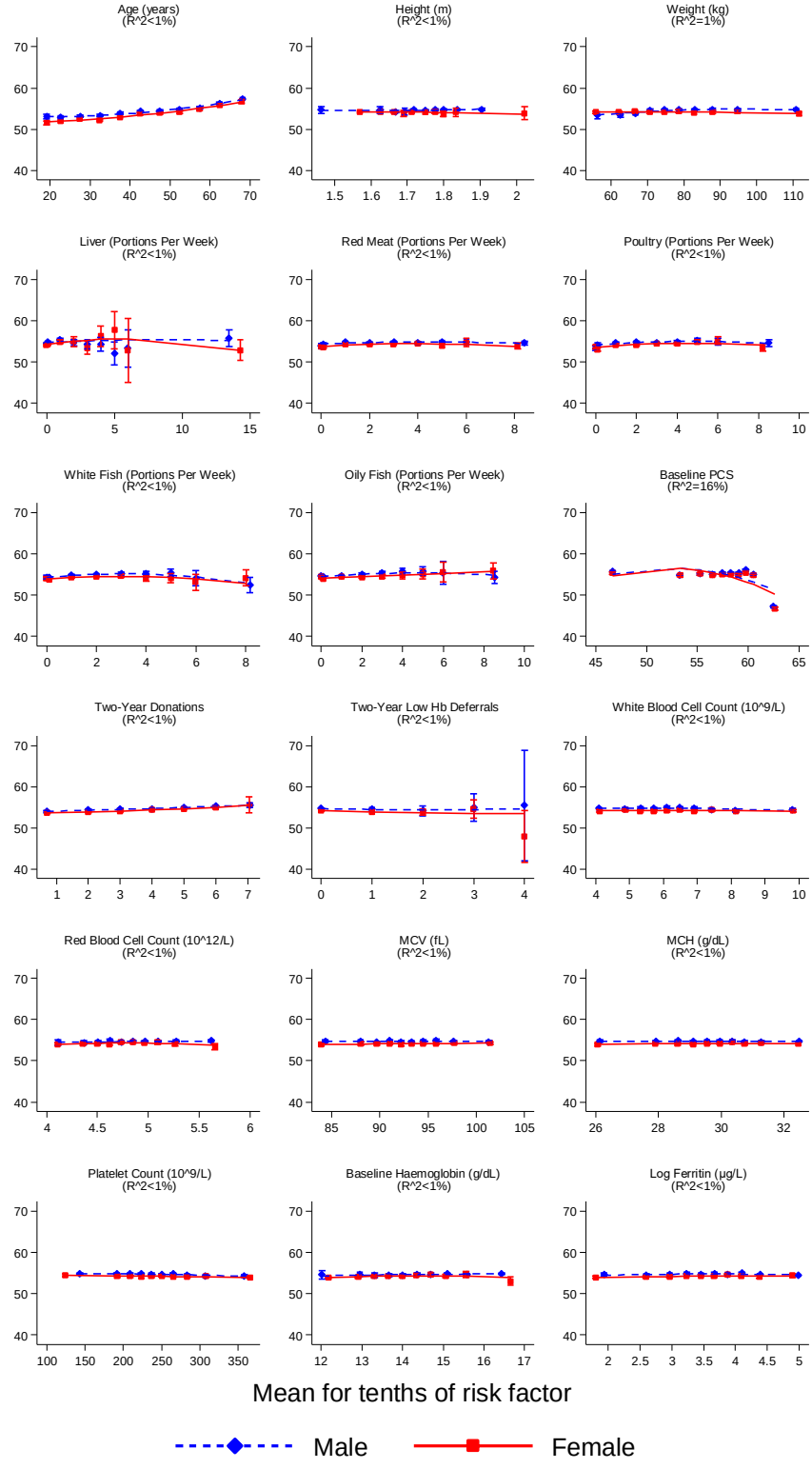



**Figure 6.3**: Cross-sectional correlates of baseline PCS and categorical variables ($R^2$ difference in brackets). Variables are plotted against PCS for men (blue) and women (red).




Response means are adjusted to age 50

**Figure 6.4**: Cross-sectional correlates of baseline MCS and continuous variables ($R^2$ difference in brackets). Variables are plotted against MCS for men (blue) and women (red).

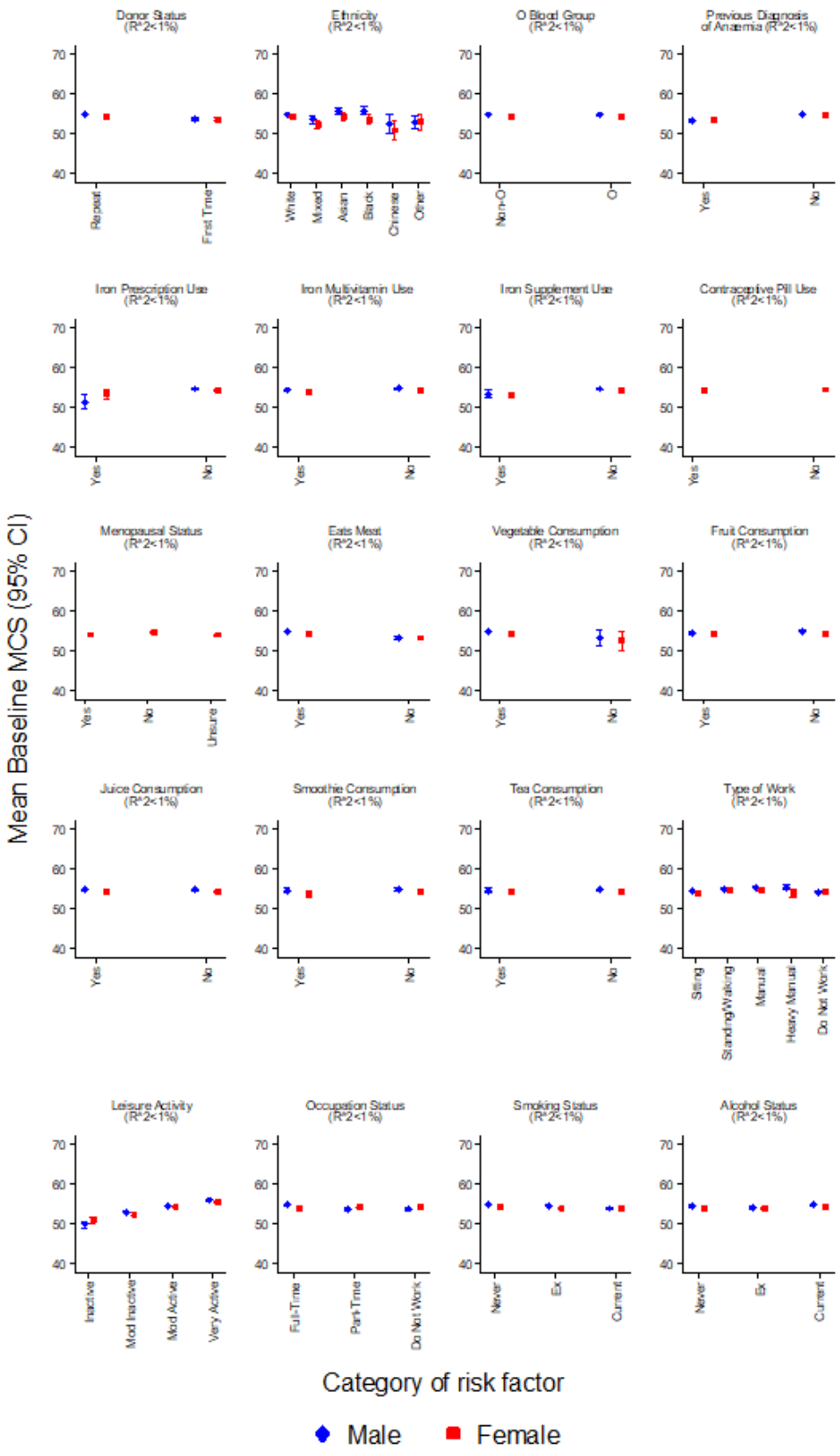



**Figure 6.5**: Cross-sectional correlates of baseline MCS and categorical variables ($R^2$ difference in brackets) Variables are plotted against MCS for men (blue) and women (red).

### 6.3.5 Trends of PCS, MCS, and their Sub-Components over Time

There was a similar trend in the PCS, MCS, and their sub-component scores over time for both sexes in all randomised groups. The PCS, Physical Functioning, Bodily Pain, General Health Perceptions, Physical Functioning, Emotional Role Functioning, and Mental Health scores in both sexes, and the MCS in women, followed a similar pattern. The six-monthly scores from the Sf-12 were ~1-2 points lower than the baseline score measured by the Sf-36, and the two-year score was comparable to the baseline score. In most scores aside from general health perceptions, women scored slightly lower than men. The Physical Role Functioning score varied little throughout the two years of follow up, and there was no difference by sex. The Vitality score followed a different trend, and fell slightly every six months in both sexes, including after two years of follow-up. Social Role Functioning and MCS in men fell between baseline and six months, but varied little from six months to two years. There was little observed difference in any score by inter-donation interval in both sexes (**Figures 6.6 – 6.7**).

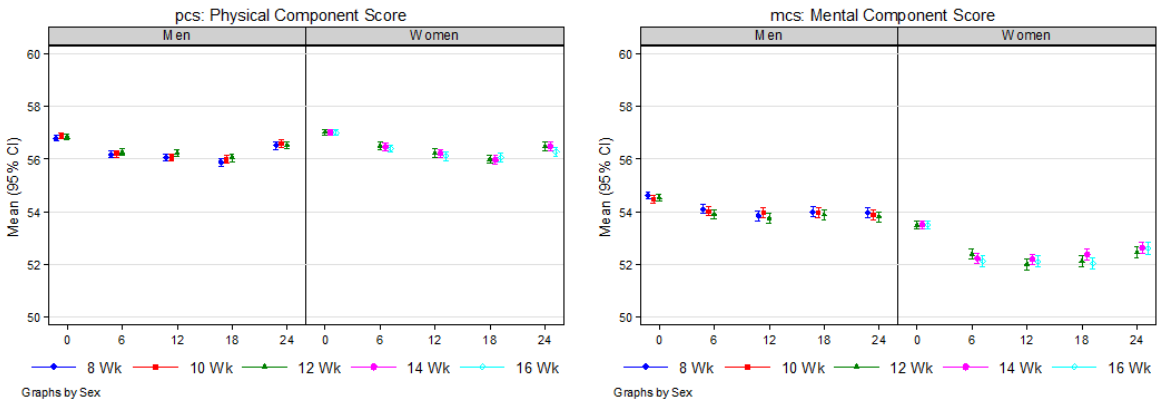



**Figure 6.6**: Mean PCS and MCS at each six month interval of the trial, recorded using the Sf-36 or Sf-12 questionnaire, stratified by randomised group

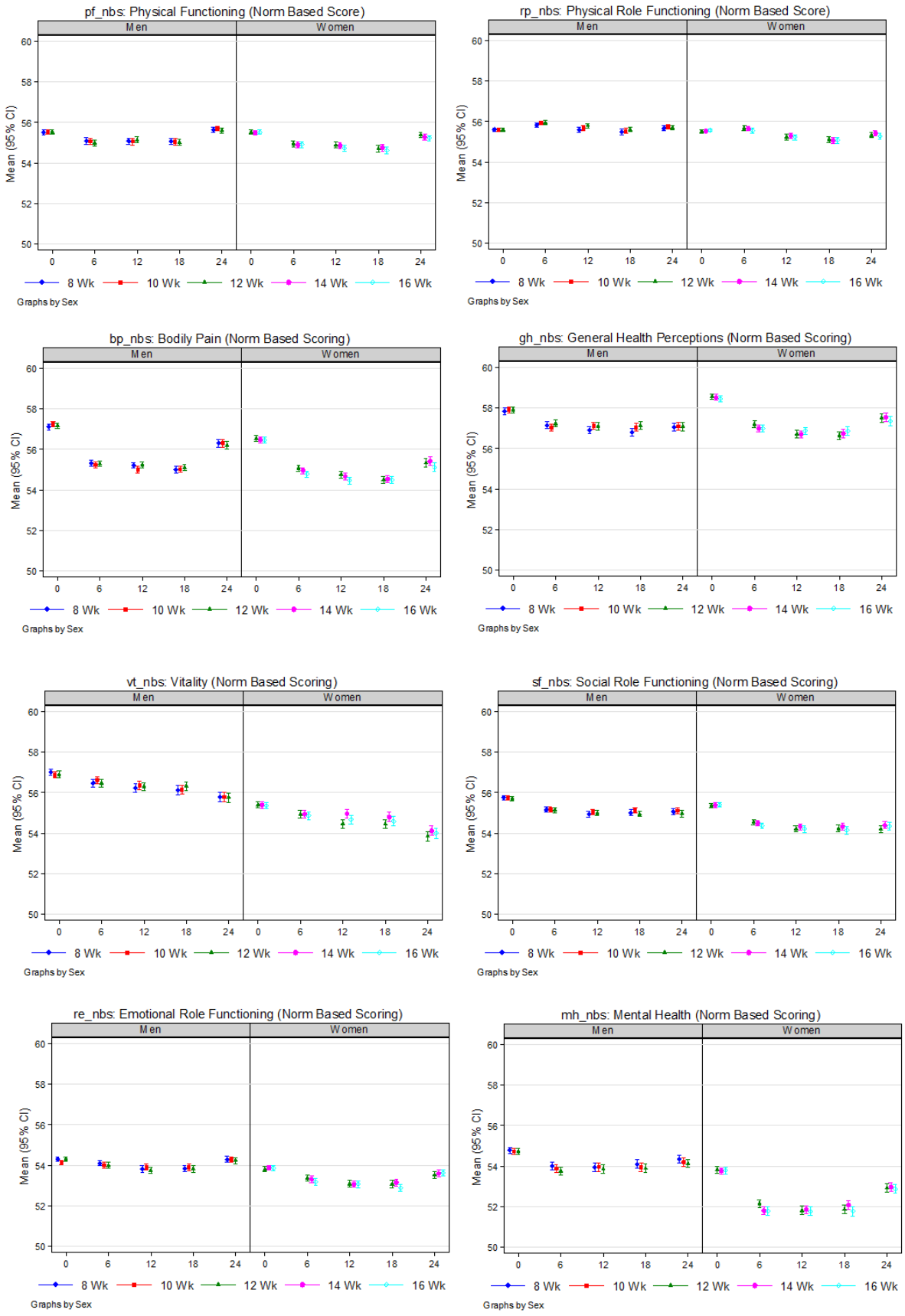



**Figure 6.7**: Mean scores of Physical Functioning, Physical Role Functioning, Bodily Pain, General Health Perceptions, Vitality, Social Role Functioning, Emotional Role Functioning, and Mental Health, recorded using the Sf-36 or Sf-12 questionnaire, stratified by randomised group

### 6.3.6 Associations between baseline variables and PCS, MCS, and their sub-components

The only sub-component for which inter-donation interval was associated with a significant change was Physical Role Functioning in men (**Table 6.4** Model 3 coefficient -0.026 95%CI [-0.052, -0.005], p=0.016). For all other sub-components in both sexes, as well as the PCS and MCS summary scores, the effect of inter-donation interval was non-significant and unchanged by addition of covariates to the model (**Table 6.4**). Modelling of sub-components adjusted for baseline characteristics, iron supplementation and donation history found in men that higher age and weight were significantly associated with lower Physical Functioning, Physical Role Functioning, Bodily Pain, General Health, and Social Role Functioning. Additionally, use of iron supplements and a previous diagnosis of anaemia were significantly associated with lower scores in all sub-components. A higher number of low haemoglobin deferrals was associated with lower score for Physical Role Functioning and Bodily Pain. In contrast, a higher number of donations in the previous two years was significantly associated with higher scores in all sub-components. Higher age was significantly associated with higher Social Role Functioning, Emotional Role Functioning, and Mental Health (**Table 6.5**).

In women, higher age was significantly associated with lower Physical Functioning, Physical Role Functioning, and Bodily Pain, and higher scores in all other sub-components. Higher weight was significantly associated with lower scores in all sub-components except for Social Role Functioning which was not significantly different by weight. Use of iron prescriptions was significantly associated with lower Physical Functioning, Physical Role Functioning, Bodily Pain, General Health and Social Role Functioning, and a previous diagnosis of anaemia was significantly associated with lower scores in all sub-components except for Physical Functioning. On the other hand, a higher number of donations in the previous two years was associated with higher scores in all sub-components. In contrast to men, the number of low haemoglobin deferrals in the previous two years did not significantly affect any sub-component scores (**Table 6.5**).

In neither sex was there a significant association between new donor status and any sub-component.

The magnitude of effect was low on all occasions, with most coefficients below 0.5 (**Table 6.5**).

**Table 6.4**: Coefficients (95% CI) of the effect of inter-donation interval on PCS and MCS scores and their sub-components in both sexes

| Symptom/Model | Men | Women |
|---|---|---|
| **Physical Functioning** | **N=21774** | **N=21575** |
| Randomised Group Only | 0.001 (-0.032, 0.035) | 0.021 (-0.012, 0.054) |
| Baseline Characteristics | 0.001 (-0.032, 0.034) | 0.012 (-0.019, 0.043) |
| Baseline Iron Variables | -0.001 (-0.034, 0.032) | 0.011 (-0.020, 0.043) |
| **Physical Role Functioning** | **N=21572** | **N=21561** |
| Randomised Group Only | -0.026 (-0.050, -0.003) | 0.005 (-0.020, 0.031) |
| Baseline Characteristics | -0.026 (-0.050, -0.003) | 0.002 (-0.024, 0.027) |
| Baseline Iron Variables | -0.028 (-0.052, -0.005) | 0.001 (-0.024, 0.026) |
| **Bodily Pain** | **N=21772** | **N=21567** |
| Randomised Group Only | -0.012 (-0.050, 0.026) | 0.030 (-0.012, 0.072) |
| Baseline Characteristics | -0.013 (-0.051, 0.024) | 0.022 (-0.019, 0.064) |
| Baseline Iron Variables | -0.016 (-0.053, 0.022) | 0.021 (-0.020, 0.062) |
| **General Health** | **N=21779** | **N=21576** |
| Randomised Group Only | -0.038 (-0.084, 0.009) | 0.001 (-0.046, 0.047) |
| Baseline Characteristics | -0.037 (-0.082, 0.009) | -0.012 (-0.057, 0.033) |
| Baseline Iron Variables | -0.039 (-0.085, 0.006) | -0.013 (-0.058, 0.032) |
| **Vitality** | **N=21769** | **N=21569** |
| Randomised Group Only | -0.001 (-0.051, 0.049) | -0.015 (-0.066, 0.037) |
| Baseline Characteristics | 0.001 (-0.049, 0.050) | -0.022 (-0.072, 0.028) |
| Baseline Iron Variables | -0.001 (-0.051, 0.048) | -0.024 (-0.073, 0.026) |
| **Social Role Functioning** | **N=21768** | **N=21575** |
| Randomised Group Only | 0.001 (-0.030, 0.032) | 0.005 (-0.029, 0.040) |
| Baseline Characteristics | 0.001 (-0.030, 0.032) | 0.003 (-0.031, 0.037) |
| Baseline Iron Variables | 0 (-0.031, 0.031) | 0.002 (-0.032, 0.036) |
| **Emotional Role Functioning** | **N=21747** | **N=21556** |
| Randomised Group Only | 0.001 (-0.033, 0.034) | 0.010 (-0.027, 0.048) |
| Baseline Characteristics | 0.001 (-0.032, 0.035) | 0.009 (-0.028, 0.047) |
| Baseline Iron Variables | 0 (-0.034, 0.033) | 0.009 (-0.028, 0.046) |
| **Mental Health** | **N=21769** | **N=21569** |
| Randomised Group Only | 0.024 (-0.025, 0.073) | 0.028 (-0.023, 0.078) |
| Baseline Characteristics | 0.025 (-0.023, 0.073) | 0.027 (-0.022, 0.076) |
| Baseline Iron Variables | 0.023 (-0.024, 0.071) | 0.026 (-0.023, 0.075) |
| **PCS** | **N=21755** | **N=21565** |
| Randomised Group Only | -0.27 (-0.060, 0.005) | 0.011 (-0.024, 0.046) |
| Baseline Characteristics | -0.028 (-0.059, 0.003) | 0 (-0.033, 0.033) |
| Baseline Iron Variables | -0.030 (-0.061, 0.001) | 0 (-0.033, 0.032) |
| **MCS** | **N=21757** | **N=21566** |
| Randomised Group Only | 0.018 (-0.029, 0.065) | 0.010 (-0.040, 0.060) |
| Baseline Characteristics | 0.019 (-0.026, 0.065) | 0.011 (-0.037, 0.059) |
| Baseline Iron Variables | 0.018 (-0.028, 0.063) | 0.010 (-0.038, 0.058) |

**Table 6.5**: Coefficients (95% CI) of baseline variables on sub-components of the PCS and MCS for men and women.

| Variable | Physical Functioning | Physical Role Functioning | Bodily Pain | General Health | Vitality | Social Role Functioning | Emotional Role Functioning | Mental Health |
|---|---|---|---|---|---|---|---|---|
| **Men** | | | | | | | | |
| Inter-Donation Interval[6] | -0.00 (-0.03, 0.03) | 0.03 (0.01, 0.05) | -0.02 (-0.02, 0.05) | 0.04 (-0.01, 0.08) | 0.00 (-0.05, 0.05) | -0.00 (-0.03, 0.03) | 0.00 (-0.03, 0.03) | -0.02 (-0.07, 0.02) |
| Age (years)[7] | -0.60 (-0.65, -0.54) | -0.37 (-0.41, -0.33) | -0.89 (-0.95, -0.82) | -0.23 (-0.31, -0.14) | 0.68 (0.59, 0.76) | 0.26 (0.20, 0.31) | 0.40 (0.34, 0.46) | 1.14 (1.06, 1.23) |
| Weight (kg)[7] | -0.51 (-0.57, -0.45) | -0.16 (-0.21, -0.12) | -0.37 (-0.43, -0.30) | -1.17 (-1.26, -1.09) | -0.59 (-0.68, -0.50) | -0.07 (-0.13, -0.02) | 0.03 (-0.03, 0.09) | 0.00 (-0.09, 0.09) |
| New Donor (Y/N) | -0.05 (-0.29, 0.19) | -0.06 (-0.22, 0.11) | 0.06 (-0.21, 0.32) | -0.18 (-0.50, 0.14) | 0.12 (-0.23, 0.47) | -0.19 (-0.41, 0.03) | -0.02 (-0.26, 0.22) | -0.20 (-0.53, 0.14) |
| Iron Prescription Use (Y/N) | -3.67 (-4.86, -2.48) | -3.34 (-4.17, -2.50) | -3.78 (-5.12, -2.43) | -3.40 (-5.02, -1.77) | -2.39 (-4.16, -0.62) | -2.43 (-3.54, -1.32) | -1.93 (-3.13, -0.73) | -2.28 (-3.98, -0.57) |
| Previous Anaemia Diagnosis (Y/N) | -0.26 (-0.53, 0.01) | -0.69 (-0.88, -0.50) | -1.06 (-1.37, -0.76) | -1.22 (-1.60, -0.85) | -1.42 (-1.82, -1.01) | -0.89 (-1.15, -0.64) | -0.73 (-1.00, -0.45) | -1.39 (-1.79, -1.00) |
| 2-Year Donations (n)[7] | 0.11 (0.05, 0.18) | 0.11 (0.07, 0.16) | 0.22 (0.15, 0.29) | 0.35 (0.27, 0.44) | 0.38 (0.29, 0.47) | 0.16 (0.10, 0.21) | 0.21 (0.15, 0.27) | 0.38 (0.29, 0.47) |
| 2-Year Low Hb Deferrals (n)[7] | -0.06 (-0.14, 0.02) | -0.06 (-0.12, -0.01) | -0.11 (-0.20, -0.02) | -0.01 (-0.12, 0.10) | -0.02 (-0.14, 0.10) | 0.00 (-0.07, 0.08) | -0.04 (-0.12, 0.03) | -0.03 (-0.15, 0.08) |
| $R^2$ | 0.023 | 0.014 | 0.027 | 0.031 | 0.023 | 0.008 | 0.013 | 0.042 |
| **Women** | | | | | | | | |
| Inter-Donation Interval[6] | 0.01 (-0.02, 0.04) | 0.00 (-0.02, 0.03) | -0.02 (-0.02, 0.02) | 0.01 (-0.03, 0.06) | 0.02 (-0.03, 0.07) | -0.00 (-0.03, 0.03) | -0.01 (-0.05, 0.03) | -0.03 (-0.07, 0.02) |
| Age (years)[7] | -0.81 (-0.87, -0.76) | -0.37 (-0.42, -0.33) | -0.77 (-0.84, -0.69) | 0.26 (0.18, 0.34) | 0.94 (0.85, 1.03) | 0.43 (0.37, 0.49) | 0.61 (0.54, 0.67) | 1.23 (1.15, 1.32) |
| Weight (kg)[7] | -0.94 (-1.00, -0.88) | -0.37 (-0.42, -0.22) | -0.77 (-0.85, -0.70) | -1.60 (-1.68, -1.52) | -1.00 (-1.09, -0.91) | -0.29 (-0.35, -0.23) | -0.20 (-0.27, -0.13) | -0.24 (-0.33, -0.16) |
| New Donor (Y/N) | -0.07 (-0.27, 0.12) | 0.05 (-0.11, 0.21) | 0.10 (-0.15, 0.36) | -0.17 (-0.45, 0.11) | 0.30 (-0.01, 0.61) | -0.11 (-0.32, 0.11) | -0.07 (-0.31, 0.16) | 0.22 (-0.09, 0.52) |
| Iron Prescription Use (Y/N) | -0.98 (-1.65, -0.31) | -0.95 (-1.49, -0.41) | -1.54 (-2.42, -0.66) | -1.80 (-2.77, -0.84) | -0.35 (-1.42, 0.72) | -0.79 (-1.52, -0.06) | -0.49 (-1.29, 0.30) | 0.07 (-0.98, 1.12) |
| Previous Anaemia Diagnosis (Y/N) | -0.11 (-0.24, 0.01) | -0.40 (-0.50, -0.31) | -0.81 (-0.97, -0.65) | -1.15 (-1.32, -0.97) | -1.82 (-2.01, -1.62) | -0.72 (-0.85, -0.59) | -0.76 (-0.90, -0.61) | -1.33 (-1.52, -1.14) |
| 2-Year Donations (n)[7] | 0.21 (0.15, 0.28) | 0.27 (0.22, 0.32) | 0.40 (0.31, 0.49) | 0.45 (0.36, 0.55) | 0.55 (0.45, 0.66) | 0.33 (0.26, 0.40) | 0.28 (0.20, 0.35) | 0.42 (0.32, 0.53) |
| 2-Year Low Hb Deferrals (n)[7] | -0.02 (-0.06, 0.03) | 0.01 (-0.02, 0.05) | 0.05 (-0.01, 0.11) | 0.06 (-0.00, 0.13) | 0.04 (-0.03, 0.12) | 0.01 (-0.04, 0.06) | -0.02 (-0.08, 0.03) | -0.01 (-0.08, 0.06) |
| $R^2$ | 0.053 | 0.016 | 0.028 | 0.057 | 0.046 | 0.018 | 0.022 | 0.044 |

[6] Per one week decrease

[7] Per 1 SD increase. SDs reported in **Table 3.1** and **Table 3.2**

## 6.4 Discussion

In this chapter, I have investigated the INTERVAL trial's secondary outcome – donor well-being measured by the physical and mental component summary scores of the Sf-36 questionnaire. I have also analysed effects of shorter inter-donation interval with the sub-components of the PCS and MCS, including assessing their associations with other baseline characteristics. This adds to previously published INTERVAL trial results which analysed PCS and MCS scores only as pre-specified outcomes.

### 6.4.1 Trends of PCS, MCS, and sub-components during the INTERVAL trial

The PCS, MCS, and sub-components displayed similar trends in most instances, with the baseline and two-year scores being similar, while the intermediary scores were lower. While it has been reported that the scores from the PCS and MCS and their sub-components are highly correlated between the Sf-36 and Sf-12 questionnaire, it is possible that the observed patterns were due to the different questionnaires. In particular, the questions in the Sf-12 questionnaire were not in the same order as they appeared in the Sf-36 (**Appendix C and Appendix D**). This may induce changes in how participants score questions as their mood and health perceptions may be influenced by the questions just answered. This pattern was not observed for Physical Role Functioning, Vitality, and Social Role Functioning in both sexes, and MCS in men only. However, in all cases the magnitude of difference was small, below 2, which is neither subjectively nor clinically meaningful. A clinically meaningful change corresponds to a difference of 3 points [244, 247, 258].

### 6.4.2 Effect modification on the relationship between inter-donation interval and well-being measures

Previous INTERVAL results did not find a significant effect of shorter inter-donation interval on PCS and MCS [74]. The analysis of sub-components in this chapter found that shorter inter-donation interval affected only Physical Role Functioning in men, with a small decrease in the score.

PCS, MCS and sub-components were nevertheless associated with a few baseline characteristics. In both sexes, higher age was associated with lower scores in Physical Functioning, Physical Role

Functioning, and Bodily Pain. This could be because higher age is often also associated with additional health complications. In contrast, higher age was associated with higher Social Role Functioning, Emotional Role Functioning, and Mental Health in both sexes. Higher weight was also associated with lower Physical Functioning, Physical Role Functioning, Bodily Pain, General Health. This is likely because higher weight is known to affect an individual's physical abilities such as increased difficulty supporting their weight and reduced stamina. In women, higher weight was also associated with lower Vitality, Emotional Role Functioning, and Mental Health.

In both sexes, the number of donations given in the past two years was significantly associated with higher scores in all subcomponents, which adds to the hypothesis that more committed donors are healthier than other populations. Use of iron supplementation or a previous diagnosis of anaemia was associated with lower scores in all sub-components in men, and most sub-components in women. This adds to the hypothesis in **Chapter 5** that those on iron supplementation represent those already at risk of poorer health, and that there is a possibility that this could be extended to include those who have experienced diagnosed anaemia. As above, the magnitudes of these associations were very small, mostly below 0.5, and none indicating clinical significance.

### 6.4.3 Implications

INTERVAL's key secondary outcome was the well-being of participants. It is important to ensure that increased donation does not affect a donor's well-being, as they may not return to donate. The results from both the primary INTERVAL findings [74], which found little effect of shorter inter-donation interval on PCS and MCS, are further supported by the extended analyses in chapter. While some differences were observed in PCS, MCS and their sub-components over time, they were of low magnitude, and not of clinical significance. The same was found of associations with other baseline characteristics. The primary implication of this chapter is that it is likely that donors' well-being would not be negatively affected by increased donation.

### 6.4.4 Strengths and Limitations

The primary strength in this analysis is the use of the longitudinal data from INTERVAL trial, which were collected routinely every six months from a very large sample. In addition, the modelling framework adopted allowed the use of all information from all available time points to minimise selection biases and increase statistical power. Norm-based scoring was also used to aid interpretability.

The primary limitation of this analysis is that the questionnaire was changed to the Sf-12 survey during the six-monthly follow-up. While the Sf-36 and Sf-12 PCS and MCS scores have been shown to be very highly correlated [255, 257, 258], the questions in the Sf-12 questionnaire did not appear in the same order in which they appeared in the Sf-36 questionnaire, and so this may affect responses. In addition, it has previously been found that participants sometimes struggle to interpret questions in the Sf-36 and Sf-12 questionnaires. Specific difficulties reported included respondents' inability to attach meaning to the distance they are asked in the walking questions (such as attaching meaning to a mile), and whether carrying groceries meant carrying a small amount from a local shop, or a heavy bag from a weekly shop, and have to give their best guess, which can be unreliable [261, 262]. In addition, as participants' health over time changes, so does their perception of health [263]. This response shift pattern can be problematic as participants are not comparing their health to a uniform baseline. Some compare to their peers, their past selves, or a hypothesised general population, and this can result in very different interpretations of the physical functioning questions in particular [262, 264, 265]. Moreover, the mean score for the PCS, MCS, and all sub-components of was above 50 at all time points for participants in the INTERVAL trial. This shows that INTERVAL represented a healthier population than the general population and so results may not be generalisable to non-donor UK populations. Results may also not be generalisable to other countries as population norms are country specific.

### 6.4.5 Conclusions

Before assigning personalised inter-donation intervals, donor wellbeing should be considered to ensure that donor retention is not impacted. Results from this chapter found that there was minimal effect of the inter-donation interval on PCS, MCS and their sub-components in INTERVAL trial participants. There were statistically significant associations found for iron-related variables, age, and weight and the sub-components, however the magnitude of associations were still low and there was no convincing evidence that the magnitude of associations would be clinically significant. It is likely that for the majority of the UK blood donor population there would be minimal impact to their well-being should a personalised donation interval recommend they come to donate more frequently than current NHSBT guidelines.

# Chapter 7 – Discussion

## Summary

The aim of this thesis was to investigate different aspects of blood donor health (including well-being, iron levels and symptoms related to iron deficiency) and to summarise key characteristics of donors who may be able to safely give blood donations more frequently. Using data from the INTERVAL trial, which had as its key aim to maximise blood donations while minimising low haemoglobin deferrals, analyses were conducted to assess the effect of more frequent donation on ability to donate blood, experience of low haemoglobin deferrals, frequency of reporting post-donation symptoms (particularly those related to iron deficiency), and blood donor well-being.

This chapter summarises the main findings from previous chapters, places them in the context of wider literature, examines the practical implications of findings, and suggests possible directions for further research.

## 7.1 Summary of Findings

### 7.1.1 Maximising Blood Donations While Minimising Low Haemoglobin Deferrals

A key aim of this thesis was to identify characteristics of blood donors which would enable NHSBT to maximise blood donations from the UK blood donor population while minimising low haemoglobin deferrals.

This thesis has identified the donor characteristics associated with more frequent donation, as well as those associated with low haemoglobin deferrals. It also assessed correlates of haemoglobin and ferritin levels, as the former determines low haemoglobin deferrals, and ferritin is a general measure of iron status which can be important for donor safety, especially when considering post-donation symptoms (**Chapter 4**). These analyses were supplemented by a systematic review of the literature (**Chapter 2**) on low haemoglobin deferrals to add to the evidence identified in a previous review, which was considered alongside the results from INTERVAL.

The systematic review confirmed previous findings and added to the evidence base of factors that affect low haemoglobin deferrals in blood donors. It confirmed the impact of previously known factors, including female sex, lower weight and older age in men on low haemoglobin deferral, and further strengthened the evidence for differences in low haemoglobin deferral by ethnic group, geographic location, and associations with ferritin levels. There was inconclusive evidence on the effect of new vs repeat donor status (**Chapter 4**).

Additional factors which may impact low haemoglobin deferral in blood donors were identified as areas in need of further investigation. These included blood group, previous platelet donation, diet, smoking, time of day, genetic data, and rhesus status. The INTERVAL trial data were analysed to further assess the role of some of these factors. Male blood donors with a non-O blood type had a higher relative risk of low haemoglobin deferral than O blood-group donors.

Correlates and moderators of the effects of randomised inter-donation interval on four outcomes were investigated using INTERVAL trial data (**Chapter 4**). These outcomes analysed included number of donations and low haemoglobin deferrals during the trial, as well as two year haemoglobin and ferritin levels. This chapter added to previously published INTERVAL results by considering outcomes which had not been previously examined and including a wider range of baseline characteristics. Baseline variables that had a significant association with the number of donations during the trial included red blood cell count, baseline MCH, haemoglobin, log ferritin, and two year history of donations for men, and age for women. For both sexes, randomised group had a highly significant effect – in men, a decrease in the inter-donation interval of one week amounted to a 0.4 unit increase in blood given over two years, and for women this was a 0.2 unit increase. Some characteristics were associated with both number of donations and low haemoglobin deferrals – number of donations in the past two years and baseline haemoglobin in both sexes, and baseline ferritin in men. Analyses of two year haemoglobin and ferritin levels found few characteristics with significant interactions effects

with randomised inter-donation interval. These were largely biomarkers which are not routinely collected.

### 7.1.2 Post-Donation Symptoms and Iron Supplementation

One key consideration when assigning inter-donation intervals to blood donors is donor retention. If blood donors experience symptoms post-donation, they may stop returning to donate even when considering committed repeat donors. GEE modelling investigated if more frequent donation could influence post-donation symptoms in donors, such as tiredness, fainting, restless legs syndrome, and pica, and whether iron supplementation could mediate the effect of randomised inter-donation interval on symptoms (**Chapter 5**). Iron supplementation has been shown to reduce symptoms on occasion including RLS, fatigue, and pica. These analyses added to previous literature by analysing a wider range of symptoms, some of which have not been studied before in a blood donor population, such as breathlessness and feeling faint.

Decreasing the inter-donation interval significantly increased a donor's risk of developing almost all symptoms studied in the trial for both sexes. The exceptions were chest pain and pica in men, and palpitations and restless legs syndrome in women, with no significant effect of randomised inter-donation interval found. While a donor's use of iron supplementation at baseline and iron during the trial were associated with symptoms, the effect of randomised group was not mediated by iron supplementation for any of the symptoms studied.

### 7.1.3 Effects of More Frequent Donation on Well-Being

An additional consideration when assigning inter-donation intervals to blood donors is the impact on their quality of life. The effect of iron deficiency without anaemia on well-being is not well established, and blood donors may be susceptible to this.

Longitudinal analyses were conducted to investigate the impact of more frequent donation on well-being – the key secondary outcome of the INTERVAL trial (**Chapter 6**). Well-being was assessed by the physical and mental component scores of the Sf-36 questionnaire at baseline and after two years, and

the Sf-12 questionnaire every six months. Previous analyses had found that the summary PCS and MCS scores were largely unaffected by inter-donation interval [74].

This chapter added to the literature by studying the sub-components which comprise the PCS and MCS summary scores in addition to the summary scores, and expanded the literature base which currently contains just one study in a blood donor population which specifically analysed PCS and MCS, which found no significant effect of blood donation on well-being [233].

Results from these analyses concurred with the previous study, finding little effect of inter-donation interval on the Sf-36 scores. This was also true of the Sf-12 scores, and the subcomponent scores of the PCS and MCS. Inter-donation interval had a statistically significant effect in Physical Role Functioning for men only, and no other sub-components were significantly affected. Other correlates of the subcomponents included weight and age, and iron-status variables. However, few of these associations were clinically meaningful.

### 7.1.4 Summary

Overall, the findings from this thesis indicate that it is possible to identify characteristics of donors who may be able to donate more frequently than current NHSBT inter-donation intervals while minimising low haemoglobin deferrals. Many of these characteristics are routinely collected. However, an increase in post-donation symptoms is a possible consequence of more frequent donation and iron supplementation may not offset the increased risk of post-donation symptoms posed by more frequent blood donation. Effects of more frequent blood donation on well-being of blood donors are likely to be minimal.

## 7.2 Strengths and Limitations

The primary strength of the results presented in this thesis is that they are based on analyses from the largest pragmatic randomised controlled trial designed to assess blood donation intervals. Inferences based on comparisons of randomised inter-donation intervals in over 45000 participants are free from confounding issues. In addition, as most donors adhered to their randomised inter-donation interval,

a clear separation between groups was achieved, and the trial represented the geographical breadth of England [74]. The trial also recorded a comprehensive set of outcomes and had almost complete data for the two main outcomes – number of donations and number of low haemoglobin deferrals [186]. In addition, the longitudinal models used in the analysis of symptoms and PCS and MCS analyses allowed for all timepoints at which data was collected to be used in the analysis, strengthening the reliability and power of the analyses.

One limitation of the analyses is the nature of self-reported data. This was relied upon in baseline data collection, in particular for dietary variables which regarded estimating the number of portions of food consumed in a typical week. This may not be reliable as many people would not stick to a regular schedule eating the same type of food with the same frequency each week, or have an accurate record of their food consumption, forcing them to make their best guess at a response. In addition, the symptoms analysed were self-reported. While some of these symptoms such as fainting are very unambiguous, others such as feeling more tired or breathless than usual are subjective, and different donors would have a different level of feeling the symptom to report that they had experienced it. Some questions in the Sf-36 questionnaire were also shown to be difficult for participants to interpret meaningfully, and it is possible that this could affect results [262]. There is also the limitation afforded by the trial setting – as donors were actively participating in a trial, compliance with the assigned inter-donation intervals is likely to be higher than if donors were donating outside of the trial. In addition, the donors who participated in the trial were likely to be more altruistic and motivated to donate than the general population. Consequently, it is possible that the observed results from this thesis are of a higher magnitude than what would be observed if the inter-donation interval were varied in routine practice in England. Moreover, as the trial could not by design be blinded, it is possible that participants on the shorter inter-donation interval groups were more sensitive of changes to their iron status and post-donation symptoms.

## 7.3 Results in Context of Other Literature

Previous analyses from INTERVAL data have shown that allocating donors to more frequent inter-donation intervals did amount to an increase in blood collected during the study period for both sexes. No difference in quality of life, physical activity, or cognitive function was observed across randomised groups, however those on the more frequent intervals did experience an increase in symptoms and low haemoglobin deferrals, and a decrease in their two year haemoglobin and ferritin [74]. These findings were consistent in the two year study, which also found that donors were more likely to be diagnosed with low iron levels by a doctor on the more frequent inter-donation groups [186].

The findings from this thesis extended the main trial findings [74], by assessing interaction effects to determine which characteristics which may be useful to define donors most able to tolerate donation at more frequent intervals, while minimising low haemoglobin deferrals and symptoms. A thorough investigation of the effect of randomised inter-donation interval on post-donation symptoms and well-being measures was also conducted.

The observational associations findings from this thesis are consistent with previous findings in blood donor populations. The Danish Blood Donor Study began 2010 and recruited blood donors in Denmark to assess why some donors are healthier than others. It has recruited over 40,000 participants [266]. It has found that ferritin is one of the most significant predictors of haemoglobin and development of low haemoglobin in blood donors [189], that donation intensity is a key predictor of iron stores in blood donors [76, 191], and that there is little association between blood donation and self-reported quality of life [233]. The RISE study from the USA recruited 2425 blood donors into sex-specific first time/reactivated donor and frequent donor cohorts. These cohorts were followed up for between 15 and 24 months to evaluate their iron status [84]. It also concluded that donation intensity is a key predictor of iron stores [84]. The Donor InSight study was conducted in the Netherlands in the form of a self-administered questionnaire on demographics, lifestyle nutrition, physical activity, medical history, reproductive factors, and donor motivation [267, 268]. While dietary variables were not

significant in the analyses from INTERVAL data, the Donor InSight study in the Netherlands found a positive association between consumption of heme iron, and a smaller association with non-heme iron, and ferritin levels, which was mediated by donors' baseline ferritin. However it concluded that dietary advice was not effective [269]. Results from this study concurred with findings in this thesis that lifestyle factors contribute little to low haemoglobin deferral levels [270].

## 7.4 Implications.

Blood donation in the UK and elsewhere is inherently an altruistic act. Donors donate blood to give something back to their community. It is the duty of the blood service to ensure that the generosity of blood donors does not come at a cost to their physical or mental wellbeing. When deciding how long a donor should wait to return for donation, the blood service need to balance the demand for blood (or the donor's medical benefit from donating) against any potential adverse consequences on donor health such as low haemoglobin or ferritin levels, post-donation symptoms, and the general impact on wellbeing. While the analyses from this thesis have shown that there is little risk afforded to donors with respect to post-donation symptoms (**Chapter 5**), or general wellbeing (**Chapter 6**) from more frequent blood donation, low iron stores remains a concern. For the blood service, the primary concern will be low haemoglobin deferral, and the demotivation that this causes in donors, even regular, committed donors. In certain groups, such as women, ethnic minorities, and those with lower haemoglobin levels at previous donation visits, this can be of particular concern (**Chapter 2**). However, there are also the consequences of iron deficiency, which may occur in the absence of a low haemoglobin deferral, as a single blood donation removes 240-260mg of iron. Consequences of iron deficiency can include restless legs syndrome, fatigue, and cognitive effects. It is therefore important to minimise donors' risks of iron deficiency, which may not be captured in haemoglobin testing solely. While few symptoms of iron deficiency were recorded in INTERVAL, it is possible that this may be differential in the general donor population, and due to the self-reported and subjective nature of this reporting, it is possible that symptom levels may be higher than reported.

The results from this thesis provide some considerations to take into account when varying the inter-donation interval for blood donors. The overarching objective is to integrate the considerations surrounding personalised donation intervals into a coherent whole – to be able to allow blood donors to donate more frequently should there be a need for more blood, while minimising the risk that the donor comes to harm. Information on factors that affect a donor's ability to donate can be considered alongside the risk of the donor experiencing a deferral, post-donation symptom, or serious adverse event. Although overall blood demand has been decreasing recently, there exist groups of blood donors that are a priority for NHSBT such as BAME donors and those of rare blood groups, so a personalised donation policy may be relevant in the shorter term or in the future as demand for blood and demographics of the general and blood donor population change.

### 7.4.1 Assignment of Personalised Donation Intervals

Results from **Chapter 2** on correlates of low haemoglobin deferrals can be readily used by the blood service to inform literature such as pamphlets given to blood donors before and after donation, and recommend that donors in groups more predisposed to low haemoglobin deferrals take measures to manage their iron status between donations, to minimise low haemoglobin deferrals in these groups.

**Chapter 4** demonstrated that there are some factors that affect a donor's ability to donate blood at more frequent intervals, such as red blood cell count and occupation status, and so if the blood service wishes to maximise blood donations from the blood donor population it is easy to consider these. Some factors, affect both donation and low haemoglobin deferral numbers. These include the number of donations in the previous two years and ferritin levels. A balance between these two effects would need to be sought when deciding personalised donation intervals. If looking to predict haemoglobin and ferritin levels as part of this process, it would be necessary to perform more haemotology analyses than is currently routine practice, which comes at an additional cost to the blood service.

In **Chapter 5**, it was found that iron supplementation did not change the effect of randomised group on a donor's likelihood to develop symptoms due to more frequent donation. The implication of this

is that, while there may be other reasons why donors may be encouraged to take iron supplementation such as increasing haemoglobin levels to minimise low haemoglobin deferrals, in more frequent donation groups, it should not be expected that this will also reduce post-donation symptoms, nor mitigate against the increased risk of developing post-donation symptoms posed by more frequent blood donation.

The results from **Chapter 6** were largely non-significant, and it is possible that the only significant effect due to inter-donation interval was found by chance. Regardless, none of the variables studied were associated with a clinically meaningful change in the sub-components of PCS and MCS. It is therefore expected that changes to a donor's overall quality of life due to more frequent donation would be minimal. This trend continued in the INTERVAL two year extension study, and thus NHSBT may look to prioritise minimising low haemoglobin deferrals and post-donation symptoms.

### 7.4.2 Blood Collection During the Coronavirus Pandemic

The findings from this thesis are relevant to clinical practice. With the current coronavirus pandemic, blood collection agencies will have additional challenges for donor recruitment and retention. In the beginning of the pandemic in China, the USA and Australia, blood donations dropped considerably due to both fear of infection and more limited donation opportunities due to lockdown restrictions [271-275]. However, focused retention and recall of donors has been effective at maintaining and improving donor numbers after this initial fall in donations [276]. In addition, blood collection agencies in China and Australia have found that donors who receive clear communication about coronavirus adaptations to the service were more likely to donate during the pandemic [273, 277], and messages of this kind have been advised as a method to improve blood donor numbers [273]. However, it is possible that first time or reactivated donors who respond to the pandemic will not remain committed donors, and countries which are reliant on replacement donors may not see the same benefits [271]. Personalised donation intervals to maximise the supply of blood from existing voluntary donors could help blood collection agencies which struggle as the pandemic progresses to maintain blood supply.

### 7.4.3 Ferritin Testing of Blood Donors

A key consideration raised from this thesis is ferritin testing of blood donors. The Netherlands recently implemented a ferritin testing policy in blood donors, testing ferritin at a donor's first, and every fifth donation [278]. Donors were deferred between 6 and 12 months based on ferritin levels, and retention rates following a low ferritin deferral were 80% in men and 60% in women. However, low haemoglobin deferral rates were lower following ferritin deferral. A small-sample study from REDS-III found that ferritin testing was viable, with no significant variation in ferritin concentrations found in plasma separated from whole blood after five days, which reduces the pressure on blood collection agencies [279]. Should ferritin-based deferral policies be considered, iron supplementation may play a role in mitigating low ferritin deferrals more than observed in low haemoglobin deferrals. The HIERS trial in the USA randomised participants to iron supplementation following blood donation, and found that both haemoglobin and ferritin recovery were more rapid in donors taking iron supplementations, especially amongst those with low ferritin [280]. It found that the greatest benefit to iron stores was up to four weeks after donation, and recommended donors take iron supplementation immediately following donation to better manage iron levels [281, 282]. Additional RCT evidence would be useful to confirm the effect of iron supplementation on haemoglobin and ferritin levels in a blood donor setting.

### 7.4.4 BAME Donors and Rare Blood Groups

Currently, NHSBT has a shortage of black donors in particular [283]. This is important, as those from the same ethnic group are more likely to be from the same blood groups, and minor blood groups from donors of a different ethnicity can trigger reactions during transfusion even if donors are matched in their main blood type [284]. Despite black donor numbers rising by 35% in three years, there are still not enough to meet the demand for transfusions amongst patients with sickle cell disease, which has a higher prevalence in the black and minority ethnic community [285, 286]. Moreover, the Ro Kell negative blood which is particularly important for treating sickle cell disease has seen demand rise by 50% since 2015/16 and black donors are around ten times more likely to possess

this subtype than white donors [287]. While previous NHSBT campaigns have been effective [288], studies have identified motivations and deterrents to blood donation among black communities. Fear, and distrust in the blood and health services were found to be deterrents to black donors [285]. Under-representation of black donors remains a problem even in majority-black countries such as South Africa, where black donors make up 24% of the donor population compared to 83% of the general population. Altruism was cited as the single most popular motivator to donate by black South Africans, and altruism-based interventions have had the largest effect sizes in encouraging donation [285, 289]. However, warm glow (helping others because it makes one feel good), reluctant altruism (helping because one does not trust others will) have also been identified as important motivators, with first time and repeat donors responding differently to different messages based on altruism, and benevolence which emphasises both the gain for the donor and the recipient [28, 290]. Personalised inter-donation intervals could be used to address the gap in supply and demand for blood from black donors which may take time to address through novel recruitment strategies that address both the motivations and deterrents to blood donation within black communities. However, the demographics of the black donor population may be different to the INTERVAL trial population, and therefore some results from INTERVAL may not be directly applicable.

## 7.5 Future Directions

Extensions of the work in this thesis could include using the results from **Chapter 4**, **Chapter 5**, and **Chapter 6** to assign personalised inter-donation intervals in practice. While the aim of maximising the blood supply in the UK blood donor population is not immediately relevant, there are rare blood groups which are a priority for NHSBT, such as the Ro subtype which continues to see increased demand despite comprising 2% of the NHSBT blood donor population [291]. Personalised inter-donation intervals more frequent than the current NHSBT guidelines could be introduced for these blood donors. It would also be possible to overcome the primary limitation of the INTERVAL trial, that of the trial effect, and assess the impact of more frequent inter-donation intervals in routine practice. Ferritin testing could also be implemented in such a pilot scheme to help minimise low haemoglobin

deferrals and maintain donor health while donating more frequently. This could be combined with results from the COMPARE study on haemoglobin testing and STRIDES trial on vasovagal reactions to ensure donor safety [292].

In addition to this, the findings from this thesis could be used to guide future research. Expanded modelling using the results from this thesis could be performed to account for relationships between biomarkers and symptoms. Related outcomes could also be analysed together, for example, by using a joint modelling approach to assess haemoglobin and ferritin levels. Moreover, consideration could be made for clustering of donors by centre, or clusters of symptoms experienced by the same individual. In addition, structural equation modelling, LCA modelling, or machine learning approaches could be used to construct a framework that treats all the outcomes from this thesis as inter-related, enabling the development of an overall "donor risk score" which balances the donor's ability to donate while taking a holistic view of the potential consequences of donation. Outcomes from such modelling could be used to develop a more comprehensive understanding of donor health, and the impact of more frequent donation on donor health.

**Chapter 5** investigated many symptoms that had little or no previous investigation. Additional studies on the effects of blood donation on some of these symptoms could be beneficial to enhance the evidence base. In addition, while iron supplementation was reported in INTERVAL, it was not a part of the trial's design. It is possible that the donors in INTERVAL who took iron supplementation were doing so due to previous history of iron deficiency or side-effects of blood donation. In addition, fewer than 10% of INTERVAL participants took iron prescription at any point during the trial. Further study of iron supplementation in UK blood donors, such as a study with equal numbers of donors taking iron supplementation as those who did not, and ensuring via minimisation algorithms that the characteristics of donors in both groups were comparable, could be used to further investigate the mediation effect of iron supplementation on symptoms in blood donors, and to overcome the limitations of the INTERVAL data when used for this purpose.

It is important to note that findings from INTERVAL represent the UK blood donor population only. International studies carried out by blood services in other countries where the characteristics of the general population in terms of age, weight, ethnicity, donation history, and population health could present different results to those obtained using the INTERVAL data. In addition, the effect of varying the inter-donation interval could be different in countries where the inter-donation interval is different than the UK, and in countries which routinely recommend or offer iron supplementation to blood donors following blood donation. Such studies would be beneficial to set international guidelines on inter-donation intervals which could be referenced by blood services internationally should they experience variation in their blood supply and demand and wish to adjust their inter-donation intervals accordingly.

## 7.6 Conclusions

The INTERVAL trial has shown that there are some subgroups of the UK population that may be able to donate blood more frequently than current minimum inter-donation intervals. These groups of donors include men and women with a higher baseline haemoglobin level and number of previous donations, as well as older women. However, increased donation did also lead to an increase in some post-donation symptoms including tiredness, breathlessness, and fainting or feeling faint. Iron supplementation was not able to mitigate the effect of randomised group, and so it is important to bear this in mind when assigning donors to shorter intervals.

At the trial's inception, it was envisioned that there would be a shortfall in the supply of blood due to the aging population and the fact that new donor numbers are falling. However, there has instead been a decrease in blood demand owing to better transfusion practices such as only using one unit of blood at a time, having lower haemoglobin thresholds to begin transfusion, and other patient blood management techniques. While this is encouraging for the blood service, it is worth noting that the majority of the most committed blood donors in the UK are from the baby boomer generation, and in 10-15 years' time, many of these donors will be unable to give blood due to health complications or

aging out of the donor population. While INTERVAL's primary goal may no longer be immediately relevant, it is therefore possible that this work can be used in the future should the supply of blood fail to meet demand.

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# Appendix A: Systematic Review Search Strategy

**THE COCHRANE LIBRARY**

#1 MeSH descriptor Blood Donors, this term only
#2 MeSH descriptor Cytapheresis explode all trees
#3 (red cell* or RBC* or blood or platelet*) NEAR/6 (donor* or donat*)
#4 (donor* or donat* or interdonat*) NEAR/10 (defer* or delay* or exclu* or reject* or "turn* away" or interval*)
#5 (#1 OR #2 OR #3 OR #4)
#6 MeSH descriptor Anemia explode all trees
#7 MeSH descriptor Ferritins, this term only
#8 MeSH descriptor Hemoglobins, this term only
#9 MeSH descriptor Iron, this term only
#10 (iron or anaemi* or anemi* or ferritin or ferrous):ti
#11 (ferropaeni* or ferropeni* or Feosol or "Fer Iron" or "Fer-Gen-Sol" or "Fer-in-Sol" or Feratab or FeroSul or (Ferra NEAR/2 Caps) OR "Ferro-Bob" OR "Slow Fe" OR "Slow Release Iron")
#12 iron NEAR/3 (store* or status or deficien* or deplet* or supplement* or tablet* or pill* or sulphate or sulfate)
#13 (ferritin or iron or haemoglobin or hemoglobin or Hb or haematocrit or hematocrit or Hct) NEAR/3 (level* or low* or below or concentration* or cutoff or rais* or increas*)
#14 ferrous NEXT (sulfate or sulphate or fumerate)
#15 (#6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14)
#16 (#5 AND #15)

**MEDLINE (Ovid)**

1. BLOOD DONORS/
2. exp CYTAPHERESIS/
3. ((red blood cell* or red cell* or RBC* or blood or platelet* or plateletpheres*) adj6 (donat* or donor*)).tw.
4. ((donor* or donat* or interdonat*) adj10 (defer* or delay* or exclu* or reject* or turn* away or interval*)).tw.
5. or/1-4
6. exp ANEMIA/
7. FERRITINS/
8. HEMOGLOBINS/
9. IRON/
10. (iron or anaemi* or anemi* or ferritin or ferrous).ti.
11. (iron adj3 (store* or storing or status or deficien* or deplet* or supplement* or tablet* or pill* or capsule* or sulphate or sulfate)).ab.
12. ((ferritin or iron or haemoglobin or hemoglobin or Hb or haematocrit or hematocrit or Hct) adj3 (level* or low* or below or concentration* or cutoff or rais* or increas*)).tw.
13. (ferrous adj (sulfate or sulphate or fumerate)).ab.
14. (ferropaeni* or ferropeni* or Feosol or Fer Iron or Fer-Gen-Sol or Fer-in-Sol or Fer-In-Sol or Feratab or FeroSul or (Ferra adj2 Caps) or Ferro-Bob or Slow Fe or Slow Release Iron).tw.
15. 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
16. 5 and 15

**EMBASE (Ovid)**

1. BLOOD DONOR/
2. APHERESIS/
3. THROMBOCYTOPHERESIS/

4. ((red blood cell* or red cell* or RBC* or blood or platelet* or plateletpheres*) adj6 (donat* or donor*)).tw.
5. ((donor* or donat* or interdonat*) adj10 (defer* or delay* or exclu* or reject* or turn* away or interval*)).tw.
6. or/1-5
7. exp IRON DEFICIENCY ANEMIA/
8. IRON DEFICIENCY/
9. HEMOGLOBIN/
10. IRON DEPLETION/
11. (iron or anaemi* or anemi* or ferritin or ferrous).ti.
12. (iron adj3 (store* or storing or status or deficien* or deplet* or supplement* or tablet* or pill* or capsule* or sulphate or sulfate)).ab.
13. (ferropaeni* or ferropeni* or Feosol or Fer Iron or Fer-Gen-Sol or Fer-in-Sol or Fer-In-Sol or Feratab or FeroSul or (Ferra adj2 Caps) or Ferro-Bob or Slow Fe or Slow Release Iron).tw.
14. ((ferritin or iron or haemoglobin or hemoglobin or Hb or haematocrit or hematocrit or Hct) adj3 (level* or low* or below or concentration* or cutoff or rais* or increas*)).tw.
15. (ferrous adj (sulfate or sulphate or fumerate)).ab.
16. or/7-15
17. 6 and 16

**CINAHL (NHS Evidence)**
1. BLOOD DONORS/
2. exp CYTAPHERESIS/
3. ((red blood cell* OR red cell* OR RBC* OR blood OR platelet* OR plateletpheres*) AND(donat* OR donor*)).ti,ab
4. ((donor* OR donat* OR interdonat*) AND (defer* OR delay* OR exclu* OR reject* OR turn* away OR interval*)).ti,ab
5. 1 OR 2 OR 3 OR 4
6. ANEMIA, IRON DEFICIENCY/
7. FERRITINS/
8. HEMOGLOBINS/
9. IRON/
10. (iron OR anaemi* OR anemi* OR ferritin OR ferrous).ti
11. (iron AND (store* OR storing OR status OR deficien* OR deplet* OR supplement* OR tablet* OR pill* OR sulphate OR sulfate)).ab
12. (Feosol OR Fer Iron OR Fer-Gen-Sol OR Fer-in-Sol OR Fer-In-Sol OR Feratab OR FeroSul OR Ferra Caps OR Ferro-Bob OR Slow Fe OR Slow Release Iron).ti,ab
13. ((ferritin or iron or haemoglobin or hemoglobin or Hb or haematocrit or hematocrit or Hct) AND (level* or low* or below or concentration* or cutoff or rais* or increas*)).ti,ab
14. (ferrous AND (sulfate OR sulphate OR fumerate)).ab
15. 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15
16. 5 AND 16

**PUBMED** *(epublications only)*
(red cell[TI] OR red cells[TI] OR blood[TI] OR platelet[TI] OR platelets[TI] OR plateletpheresis[TI] OR defer*[TI] OR delay*[TI] OR interval* OR reject*[TI] or exclu*[TI] OR iron[TI] OR ferritin[TI] OR anemi*[TI] OR anaemi*[TI])
AND (donor*[TI] OR donat*[TI] OR interdonat*) AND (publisher[sb] NOT pubstatusnihms)

**WHO INTERNATIONAL CLINICAL TRIALS REGISTRY PLATFORM (including ClinicalTrials.gov, EU Clinical Trials Register & ISRCTN)**

((donor OR donors OR donate OR donated OR donation OR donations OR donating OR interdonation) [in Title] AND
((iron OR ferritin OR ferrous OR interval OR intervals OR deferral OR deferred) [in Interventions] OR (anemia or anemia or anaemic or anemic or iron deficient or iron deficiency or low hemoglobin OR low hematocrit) [in Condition]))

**TRANSFUSION EVIDENCE LIBRARY**
((red blood cell* OR red cell* OR RBC* OR blood OR platelet* OR defer* OR delay* OR exclu* OR reject* OR turn* away OR interval* OR iron OR ferritin OR anemi* OR anaemi*) [In Search All Text] AND (donor* OR donat* OR interdonat*)[Title or Keywords])

## Appendix B: Characteristics of Included Studies

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Afzal 2016 | Singapore | RBC | n/r | 12.5/12 | 88140 | 57 | 14.12 | 3.74 | 27.88 | One (undefined) year | New and repeat blood donors at blood donation centre over one year. Data collected on new/repeat donor status and haemoglobin levels |
| Agnihotri 2010 | India | RBC | CuSO4 and Hemocue (men), hemocue (women) | 12.5 | 6032 [6357] | 90.00 | 6.8 | 2.7 | 47.2 | January 2008 – June 2009 | Voluntary and replacement donors recruited over one and half years. Data collected on age, and voluntary donor status |
| Agnihotri 2014 | India | RBC | Hemocontrol | 12.5 | 7534 [8033] | 92.19 | 7.09 | 4.56 | 34 | 1.5 (undefined) years | Blood donors who visited donation centre over 1.5 years had data analysed. Haemoglobin and weight data collected |
| Al Shaer 2017 | UAE | RBC | Hemocue | 13.5/12.5 | 128054 [142431] | 84.34 | 10.33 | 5.25 | 31.02 | 01/01/2010 - 30/06/2013 | Allogenic whole blood donors aged between 17 and 65. Data collected on sex, age, ethnicity, and donor status. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Almeida 2013 | Brazil | RBC | Hemocue (pre-2003), HemataSTAT II (2003 onwards) | 13.0/12.5 | 385357 | 65.47 | 5.7 | 1.53 | 13.61 | January 1996 – December 2006 | Donors who had more than one visit to the centre and who were not deferred on their first visit due to the screening. Donors included in study until first low HCT deferral. Data collected on age (over vs under 50), baseline HCT level, and time between donations. |
| Arslan 2007 | Turkey | RBC | Hemocue | 13.5/12.5 | 83899 [94919] | 89.5 | 3.4 | 2.1 | 15.2 | 2001-2005 | Hospital blood bank donors aged 18-65. Data collected on education level and voluntary donor status |
| Baart 2012 | The Netherlands | RBC | Hemocue | 13.5/12.5 | 220946 [220946] [5] | 50.9 | 5.8 | 4.1 | 7.7 | 2007-2009 | Previous donors who visited a blood collection centre whose previous two donations were whole blood. Data collected on seasonality, age, previous Hb levels, time since donation, donation history and previous deferral, BMI, and blood volume. |
| Baart 2014 | Ireland | RBC | Capillary Hemocue | 13.0/12.0 | 45301 | 57.22 | 4.97 | 2.4 | 8.4 | 2008 - 2010 | Donors who visited any blood donor centre between 2008 and 2010 whose last two donations were RBC. Data collected on seasonality, age, previous Hb levels, time since donation, donation history and previous deferral. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Baart 2016 | Switzerland | RBC | Hemocue | 13.0/12.0 | 53722 | 60.41 | 5.82 | 3.28 | 9.69 | 2011 - 2013 | Donors who visited any blood donor centre between 2011 and 2013 whose last two donations were RBC. Data collected on seasonality, age, previous Hb levels, time since donation, donation history and previous deferral. |
| Backman 2016 | Finland | RBC, P, A | Fingerstick analysed with Hemocue | 13.5/12.5 | 336054 | 49.8 | 2.6 | 1.4 | 3.9 | January 2010 – December 2014 | Database of all donation attempts from January 2010 to December 2014. Data collected on blood group, rhesus status, seasonality, and first time vs repeat donors. |
| Bahadur 2011 | India | RBC | CuSO4 and Hemocue | 12.5 | 6152 [6817] | 98.3 | 2.0 | 1.2 | 34.2 | January – December 2009 | Donations were replacement (99.5%) and voluntary (0.5%). |
| Bakrim 2018 | Morocco | RBC | Sysmex | 13/12 | 15323 | 54.44 | 8.48 | 3.05 | 14.46 | November 2014 - May 2016 | Donors aged between 18 and 60 who attended donor centres or mobile blood drives. Data collected on sex, type of work, age, province, and donor status. |
| Baquero 2018 | Columbia | RBC, P, E | n/r | 13.5/13 | 139277 [179851] | n/r | 5.63 | n/r | n/r | 2011-2017 | Repeat donors who were deferred multiple times for low haemoglobin during study period. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Bashwari 2005 | Saudi Arabia | n/r | n/r | 40/38% HCT | 28398 [33900] | n/r | 3.54 | n/r | n/r | 01/01/1996 – 31/12/2003 | All prospective blood donors at university hospital between 01/01/1996 and 31/12/2003. |
| Bischke 2011 | Denmark | n/r | n/r | 13.5/12.5 | 219 [219] | 65.3 | 16.4 | 7.0 | 30.0 | n/r | Donors failing previous Hb test would have been offered iron tablets. |
| Bryant 2009 | USA | RBC | Fingerstick | 12.5 | 3549 [3730] | 53.0 | 9.2 | n/r | n/r | 10/27/2008 – 4/10/2009 | Consented donors >18yrs old. Data reported on ethnicity, age, and whether the donor stood or sat before donation. |
| Burkitbayev 2017 | Kazakhstan | RBC | Hemocue | 12/11 | 130887 | n/r | 3.5 | n/r | n/r | January 2015 - June 2017 | All donors who reported to donate during the study period. |
| Cable 2012 | USA | RBC, 2RBC | Copper suphate | 12.5 | 9633 [9901] | 48.45 | 9.54 | 3.13[5] | 15.71[5] | 2007 - 2009 | Donors who successfully donated at enrolment visit recruited to study. Grouped by gender and first time/repeat donor. Data collected on diet, smoking and reproductive history, gender, age (categorised), blood centre and 2 year previous donations (categorised) |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Cano 2012 | USA | n/r | n/r | n/r | n/r | n/r | n/r | n/r | n/r | 12 (undefined) years | Data analysed over a period of 12 months at a donor centre to see if temperature affects low HCT deferral rate. |
| Charles 2010 | Trinidad/Tobago | RBC | Copper sulphate | 13.5/12.5 | 8199 [11346] | 66.6 | 10.9 | 3.4 | 24.7 | 2005 | Data collected on age and voluntary donor status. |
| Chaudhary 1995 | India | RBC | CuSO4 | 13.5/12.5 | 12363 [14269] | 91.3 | 3.5 | 1.3 | 30.3 | 01/10.1992 – 31/12/1993 | All donors were unpaid voluntary relatives aged 18-60. Data also collected on medical history |
| Codaty 2013 | India | RBC | n/r | 12.5 | 3086 | n/r | 1.99 | n/r | n/r | 01/01/2012 – 31/12/2012 | Blood donors who attended blood bank between 01/01/2012 – 31/12/2012 |
| Cortes 2005 | Colombia | RBC | Hemocue | 13.5/13.0 | 210 | 59.5 | 7.6 | 0 | 18.8 | April – June 2004 | 300 donors presenting to a blood centre between April – June 2004, differentiated by altitude of city of residence. |
| Custer 2004 | USA | RBC | Copper sulphate | 12.5 | 4987704 [5607922] | 50.2 | 6.0 | n/r | 11.0 | 2004 | Data collected on donation history, age, ethnicity, and education levels |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Custer 2012 | USA | RBC, 2RBC, MC | n/r | 12.5 | 1064855 | n/r | 4.8 | n/r | n/r | September 2009 - July 2011 | Donors with at least one successful donation and one additional presentation between September 2009 and July 2011. Gives odds ratios for deferral by donation type, haemoglobin levels, IDI, previous donations, and age. |
| Custer 2012/ Mast 2010 | USA | RBC/P | Copper sulphate and Hemocue | 12.5 | 4987704 [5607922] | 48.9 | 8.7 | 1.1 | 15.9 | January 2006 – December 2009 | Donors from REDS study. Data collected on age, ethnicity, donor status, donor site, previous donation, education level. |
| Custer 2014 | USA | RBC, 2RBC, MC | Capillary fingerstick | 12.5 | 135040 | 50.37 | 5.09 | 0.004 | 9.94 | 01/08/2009 – 31/07/2012 | Donors who donated blood on their index visit and also donated at least once between 01/08/2009 – 31/07/2012). Information on sex, donation interval, baseline Hb, ethnicity, age and BMI (all categorised) |
| da Silva 2012 | Brazil | RBC | Cyanmethemoglobin | 13.0/12.5 | 200 | 27 | n/r | 38.89 | 52.05 | August 2005 - March 2006 | Low Hb deferred donors between August 2005 and March 2006 matched for gender with successive blood donors. Data collected on electrophoretic profile and A2 dosage, as well as measurement method. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| De Clippel 2017 | Belgium | RBC | Venuous Hb, compolab, haemospect | 13.5/12.5 | 1483 | 33.31 | 16.72 | 11.94 | 19.11 | 20/01/2015 – 31/03/2015 | Prospective study over five donor centres equipped with Compolab measurement device and compared with others. Data collected on sex and first time/repeat donors. |
| De Kort 2019 | The Netherlands | RBC | Hemocue | n/r | 131215 [138398] | 43.74 | 5.44 | 3.14 | 7.24 | 2015 | A single random visit in the study period for each donor was included in the study. Data collected on season, time of day, blood group, age, and number of visits in past 5 years |
| Delage 2012 | Canada | 2RBC | Blood analyser | n/r | 1163 | n/r | 2.32 | n/r | n/r | August 2009 onwards | Donors from August 2009 onwards, gives associations with low Hb deferral and ferritin, haemoglobin, age and donation frequency |
| Di Angelantonio 2017 Grieve 2018 | UK | RBC | Copper sulphate/gravimetric or venous blood/Hemocue | 13.5/12.5 | 45042 | 49.64 | 28.9 | 27.86 | 29.93 | Donor recruitment between 11/06/2012 - 15/06/2014. Two year follow-up | RCT which randomised donors who were aged 18, fulfilled criteria for donation, had an e-mail address, and willing to attend static donor centre to donate blood at different frequencies for two years. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Di Lorenzo 2009/2011 | Brazil | n/r | n/r | [40%/38 %] | 265173 [335109] | 66.0 | 2.8 | 0.6 | 5.5 | 2006 | Donors presenting in one of 18 blood centres or 2 hospital units. Data collected on age, ethnicity, previous donations, sickle cell trait, human development index, and size of city. |
| Duffy 2014 | USA | n/r | n/r | 12.5 | 30738 | n/r | 16.1 | n/r | n/r | July-Decmber 2012, July-December 2013 | Two study periods defined. July-Decmber 2012 where minimum inter-donation interval (IDI) is 8 weeks, and July-December 2013 where IDI is increased to 12 weeks. |
| Duffy 2015 | USA | RBC | n/r | n/r | 27347 | n/r | n/r | n/r | n/r | 2010 - 2014 | Donor database compared between 2010 and 2014 where IDIs are 8 and 12 weeks respectively. |
| Eder 2010 | USA | n/r | Mixed | 12.5 | 7546213 [7871268] | 49.3 | 7.7 | 1.4 | 13.9 | 2008 | American Red Cross donors. Data collected on donor status and age. |
| Gandhi 2012 | USA | RBC | n/r | 12.5 | 35053 [35053] [5] | 46.7 | 12.4 | 3.8 | 20.1 | 01/02/2010 – 31/01/2011 | Two hospital blood donation sites and one fixed site collection unit. Data collected on age, donation and deferral history, blood type, and disease history |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Girish 2012 | India | RBC | n/r | 12.5 [11] | 8732 [9113] | 97.3 | 1.1 | 0.7 | 16.6 | 01/10/2009 – 31/21/2010 | One fixed site collection unit. Data collected on age, and repeat donor status. |
| Goldman 2013 | Canada | RBC | n/r | 12.5 | 600 | 43 | 8.33 | 1.63 | 13.74 | January - May 2012 | Donors recruited between January and May 2012, with oversampling of younger, first-time donors. |
| Goldman 2019 | Canada | RBC | Portable hemaglobinometer | 12.5 (13)/12.5 | 941488 | n/r | n/r | n/r | n/r | 01/01/2016-30/06/2018 | Donors who presented to blood banks during the study period. In this time the minimum haemoglobin requirement for men was raised from 12.5 to 13 g/dL and the minimum donation interval for women was gradually lengthened from 56 days to 84. |
| Gomez-Simon 2014 | Spain | RBC | n/r | 13.5/12.5 | 276605 | 56.2 | 24.7 | 0.99 | 6.23 | September 2008 - September 2013 | All donor visits to mobile blood collection sites between September 2008 and September 2013. Two-step screening process used for deferrals. Data collected on age and gender. |
| Goncalez 2013 | Brazil | RBC | Capillary fingerstick or Hemocue | 13.0/12.5 | 787228 [963519] | 65.9 | 5.2 | 0.9 | 13.5 | 01/08/2007 – 31/12/2009 | Three Brazilian blood banks, data collected on repeat donor status, ethnicity, age, socio-economic status, education, and donation site. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Gorakshakar 2013 | India | n/r | Automated counter/copper sulphate | n/r | 500 | 79.8 | 15.6 | 8.4 | 43.7 | n/r | 500 blood donors from Mumbai area included in study. Data collected on age, sex, number of donations in the previous year, past and present illness, whether ready to take iron supplement collected and used to predict number of donations. |
| Gulen 2006 | Turkey | RBC/P | Capillary | [40%] | 1683 [2207] | n/r | 5.1 | n/r | n/r | July – December 2002 | Donors recruited at a blood centre at a children's hospital. |
| Hillgrove 2011 | Australia | RBC | Hemocue | 12.8/11.8 | 69686 [69686] | 47.1 | 1.5 | 0.5 | 2.3 | October – November 2004 | All donors attending in two states (New South Wales and South Australia). Data collected on age, donation and deferral history. |
| Hoekstra 2007 | Netherlands | RBC | Cell counter on venous sample, Hemocue | 13.5/12.5 | 520236 [520236] | 59.6 | 4.7 | 2.5 | 8.0 | January 2002 – December 2004 | Participants had to have donated twice during the study period. Data collected on weight and temperature. |
| Kagu 2010 | Nigeria | n/r | Hemocue | 12.5 | 3724 [4032] | n/r | 10.9 | n/r | n/r | April 2007 – April 2009 | Data collected on repeat donor status and donation site. |
| Kamel 2011 | USA | RBC | n/r | 12.5 | 1051720 [1051720] | 50.2 | 6.1 | 0.6 | 11.2 | 2010 | |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Kate 2013 | India | RBC | n/r | 12.5 | 1990 [2172] | 96.04 | 4.12 | 1.59 | 20.76 | January 2011 - December 2012 | Voluntary and replacement donors from January 2011 and December 2012. Information on low Hb deferral by sex and age |
| Khuankaew 2014 | Thailand | RBC | n/r | 13.0/12.5 | 55000 [58693] | n/r | 6.72 | n/r | n/r | 2013 | Donor data analysed from the year 2013. |
| Klausa 2013 | Poland | RBC | n/r | 13.5/12.5 | 121 | 23.14 | 28.1 | 21.43 | 30.11 | 25-10-2012 - 04/01/2013 | Survey of donors from 25-10-2012 and 04/01/2013 on dietary preferences. Deferral information on sex, first time vs repeat donors, and donors applying a special diet. |
| Konings 2013 | The Netherlands | n/r | n/r | n/r | 917 | 66.09 | 35.11 | 35 | 35.5 | 2009 - 2013 | Donors from southeast region of Sanquin Bloodsupply dataset between 2009 and 2013 analysed. |
| Kouao 2012 | Ivory Coast | RBC | Hemocue | 11.0 | 22516 [24363] | 75.0 | 3.4 | n/r | n/r | 01/01/2006 – 31/12/2008 | Hospital blood bank. Data collected on weight, repeat donor status, and blood pressure. |
| Kwenti 2016 | Cameroon | RBC | Hemoglobinometer | 13.0/12.0 | 1896 | 91.35 | 31.43 | 29.39 | 50 | 01/01/2014 - 31/12/2014 | Blood donors recruited between 01/01/2014 and 31/12/2014. Blood group and rhesus status collected, gender and age used as categorical variables. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Lau 1988 | USA | n/r | C + Hct[C] | [41%/38 %] | 140197 [145094] | n/r | 4.6 | n/r | n/r | 1981 - 1984 | Blood centre attendees. Data collected on age, donation history, exercise, occupation, previous deferral, past iron deficiency, and obstetric history |
| Lee 2013 | Hong Kong | RBC | Hemocue | 13.0/11.5 | 801 | 49.13 | 24.47 | 25.06 | 23.89 | 27/07/2009 - 24/08/2009 | Consenting donors who donated between 27/07/2009 and 24/08/2009. Data on donor weight, IDI, previous donations, and biomarkers. |
| Lee 2016 | Hong Kong | n/r | n/r | 13.0/11.5 | 90643 | 43.28 | 11.42 | 8.06 | 13.99 | 2014 - 2015 | All first time donors in 2014 and 2015 included. Age collected as categorical variable. |
| Lim 1993 | Singapore | n/r | n/r | 12.5/12.0 | 242167 [278401] | n/r | 1.6 | n/r | n/r | 01/01/1988 – 31/12/1991 | Attended Singapore Blood Transfusion Service. Data collected on race and menstruation history. |
| Magnussen 2014 | Denmark | RBC | Sysmex XE 2100 | 13.5/12.5 | 1735 | 40 | 2.48 | 0.003 | 3.94 | October 2013 - January 2014 | First time donors between October 2013 and January 2014.Age presented as categorical variable. |
| Magnussen 2015a | Denmark | RBC | Sysmex XE 2100 | 13.5/12.5 | 71450 | 46.5 | 2.57 | 0.006 | 3.5 | February 2012 - February 2015 | Donors analysed between February 2012 and February 2015. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Magnussen 2015b | Denmark | RBC | Venuous analysed with Sysmex | 13.5/12.5 | 193288 | 52 | 2.57 | 0.55 | 3.5 | 01/02/2012 – 01/02/2014 | |
| Malard 2018 | Caribbean | RBC | Sysmex | 13/12 | 20368 | n/r | n/r | 3.4 | 15.9 | 2015 | All donor candidates who presented to a collection site in 2015. Data collected on donation interval, previous haemoglobin level, and number of previous donations |
| Mangwana 2013 | India | RBC | n/r | n/r | 19878 [22404] | 93.88 | 4.39 | 1.91 | 34.4 | 01/01/2007 - 30/06/2010 | Donors who attended from 01/01/2007 and 30/06/2010 |
| Marks 2014 | Australia | RBC | Fingerprick/Venuous | n/r | 282 | 0 | 4.96 | n/r | n/r | from March 2009 - October 2010 | Female donors only, who had given at least one blood donation in the past two years recruited from March 2009 to October 2010 into trial receiving iron or placebo pills post-donation. Primary outcome was ferritin levels after 12 weeks. Age and Hb levels recorded. |
| Mast 2015 | USA | RBC | n/r | n/r | 393 | n/r | n/r | n/r | n/r | n/r | Frequent blood donors from 3 centres assigned to intervention groups of iron/placebo pills, iron status letters, and no action, and followed up for three years. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Mathur 2012 | India | RBC | CuSO4 and Hemocue | 12.5 | 30948 [35339] | n/r | 14.19 | n/r | n/r | September 2005 - July 2006 | Voluntary donors who attended between September 2005 and July 2006. Compares CuSO4 method with Hemocue. |
| Meinia 2018 | India | RBC | n/r | 12.5 | 2106 [2195] | 89.61 | 0.76 | 0.16 | 6.07 | June 2015 – May 2016 | Donors who presented during the study period. |
| Mirrezaie 2011 | Iran | n/r | Hemocue | 12.5 | 2000 | 70.0 | 16.3 | 10.0 | 31.0 | n/r | Randomly selected donors. |
| Munasinghe 2011 | Sri Lanka | RBC | n/r | n/r | 6964 [7609] | n/r | 3.5 | n/r | n/r | 2008-2010 | Data collected on repeat donor status. |
| Muon 2018 | Portugal | RBC | Spectrophomotery | 13.5/12.5 | 739576 | 48.94 | u/c[5] | u/c[5] | u/c[5] | 2007-2016 | Donors who presented during the study period. Data collected on sex and time between donations |
| Nadarajan 2010 | Malaysia | n/r | Copper sulphate and Hemocue | n/r | 84989 [93807] | n/r | 2.1 | n/r | n/r | 2006 - 2008 | Data collected on repeat donor status and donation interval |
| Nasserinejad 2013 | The Netherlands | RBC | Fingerstick | 13.5/12.5 | 15626 | 45.17 | 30.6 | 20.64 | 38.82 | 01/01/2007 – 31/12/2009 | Includes donors whose first visit was between 01/01/2007 – 31/12/2009 and who donated at least twice during this period. Data collected on age and gender, as well as season (summer vs winter) |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Nasserinejad 2015 | The Netherlands | RBC | Photometer | 13.5/12.5 | 5388 | 35.3 | 27.39 | 18.4 | 32.3 | 01/01-2005 - 31/12/2012 | New donors whose first visit was between 01/01-2005 and 31/12/2012 and who made at least one visit after the first donation |
| Nasserinejad 2016 | The Netherlands | RBC | n/r | 13.5/12.5 | 4461 | 34.79 | 9.26 | 5.28 | 11.38 | 01/01-2005 - 31/12/2012 | New donors whose first visit was between 01/01-2005 and 31/12/2012 and who made at least one visit after the first donation. Data collected on age and gender, as well as season (summer vs winter) |
| Newman 2013 | USA | RBC | n/r | n/r | 2247 | 49.4 | 4.51 | 1.09 | 8.11 | 06/06/2008 - 07/07/2008 followed for 3.75 years | 500 donors with a successful first donation between 06/06/2008 and 07/07/2008 and with at least two donations followed for 3.75 years |
| Ngoma 2013 | Japan | RBC | n/r | 12.5/12.0 | 218376 [231361] | 51.61 | 5.85 | n/r | n/r | March 2010 - March 2011 | Donors who attended two blood centres between March 2010 and March 2011. Information given on deferral by age (categorical), gender, location, and first time vs repeat donors. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Ngoma 2014 | Japan | RBC | n/r | 12.5/12.0 | 21788 [24778] | 43.55 | 11.91 | 0.006 [6] | 18.08 [6] | March 2010 - March 2011 | Students aged 16-24 who donated in two blood centres from March 2010 to March 2011. Information given on deferral by age (categorical), gender, location, and first time vs repeat donors. |
| O'Meara 2011 | Switzerland | RBC | Cell counter | 13.3/12.3 | 160612 [160612] | 46.2 | 2.4 | n/r | n/r | 1996 - 2009 | Optional iron supplementation offered from 2004 onwards. Data collected on age. |
| Oumeziane 2013 | UAE | RBC | Hemocue | 13.5/12.5 | 88509 [93230] | 90.44 | 4.18 | 2.4[6] | 21[6] | 2010-2012 | All prospective donors from 2010-2012) |
| Patiakas 2013 | Greece | RBC | n/r | n/r | 14358 [16580] | n/r | 2.67 | n/r | n/r | 2007 - 2013 | Blood donors in past six years questioned. Compared donors who donated in hospital and out of hospital. |
| Perez 2018 | USA | RBC, P, Ps, A | n/r | 12.5 (13)/12.5 | 1351593 [1448757] | 49.94 | 8.29 | 7.81 | 8.77 | 01/06/2015 - 30/11/2015, and 01/06/2015 - 30/11/2015 | Donors who presented during both study periods. Data collected on age, ethnicity, donor status and blood group |
| Pierelli 2011 | Italy | n/r | Capillary blood analyser. | 13.5/12.5 | 13196 [13347] | n/r | 3.0 | n/r | n/r | November 2008 – December 2010 | Hospital blood collection unit. Data collected on first time and reactivated donors, and mean corpuscular volume. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Prados Madrona 2014 | Spain | RBC | Hemocue | 8.4/7.8 mmol/L | 19916 | 48.68 | 24.01 | 7.16 | 0.4 | 2005-2009 | All donor presentations from 2005-2009. Age collected as categorical variable |
| Py 2014 | France | RBC | n/r | 13.0/12.0 | 2335869 | 53.71 | 2.13 | 0.74 | 3.33 | 2010 | Retrospective analysis of data from 2010. |
| Rabeya 2008 | Malaysia | RBC | n/r | 13.5/12.5 | 4001 [4138] | 70.0 | 2.3 | 0.9 | 5.6 | January 2006 – December 2006 | Data collected on donation history. |
| Raka 2010 | Macedonia | RBC | n/r | n/r | 21331 [21915] | 79.3 | 4.6 | n/r | n/r | 2009 | |
| Raouf 2016 | UAE | RBC | n/r | n/r | 62300 [74087] | n/r | 4.21 | n/r | n/r | 4 (undefined) years | Four year retrospective study, comparing deferrals at fixed and mobile sites |
| Rosochova 2011 | Switzerland | RBC | Hemocue | 13.5/12.5 | 19296 | n/r | 2.0 | n/r | n/r | 12 (undefined) months | |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Salvin 2014 | Australia | RBC, A | Capillary | 13.0/12.0, 12.5/11.5 (A) | 3049 | 52.18 | 4.2 | 1.79[7] | 5.93[7] | 4 consecutive days | Donor centres selected for inclusion based on state's relative contribution to blood supply. At selected centres over 4 consecutive days RBC donors were recruited, apheresis donors recruited in one random day of the same week. Height, weight, and previous donor history collected. |
| Samir 2015 | Egypt | RBC | n/r | 13.0/12.0 | 32412 [40765] | 74.36 | 10.52 | n/r | n/r | 2014 | Retrospective analysis of donors in 2014. Presents graphical results of deferral by month. |
| Saunders 2018 | UK | RBC | Copper sulphate | 13.5/12.5 | 1195 | 48.95 | 8.87 | 4.44 | 13.11 | n/r | Single donation from each donor selected. |
| Sebok 2007 | USA | n/r | CoSU4 followed by haemocrit analysis if this failed | 12.5 | 23100000 [24300000] | n/r | 7.8 | 1.0 | 13.8 | 2002-2004 | Data collected on age, season, and temperature variability. |
| Sharma 2013 | India | RBC | n/r | n/r | 18364 [19125] | 95.91 | 2.64 | 1.62 | 26.94 | January – June 2011 | Donor data from January – June 2011 analysed. Information on voluntary vs replacement donors given. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Shaz 2010 | USA | n/r | CoSU4 + spun haematocrit | 12.5 | 547261 [576317] | 52.8 | 8.9 | 1.2 | 15.7 | 2004-2008 | Voluntary donors who presented during the study period. Data collected on age and race |
| Sohail 2017 | Pakistan | n/r | n/r | n/r | 40220 [54292] | 89.43 | 7.41 | 2.96 | 38.45 | December 2013 – December 2016 | Voluntary blood donors who presented during the study period. |
| Sorensen 2019 | Denmark | RBC | Sysmex | 13.5/12.5 | 15567 | 53.53 | n/r | n/r | n/r | Unclear | Donors who had donated more than twice. Information on genetic data presented with a model for risk of low haemoglobin deferral. |
| Spencer 2016 | USA | RBC | n/r | 12.5 | 5017107 | 45.22 | 10 | 1.53 | 20 | 2006 - 2009 | Donors attending six donor centres between 2006 and 2009. Odds ratios given for donor deferral by race, age, weight, two year donation history, location and time between 2RBc and RBC donation. |
| Steele 2013 | USA | n/r | n/r | 12.5 | 1009127 [1219805] | 46.47 | 7.9 | 0.006 | 14.64 | 2011 | Donors who made their first donation in 2011 analysed. Gives analysis of deferral by gender and race. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Stotzer 2013 | Germany | RBC | n/r | n/r | 863 | 25.96 | 2.34 | n/r | n/r | n/r | Prospective study comparing iron replacement drug used by the blood service with a food supplement and no supplementation. |
| Sumnig 2015 | Germany | RBC | Capillary/Venuous | 13.0/12.5 | 553 [610] | 51.64 | 11.75 | 3.81 | 22.27 | n/r | Consecutive consenting donors were tested using two measurement methods. Deferral data given by sex and measurement method. |
| Sundar 2010 | India | RBC | Copper sulphate, cyanmethoglobin method | 12.5 | 16132 [16706] | 88.7 | 1.4 | 0.3 | 10.2 | January 2005 – December 2007 | Donors no more than 60 years old. Data collected on age and voluntary donor status. |
| Svirnoskaya 2012 | Belarus | RBC | Hemocue | 13.5/12.5 | 2031 [2902] | 82.01 | 4.87 | n/r | n/r | 2009 - 2010 | Donors who made their 20th donation between 2009 and 2010. |
| Timova 2014 | Macedonia | RBC | n/r | 13.5/12.5 | 2001 [2054] | n/r | 4.4 | n/r | n/r | 2013 | Donors who attempted donation in 2013 |
| Timova 2015 | Macedonia | RBC | n/r | 13.5/12.5 | 2179 [2255] | n/r | 3.44 | n/r | n/r | 2014 | Donors who attempted donation in 2014 |
| Tondon 2008 | India | P | Electronic cell counter | 12.5 | 1165 [1515] | n/r | 5.0 | n/r | n/r | January 2004 – November 2006 | Blood banks and mobiles, primarily first time donors. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Ugwu 2018 | Nigeria | RBC | Hemocue | 13.5/12.5 | 3139 [3377] | 89.16 | 19.18 | 13.78 | 51.09 | 01/05/2016-30/04/2017 | Blood donors at a tertiary care hospital. Data collected on age, education, employment status, and occupation |
| Valerian 2018 | Tanzania | RBC | n/r | n/r | 12934 [14377] | 79.13 | 2.98 | 1.59 | 8.13 | 01/01/2016-31/12/2016 | All donors who presented at the blood bank. Data collected on age, type of donor, and location of residence |
| van den Berg 2016 | South Africa | RBC | n/r | n/r | 4412 | n/r | 7.46 | n/r | n/r | 2 days | Consecutive sampling. Odds ratios of deferral risk by race, gender, age, and ferritin levels reported. |
| Van den Berg 2019 | South Africa | RBC | copper sulphate gravimetric or capillary POC | 12.5 (13.5)/12.5 | 4412 | 4.62 | 52.65 | 1.21 | 8.43 | August – October 2014 | Donors over the age of 18. Data reported on age, ethnicity, location, donor type, previous donation history, haemoglobin levels, and ferritin. |
| Vasudev 2016 | India | RBC | Hemocue | n/r | 7090 [7253] | 91.41 | 2.62 | 1.17 | 18.55 | 6 (undefined) months | Donor records from six months analysed. |
| Wichmann 2013 | Germany | RBC | Sysmex | 13.5/12.5 | 4312 [4388] | 56.93 | 2.76 | 1.09 | 5.16 | n/r | Two mobile blood sites equipped with Hemospect and donors asked to give both invasive and non-invasive blood measurement. |

| Study | Country of Study | Donation Type[1] | Hb screen[2] | Hb Threshold (M/F)[2] | Donation Attempts[3] | % Male | Hb Deferrals (%)[4] | | | Study Period | Description of Study Participants and Data Collected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | All | M | F | | |
| Wilkinson 1982 | Ireland | n/r | CuSO4 | 12.25 | 1763903 [1763903] | 65.7 | 5.7 | 1.0 | 14.6 | 1961 - 1980 | Aged 18-65. Data collected on repeat donor status. |
| Wilson 2012 | USA | n/r | Hemocue/ UltraCrit | n/r | 47488 | 47.43 | 8 | 1.03 | 14.02 | October 2008 - March 2010, September 2010 for 18 months | Donor data from September 2010 for 18 months (Ultracrit) and October 2008 to March 2010 (Hemocue) analysed comparing monthly rate of deferral by measurement method. |
| Zanella 1989 | Italy | n/r | CuSO4 and automated cell counter | 13.5/12.5 | 14641 | 63.3 | 4.3 | 1.2 | 9.7 | 1977 - 1987 | Donors who made their first donation during the study period. Data collected on age, annual frequency of donations, Hb concentration at first visit. |
| Ziemann 2006 | Germany | RBC | Automated haemotology analyser | 13.5/12.5 | 81913 | 57.5 | 6.4 | n/r | n/r | May 2003 – November 2005 | Consecutive donors. Data collected on donation interval and iron supplementation. |

n/r = not reported; [1] RBC = red blood cells; P = platelets, A = apheresis, 2RBC = double red cell, MC = multicomponent, E = Erythroperesis, Ps = Plasma; [2] g/dL (values in parentheses are % haematocrit thresholds); [3] number of donation attempts excluding deferrals due to reasons other than low Hb [total number of donation attempts]; [4] where possible, the low Hb deferral rate is calculated as a percentage of the combined total number of Hb deferrals and accepted donations, i.e. deferrals due to other reasons were excluded in the calculation of the low Hb deferral rate; [5] Data presented in paper does not add up to totals presented; [6] the number of deferrals due to reasons other than low Hb could not be determined; the deferral rate is therefore given as a percentage of all donation attempts, including those deferred due to other reasons; 5 sex-specific deferral data available for RBC donors only

# Appendix C: Sf-36 questionnaire as used in INTERVAL

## INTERVAL

### 1. YOUR HEALTH AND WELL-BEING



**1a. In general, would you say your health is:**

- ○ Excellent
- ○ Very good
- ○ Good
- ○ Fair
- ○ Poor
- ○ Don't know/prefer not to answer

**1b. Compared to one year ago, how would you rate your health in general now?**

- ○ Much better now
- ○ Somewhat better now
- ○ About the same
- ○ Somewhat worse now
- ○ Much worse now
- ○ Don't know/prefer not to answer

**1c. The following questions are about activities you might do on a typical day. Does your health now limit you in these activities? If so, how much?**

| | Yes, limited a lot | Yes, limited a little | No, not limited at all | Don't know/ prefer not to answer |
|---|---|---|---|---|
| Vigorous activities (e.g. running, lifting heavy objects, strenuous sport) | ○ | ○ | ○ | ○ |
| Moderate activities (e.g. housework, golf) | ○ | ○ | ○ | ○ |
| Lifting / carrying groceries | ○ | ○ | ○ | ○ |
| Climbing several flights of stairs | ○ | ○ | ○ | ○ |
| Climbing one flight of stairs | ○ | ○ | ○ | ○ |
| Bending, kneeling, or stooping | ○ | ○ | ○ | ○ |
| Walking more than one mile | ○ | ○ | ○ | ○ |
| Walking several hundred yards | ○ | ○ | ○ | ○ |
| Walking one hundred yards | ○ | ○ | ○ | ○ |
| Bathing or dressing yourself | ○ | ○ | ○ | ○ |

## INTERVAL

**1d. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?**

| | Yes | No | Don't know/prefer not to answer |
|---|---|---|---|
| Cut down on the amount of time you spent at work / on other activities | ○ | ○ | ○ |
| Accomplished less than you would like | ○ | ○ | ○ |
| Were limited in the kind of work / other activities you can manage | ○ | ○ | ○ |
| Had difficulty performing the work / other activities (e.g. it took extra effort) | ○ | ○ | ○ |

**1e. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?**

| | Yes | No | Don't know/prefer not to answer |
|---|---|---|---|
| Cut down on the amount of time you spent at work / on other activities | ○ | ○ | ○ |
| Accomplished less than you would like | ○ | ○ | ○ |
| Did work / other activities less carefully than usual | ○ | ○ | ○ |

**1f. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family friends, neighbours, or groups?**

- ○ Not at all
- ○ Slightly
- ○ Moderately
- ○ Quite a bit
- ○ Extremely
- ○ Don't know/prefer not to answer

**1g. How much bodily pain have you had during the past 4 weeks, ?**

- ○ None
- ○ Very mild
- ○ Mild
- ○ Moderate
- ○ Severe
- ○ Very severe
- ○ Don't know/prefer not to answer

**1h. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?**

- ○ Not at all
- ○ A little bit
- ○ Moderately
- ○ Quite a bit
- ○ Extremely
- ○ Don't know/prefer not to answer

# INTERVAL

**1i. The following questions are about how you feel and how things have been for you during the past 4 weeks. Please tick the answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks....**

| | All of the time | Most of the time | Some of the time | A little of the time | None of the time | Don't know/ prefer not to answer |
|---|---|---|---|---|---|---|
| Did you feel full of life? | ○ | ○ | ○ | ○ | ○ | ○ |
| Have you been very nervous? | ○ | ○ | ○ | ○ | ○ | ○ |
| Have you felt so down in the dumps that nothing could cheer you up? | ○ | ○ | ○ | ○ | ○ | ○ |
| Have you felt calm and peaceful? | ○ | ○ | ○ | ○ | ○ | ○ |
| Did you have a lot of energy? | ○ | ○ | ○ | ○ | ○ | ○ |
| Have you felt downhearted and low? | ○ | ○ | ○ | ○ | ○ | ○ |
| Did you feel worn out? | ○ | ○ | ○ | ○ | ○ | ○ |
| Have you been happy? | ○ | ○ | ○ | ○ | ○ | ○ |
| Did you feel tired? | ○ | ○ | ○ | ○ | ○ | ○ |

**1j. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?**

- ○ All of the time
- ○ Most of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time
- ○ Don't know/prefer not to answer

**1k. How TRUE or FALSE is each of the following statements for you**

| | Definitely true | Mostly true | Don't know | Mostly false | Definitely false | Prefer not to answer |
|---|---|---|---|---|---|---|
| I seem to get ill more easily than other people | ○ | ○ | ○ | ○ | ○ | ○ |
| I am as healthy as anybody I know | ○ | ○ | ○ | ○ | ○ | ○ |
| I expect my health to get worse | ○ | ○ | ○ | ○ | ○ | ○ |
| My health is excellent | ○ | ○ | ○ | ○ | ○ | ○ |

# Appendix D: Sf-12 as used in INTERVAL

## QoL6m

### 1. YOUR HEALTH AND WELL-BEING



**1a. In general, would you say your health is:**

- ○ Excellent
- ○ Very good
- ○ Good
- ○ Fair
- ○ Poor
- ○ Don't know/prefer not to answer

**1b. The following questions are about activities you might do on a typical day. Does your health now limit you in these activities? If so, how much?**

| | Yes, limited a lot | Yes, limited a little | No, not limited at all | Don't know/ prefer not to answer |
|---|---|---|---|---|
| Moderate activities (e.g. housework, golf) | ○ | ○ | ○ | ○ |
| Climbing several flights of stairs | ○ | ○ | ○ | ○ |

**1c. During the past 4 weeks how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?**

| | All of the time | Most of the time | Some of the time | A little of the time | None of the time | Don't know/prefer not to answer |
|---|---|---|---|---|---|---|
| Accomplished less than you would like | ○ | ○ | ○ | ○ | ○ | ○ |
| Were limited in the kind of work or other activities | ○ | ○ | ○ | ○ | ○ | ○ |

**1d. During the past 4 weeks how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?**

| | All of the time | Most of the time | Some of the time | A little of the time | None of the time | Don't know/prefer not to answer |
|---|---|---|---|---|---|---|
| Accomplished less than you would like | ○ | ○ | ○ | ○ | ○ | ○ |
| Did work / other activities less carefully than usual | ○ | ○ | ○ | ○ | ○ | ○ |

**1e. During the past 4 weeks, how much did pain interfere with normal work (including both work outside the home and housework)?**

- ○ Not at all
- ○ A little bit
- ○ Moderately
- ○ Quite a bit
- ○ Extremely
- ○ Don't know/prefer not to answer

## QoL6m

**1f. These questions are about how you feel and how things have been with you during the last 4 weeks. For each question, please tick the answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...**

| | All of the time | Most of the time | Some of the time | A little of the time | None of the time | Don't know/ prefer not to answer |
|---|---|---|---|---|---|---|
| Have you felt calm and peaceful? | ○ | ○ | ○ | ○ | ○ | ○ |
| Did you have a lot of energy? | ○ | ○ | ○ | ○ | ○ | ○ |
| Have you felt downhearted and low? | ○ | ○ | ○ | ○ | ○ | ○ |

**1g. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?**

- ○ All of the time
- ○ Most of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time
- ○ Don't know/prefer not to answer