

IRON STATUS IN A POPULATION WITH MILD
HEMOPHILIA A:
ASSOCIATIONS AND IMPACTS

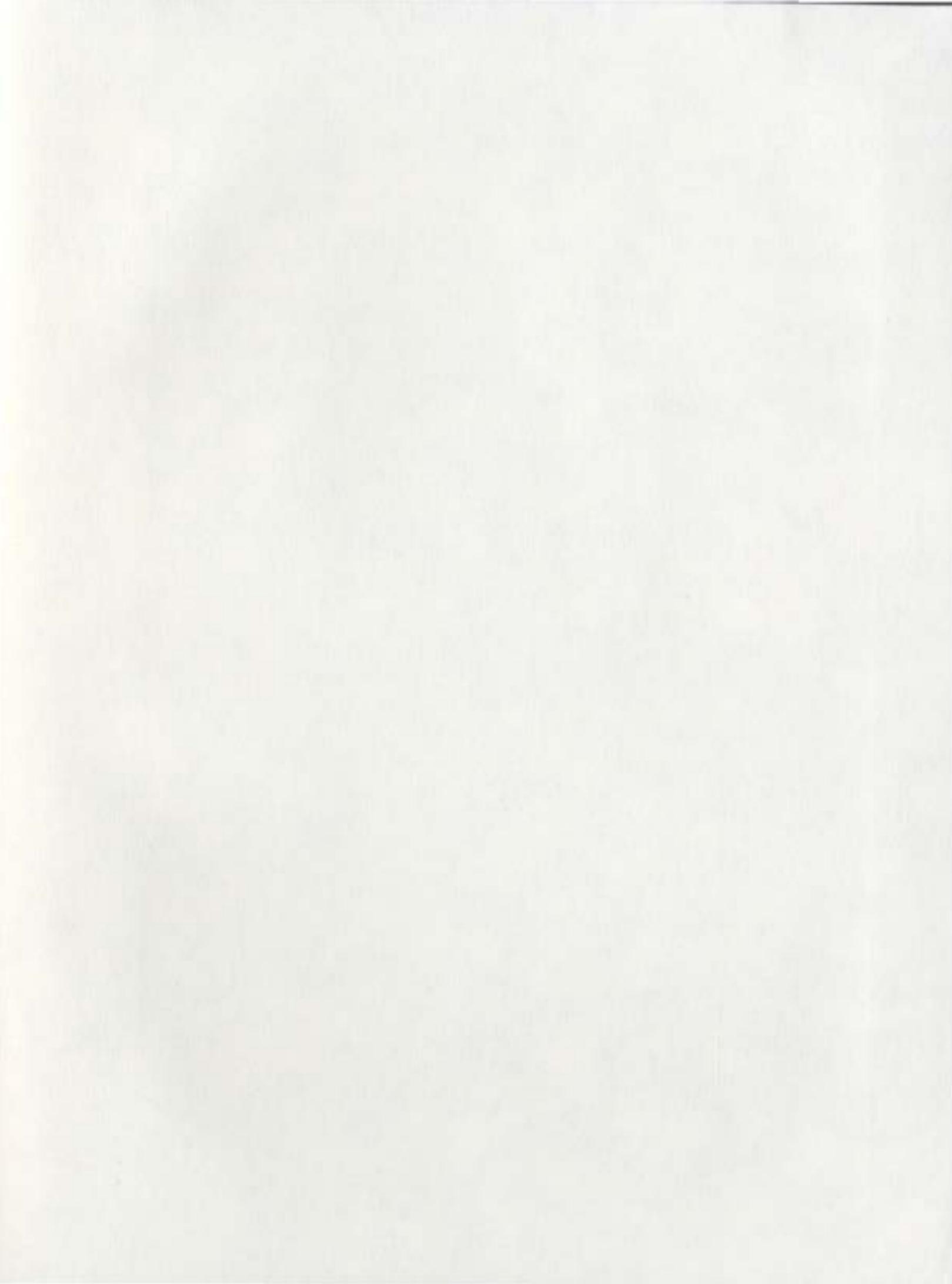
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MARC COLIN KAWAJA





**IRON STATUS IN A POPULATION WITH MILD HEMOPHILIA A:
ASSOCIATIONS AND IMPACTS**

by

© Marc Colin Kawaja

A thesis submitted to the
School of Graduate Studies
in partial fulfillment of the
requirements for the degree of
Master's in Clinical Epidemiology.

Faculty of Medicine
Memorial University

February 2006

St. John's

Newfoundland



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395 Wellington Street
Ottawa ON K1A 0N4
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Ottawa ON K1A 0N4
Canada

Your file *Votre référence*
ISBN: 978-0-494-19371-6
Our file *Notre référence*
ISBN: 978-0-494-19371-6

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Thesis Abstract:

Iron status, its associations and its impact on quality of life, was investigated in a cohort of patients residing in Newfoundland & Labrador. The population included individuals who were either affected with mild hemophilia A or were carriers of the Val2016Ala allele mutation that underlies the disease. The clinical impact of mild hemophilia A on serum ferritin, menstrual blood losses, quality of life and Body Mass Indices (BMIs) were investigated as part of a 2-year cross-sectional cohort study. The methodology included serum ferritin concentrations, complete blood counts (CBCs), the pictorial blood loss assessment chart (PBAC), bleeding histories, MOS short-form survey (SF-36) and BMIs. Iron depletion was prevalent in women (62% with ferritin levels $<50 \mu\text{g/L}$, 33% $<20 \mu\text{g/L}$), particularly in those who were menstruating (80% $<50 \mu\text{g/L}$, 47% $<20 \mu\text{g/L}$). Women in general self-reported a lower mean General Health Scale score (63.9, 59.9-67.9 vs. 70.6, 69.5-71.7) and a higher mean Role Emotional Scale score (89.3, 85.8-92.8 vs. 79.5, 77.7-81.3) than norms for the general U.S. female population. Men with a history of severe bleeding had significantly lower ferritin levels than men without a history of severe bleeding ($123.4 \mu\text{g/L}$, 63.6-186.3 vs. $189.8 \mu\text{g/L}$, 105.9-301.5; $p < 0.05$). Mutation status did not influence iron status in either sex or menstrual blood loss in women as measured by the PBAC. Serum ferritin level was not associated with PBAC score, but was related to women's BMI ($r = 0.235$, $p < 0.01$). Neither the means of the eight SF-36 domains, health transition scale, nor the two component summary measures were significantly lower for mildly iron-deficient or iron-deficient women. The SF-36 is a general measure of various quality of life domains and may not have been sensitive enough to measure the effects iron deficiency could potentially have on women's cognition and fatigue. A study using measures more sensitive to these effects would better investigate the impact of iron depletion and iron deficiency. Further research is also required to determine whether the low ferritin levels observed in women could possibly be a result of inadequate dietary intake of iron or insufficient iron absorption.

Acknowledgements

Dedication:

I would like to dedicate this thesis to my family who have always supported me in my work.

Acknowledgements:

This would not have been possible without the continuous support, guidance and leadership of my co-supervisors, Dr. Mary-Frances Scully and Dr. Gerry Mugford, and my supervisory committee member, Dr. Brendan Barrett. I have acquired a great deal of understanding in the area of hematology through Dr. Scully's supervision and Dr. Barrett has given me a great deal of understanding with regards to research and what it has to offer through his outstanding supervision these past years. I would also like to highlight the unwavering support throughout this process provided by Dr. Gerry Mugford. The feedback and guidance provided by Dr. Kirsty Tompkins was also appreciated.

I would like to extend a special thanks to the clinical impact of mild hemophilia A research team which includes: Dr. Mary-Frances Scully, Dr. Brendan Barrett, Meghan Walsh, David MacGregor, Michelle Hendry, Andrea Hann, Marilyn Harvey, and Charlotte Sheppard. I would also like to thank Andrea Kavanaugh for designing our databases, and Rebecca Mulcahy and Susan Stuckless for assistance with data entry and analysis.

And finally, I would like to thank Bayer Canada Inc. in supporting the clinical impact of mild hemophilia A project and the Canadian Hemophilia Society, Novo Nordisk Canada Inc., and the Faculty of Medicine at Memorial University of Newfoundland for supporting my thesis work.

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Chapter 1
Thesis Overview

1.0 Thesis Overview

This thesis will be presented in six chapters. Chapter 1 gives an overview of the chapter contents along with the co-authorship statement.

The intent of chapter 2 is to provide an overall view of iron deficiency, its associations and impacts, and the possible role it may play in individuals with mild hemophilia A. Detailed information regarding specific associations and impacts among iron-deficient individuals including mutation status, blood loss and cognition and fatigue is provided. Moreover, a review of relevant literature regarding prevalence of mild hemophilia A and iron deficiency is incorporated. Finally, the rationale for conducting this investigation is presented.

Chapter 3 includes the research questions of our study. The exact testable questions consisting of our primary and secondary objectives regarding our investigation is specified.

Chapter 4 includes our methods which discusses the study design, population, patient recruitment and assessment. To finish, a discussion of our outcomes, statistical analysis, data management and ethical considerations is presented.

In chapter 5, the baseline description of our subjects is provided. The results of the analysis of both the primary and secondary outcomes along with level of statistical relevance are offered.

And finally, in chapter 6, our discussion and implications of our results in terms of our outcomes are presented. Conclusions that may be reasonably drawn from the outcomes of the study along with associated strengths and weaknesses are provided. Direction for future studies is also included.

1.2 Co-Authorship Statement

i) Design and identification of the Master's project

Marc Kawaja, Dr. Mary-Frances Scully, and Dr. Brendan Barrett were responsible for the design and identification of the research topic.

ii) Practical aspects of the research

The collection of data used in this thesis was completed as part of a study examining the clinical impact of mild hemophilia A. Data collection was performed by Dr. Mary-Frances Scully, Meghan Walsh, David MacGregor, Michelle Hendry, Andrea Hann, Marilyn Harvey, Charlotte Sheppard, Rebecca Mulcahy and Marc Kawaja. Data entry into Access database was completed by Susan Stuckless, Rebecca Mulcahy, Marc Kawaja, and Meghan Walsh.

iii) Data analysis

Marc Kawaja completed the data and statistical analysis for the study in this thesis. Dr. Brendan Barrett and Dr. Gerry Mugford were consulted regarding the statistical methodology.

iv) Manuscript preparation

Marc Kawaja completed the manuscript with relevant revisions made by Dr. Brendan Barrett, Dr. Gerry Mugford, Dr. Mary-Frances Scully, and Dr. Kirsty Tompkins.

Chapter 2

Introduction

2.0 Overview

Initially, as part of a two-year cross-sectional cohort study aimed at defining the clinical impact of mild hemophilia A, a kindred with the same Factor VIII mutation underlying the disease who predominantly resides in the province of Newfoundland and Labrador, Canada was identified. Many adult women enrolled were subsequently identified as having low iron stores and literature suggests that iron depletion can have a significant effect on cognition and fatigue. This thesis is specifically focused on the burden of sub-optimal iron status in a population including this kindred of patients and its associations.

While there is little evidence to suggest that iron deficiency may be a complication in males, there is reason to believe that female carriers of mild hemophilia A could be at risk for having low iron stores (Kadir, Economides, Sabin, Pollard, & Lee, 1999). In general, mild hereditary bleeding disorders can have a significant impact on women - namely, prolonged and heavy menstrual bleeding - and signs of iron deficiency are commonly present in women who experience more than 60 ml of blood loss per month. Studies of iron status among adult women in Canada are also greatly lacking and are essentially non-existent for women in Newfoundland and Labrador. In recognition of this need, we are documenting the prevalence of iron deficiency and iron depletion in a rural population including affected men and women who are symptomatic and asymptomatic obligate carriers of the Val2016Ala allele mutation that underlies mild hemophilia A. We are also investigating the association of iron status with blood loss and

other potentially influential variables, and the potential impact sub-optimal iron status may have on various quality of life parameters.

2.1 Mild Hemophilia A

2.1.1 Background

Hemophilia is the second most common hereditary bleeding disorder in the world after von Willebrand's disease (vWD). Hemophilia A and B are sex-linked recessive disorders with defining phenotypes of cofactor VIII and IX deficiencies, respectively. The severity of bleeding is generally accepted to correlate with the level of factor activity and depending in part on the genetic mutation, the level of factor VIII activity can vary. According to the International Society on Thrombosis and Haemostasis (ISTH) standards, severe hemophilia is defined by factor levels less than 1% (0.01 U/mL), moderately severe hemophilia is diagnosed if the diagnostic factor levels are between 1 and 5% (0.01 to 0.05 U/mL) and mild hemophilia is characterized by a factor activity between 5 and 40% (0.05 to 0.40 U/mL) (White, Rosendaal, Aledort, Lusher, Rothschild, & Ingerslev, 2001).

Determining, with precision, the true global prevalence of mild hemophilia A to use as a reference standard is difficult. The observed prevalence of hemophilia is dependent on the incidence at birth and the duration or mortality of the diseases, both of which have changed, and will continue to change, as a result of improved screening procedures and treatment, respectively. The prevalence of co-morbid conditions such as HIV/AIDS and Hepatitis C, as well as the availability of screening procedures and treatment, also differ from one country to another, affecting national mortalities and the

ensuing national prevalence. Considering these limitations, the overall prevalence of hemophilia A in many Western countries is estimated to be around 1 in 5,000 (Rosendaal & Briët, 1990).

2.1.2 Mild Hemophilia A in Newfoundland

The number of people registered with hemophilia A in Canada is approximately 1 in 10,000 (Canadian Hemophilia Registry, 2004) and exclusively in the province of Newfoundland & Labrador 1 in 5000 persons are registered as having hemophilia A (Newfoundland and Labrador Hemophilia Registry, personal communication, December, 2004). What is strikingly different in the province of Newfoundland & Labrador is the unusually high proportion of mild cases of hemophilia A as determined by Factor VIII activity levels. While the most recent comprehensive national audit, performed by the Canadian Hemophilia Registry during March to July 2004, reports 1330 of 2221 (59.9%) factor VIII hemophiliacs as mild, in the province of Newfoundland and Labrador the prevalence is much higher with 104 of 114 (91.2%) registered patients with hemophilia A being classified as mild. Eleven of the 104 registered patients are female with factor VIII levels less than 30% and are considered to have mild hemophilia rather than just simply being labelled as “carriers” (Newfoundland and Labrador Hemophilia Registry, personal communication, December, 2004).

This unusual pattern of disease is likely a result of a founder effect. Dr. Yagang Xie’s laboratory at Memorial University of Newfoundland working in collaboration with Dr. David Lillicrap’s laboratory at Queen’s University and Dr. R. Bagnell’s laboratory at King’s and St. Thomas’ Medical School in England have identified a single nucleotide

polymorphism in the factor VIII genes, resulting in an amino acid change from valine (GTG) 2016 to alanine (GCG), as the underlying defect in this kindred. All affected males and obligate female carriers in the population carried the same mutant allele. This factor VIII mutation was found to be a molecular event independent of a Val2016Ala mutation localized in South East England via the identification of distinct factor VIII polymorphic haplotypes between the Newfoundland and English populations (Xie, 2002). The initial small population size, minimal immigration, and stagnated population growth in the New World Island region of Newfoundland and Labrador, likely facilitated the enrichment of the Val2016Ala allele.

2.1.3 Burden of Mild Hemophilia A

Although mild hemophilia has been reported in the literature since 1953, there is a paucity of studies with adequate power in the literature addressing bleeding in mild hemophiliacs. The largest cohort study includes 55 patients and is confined to patients aged less than 17 years of age. Bleeding episodes extracted from medical records mostly occurred in muscle/soft tissue or joints and were associated with trauma (Venkateswaran, Wilimas, Jones, & Nuss, 1998). Anecdotal evidence from small case series also suggest that patients with mild hemophilia, although they do not tend to bleed spontaneously, can have the same spectrum of significant bleeding disorders as patients with moderate and severe hemophilia if they experience trauma or surgery (Pappas, Salzman, Britten, & Riseborou, 1964; Coy, Bivins, & Belin, 1974; Beall, O'Leary, & Pierce, 1974). A number of case reports in the literature have used the term occult hemophilia to refer to mild hemophilia A and B (Aggeler, Hoag, Wallerstein, & Whissell, 1961; Kitchens, 1980). It

is generally felt that in these disorders, bleeding is less severe, does not occur spontaneously, and only occurs in response to trauma or surgery. However, no published cohort studies assessing long term musculoskeletal complications in middle and late adulthood of patients diagnosed with mild hemophilia A were found.

Preliminary clinical evaluation of a large cohort of patients with mild hemophilia A in the province of Newfoundland and Labrador has shown that the diagnosis is often delayed and certainly some patients in this cohort appear to have experienced significant musculoskeletal problems potentially related to undiagnosed hemophilia A. Ideally patients diagnosed with mild hemophilia A should undergo a response trial of Desmopressin (DDAVP). Patients who do respond well should receive early treatment with DDAVP for mild to moderate musculoskeletal injury and receive infusions with Recombinant Factor VIII for more severe injury.

In patients with severe hemophilia A, the most likely cause of death prior to contamination of the blood supply with the HIV and hepatitis C virus was bleeding into the central nervous system (Andes, Wulff, & Smith, 1984; Eyster, Gill, Blatt, Hilgartner, Ballard, Kinney, 1978). Whether or not intracranial bleeding is an important issue for patients with mild hemophilia A has not been well documented. Another important type of bleeding in severe hemophilia is spontaneous gross hematuria, which is generally a painless benign condition unless accompanied by large clot formation. An increased prevalence of hypertension has also been reported in this patient population (Rosendaal et al., 1990). The prevalence of these disorders in patients with mild hemophilia A is currently unknown as is the prevalence of other problems such as epistaxis, gastrointestinal bleeding and bleeding with dental work.

2.1.4 Iron Deficiency in Male Hemophiliacs

Iron deficiency is generally not considered a complication of hemophilia in men despite it commonly being a result of the occult or overt blood losses previously mentioned. The literature exploring the state of iron balance in male hemophiliacs is sparse, although one small case series questioned whether intermittent occult blood loss from microscopic subclinical hematuria might predispose moderate to severe hemophiliacs to iron deficiency. Lottenberg et al. examined bone marrow hemosiderin levels, radioactive iron kinetics, serum ferritin measurement, serum iron, and iron-binding capacity, seeking to measure urinary and stool blood loss in 8 male hemophiliacs. All the patients had factor levels in either the moderate (n=1) or severe range (n=7) as according to ISTH standards, with one patient having severe factor IX deficiency and the remainder having factor VIII deficiency. Patients with active genitourinary bleeding were excluded. The authors did not observe any overt bleeding episodes and found it difficult to persuade some subjects to return for follow-up observations. Four of the hemophiliac bone marrow hemosiderin estimates had no visible iron and the differences between age-matched, non-anemic, Hodgkin's or non-Hodgkin's lymphoma (stage I or IIA) controls and hemophiliac samples was significant ($p < 0.001$) (Lottenberg, Kitchens, Roessler, & Noyes, 1981).

The authors state that serum iron values, percent saturation, and serum ferritin levels were not suggestive of iron deficiency and suggest that in combination with an observed decline in total-body radioactive iron, this evidence is consistent with normal body iron balance. Lottenberg et al. suggest 2 hypotheses for what they consider a discrepancy between the bone marrow samples and the other measures of iron status: (i)

the external blood loss that went undetected must have been minimal or (ii) the external blood loss was insignificant, but as internal tissue bleeding occurs, iron is subsequently deposited in hemosiderin-laden macrophages in synovia and other connective tissue and is not redistributed to the marrow, reducing bone marrow stores, but leaving ferritin levels and therefore erthropoiesis maintained. However, the authors used a serum ferritin cut-off of $< 12 \mu\text{g/L}$ as indicative of iron deficiency and literature suggests that this cut-off is low and may exclude patients who have adverse health consequences associated with higher serum ferritin measures. One extensive diagnostic review suggested a cut-off of $< 40 \mu\text{g/L}$ as optimal (Guyatt, Oxman, Ali, Willan, McIlroy, & Patterson, 1992). The 4 patients with bone marrow hemosiderin samples showing no visible iron had serum ferritin values of 12, 18, 40 and $66 \mu\text{g/L}$ (Lottenberg, Kitchens, Roessler, & Noyes, 1981). Taken together, this weakens the validity of the author's implication that there is a discrepancy between these measures and the conclusion that all the patients had normal body iron stores.

One other study systematically evaluated anemia and iron status in a large population of children and young adult men with hemophilia. In this cross-sectional cohort study 94 boys ($n=74$) or young men ($n=20$) were prospectively evaluated during routine comprehensive hemophilia clinic visits. As per ISTH standards, 49 were severe, 28 moderate, and 17 mild. Seventy-eight patients were factor VIII deficient, 14 factor IX deficient, and 2 had another deficiency. Thirty-four were HIV positive and none of the patients were receiving iron supplementation at the time of the study. Slightly reduced hemoglobin values, expressed as age-related percentiles, were observed in hemophiliacs of all ages. Twenty-nine of the patients (31%) were considered frankly anemic with

values less than the third percentile for age. While none of the adult patients had frank anemia, 75% of them had hemoglobin values less than the mean value for age. The mechanism of the reduced hemoglobin in the entire study population or in individual patients with the lowest hemoglobin values was not clear and mild sub-clinical iron deficiency or the anemia of chronic disease were not proven to be part of the anemic pathophysiology. Only 5 patients had serum ferritin values $< 20 \mu\text{g/L}$. The authors concluded that slightly decreased hemoglobin values are common in patients with hemophilia, but the mechanism of hemoglobin reduction does not appear to be iron deficiency or impaired iron reutilization (Buchanan & Holtkamp, 1988).

The paucity of literature that has evaluated iron status in male hemophiliacs suggests that iron deficiency is likely not a complication of the disease. Furthermore, only a few of the hemophiliacs in these studies were of mild severity. Therefore, in the present study, it is not expected that the prevalence of iron deficiency in males with mild hemophilia will be significantly different from normal males. However, given the scarcity of the literature on this topic these groups will still be compared. While there is little evidence to suggest that iron deficiency may be a complication in males, there is reason to believe that female carriers of mild hemophilia A may be at risk of being iron deficient (Kadir et al., 1999).

2.2 Hemophilia in Women

2.2.1 Background

Due to its X-linked inheritance pattern, hemophilia occurs rarely in females. However, case-reports have suggested that severely affected girls and women suffer from

the same bleeding problems as their male counter-parts and also experience very severe, sometimes life-threatening menorrhagia (Lusher & McMillian, 1978; Ulutin, Muftuoglu, & Palamar, 1965 Joist, Bouhasin, & Roodman, 1977; Merskey, 1951; De La Chappelle, Ikkala, & Nevanlinna, 1961). Wahlberg et al. studied a group of carriers and non-carriers of hemophilia A with family histories of both the severe and moderate phenotypes. Through combining information from pedigree data and discriminant analysis data, 43 possible carriers were classified as either carriers or non-carriers with about 95% confidence. The median (range) factor VIII activity (one-stage assay), expressed as percentage of normal pooled plasma, of all carriers (n=38) (obligate and classified combined) and those classified as non-carriers (n=16) was 62(25-120) and 88(38-124), respectively ($p < 0.001$). The carrier group also had significantly more bleeding symptoms than non-carriers ($p < 0.001$) (Wahlberg, Blombäck, & Brodin, 1982).

It has been suggested more recently that mild hemophilia may be more common in females than was once originally thought (Venkateswaran, Wilimas, Jones, & Nuss, 1998). Eyster et al. studied two unrelated families with mild hemophilia A and identified 6 obligate carriers with low factor VIII procoagulant activity. Both families had successive generations of males affected with mild hemophilia A with factor VIII procoagulant activities ranging from 0.11-0.18 U/ml in the first family and 0.059-0.10 U/ml in the other. The factor VIII procoagulant values of 2 of the 5 carriers in the first family was 0.41 and 0.59 and the values in 4 of the 6 carriers in the other family was 0.36, 0.35, 0.20, and 0.23 U/ml. Based on their findings the authors emphasize that factor VIII levels should be determined on all obligate or suspected carriers prior to surgery or

dental extractions so as to identify individuals at risk for postoperative bleeding (Eyster, Ladda, & Bowman, 1977).

2.2.2 Prevalence of mild bleeding disorders

In general, mild hereditary bleeding disorders can have a significant impact on women; namely, prolonged and heavy menstrual bleeding (Kadir, Economides, Sabin, Owens, & Lee, 1998; Kadir et al. 1999). Kadir et al. demonstrated that when 150 women, referred for menorrhagia assessment, were carefully screened, 17% were diagnosed with a congenital bleeding disorder (Kadir et al., 1998). Similarly, using a pictorial blood loss assessment chart (PBAC), to assess menstrual blood loss in women with bleeding disorders, menorrhagia (PBAC score > 100) was putatively confirmed in patients with von Willebrand's disease (vWD), hemophilia carriers, and factor XI deficiency, with a prevalence of 73%, 57%, and 59%, respectively. In comparison, menorrhagia was confirmed in only 29% of women in the control group ($p=0.001$). There was no difference in the PBAC scores of hemophilia carriers with FXI or FVIII levels ≤ 3.0 IU/ml compared to those with higher levels, however the number of women with levels ≤ 3.0 IU/ml who had completed PBAC were very small. Also, while the authors demonstrate that a significant number of carriers of hemophilia A or B have verified menorrhagia, they caution that the response rate was particularly low in this group (26%), possibly due to the fact that they suffer less problems compared to the other groups that demonstrated higher response rates (vWD, 69%; FXI, 47%; Control, 60%). The findings may therefore represent an overestimate because the large number of non-responders might have had normal menstruation (Kadir et al., 1999).

2.2.3 Menstrual Blood Loss

These publications by Kadir et al. have generated a global interest in the issue of women with menorrhagia and bleeding disorders. Menorrhagia is the term used to describe excessive menstrual bleeding. Metrorrhagia is the term used to describe menstrual bleeding lasting longer than 7 days of interval bleeding and is another common symptom in women with congenital bleeding disorders. A normal cycle will last less than 7 days and occurs regularly at intervals between 21 and 37 days. Regardless of the length of flow, 70% of blood loss will occur by the second day and 90% by the third day. Total blood loss of 20-80mL, representing 10-35mg of iron is considered to be normal. The mean menstrual blood loss for a normal period is approximately 40mL. Signs of iron deficiency are present in a significant proportion of women who lose more than 60mL of blood with each menstrual flow (Swartz & Butler, 1992). In a large population study Hallberg and associates attempted to define the upper normal limit of the menstrual blood loss. Using the accurate and reliable alkaline hematin method to measure menstrual losses, and various parameters reflecting the iron state of the body, Hallberg et al. showed that signs of iron deficiency are present in women who experience more than 60 ml of blood loss and occurs in 67% of women whose menstrual exceeds 80 ml. Based on these findings, the upper normal limit of the menstrual blood loss is situated between 60-80 ml and blood loss above 80 ml is regarded as pathological (Hallberg, Hogdahl, & Nilsson, 1966).

The combination of menorrhagia and metrorrhagia can occur and is particularly problematic. Therapeutic interventions such as dilatation and curettage will often worsen the problem since scraping the lining of the uterus disrupts any platelet plugs or fibrin

clots that may be in place (Paper, 2000). In addition, it is increasingly recognized that women with hereditary bleeding disorders that are mild may suffer from dysmenorrhea and mid-cycle pain. They may also have problems with conception, fertility and have an increased prevalence of post partum hemorrhage (Aledort, 2000; Seeler, 1999). Discovering they are obligate carriers may also potentially have a negative psychological effect on women and there is a need to properly describe and quantify the potential impact of a mild bleeding disorder on their quality of life (Kadir, Sabin, Goldman, Pollard, Economides, & Lee, 2000; Ranta, Lehesjoki, Peippo, & Kaariainen, 1994; Varekamp, Suurmeijer, Brocker-Vriends, van Dijck, Smit, Rosendaal, & Briet, 1990). In this study we are interested in documenting the prevalence of iron deficiency, its association with menstrual blood loss, contraceptive usage and body mass index (BMI), and the impact it may potentially have on various quality of life parameters, in a population including women who are symptomatic and asymptomatic obligate carriers of the Val2016Ala allele mutation that underlies mild hemophilia A.

2.3 Iron Deficiency in Women

2.3.1 Iron Compartments, Transport and Depletion Levels

Measures of iron status reflect changes in different body iron compartments and are affected at different levels of iron depletion. Therefore a brief overview of body iron compartments, iron transport and distinctions between different levels of depletion are necessary. This is not only essential in order to define different iron deficient states and the associated clinical impacts, but to also conceptualize each laboratory measure in terms of iron status.

Iron status can be characterized in relation to the amount of iron held in the storage and functional areas of the body. The former is located primarily in the parenchymal cells of the liver and the reticuloendothelial cells of the bone marrow, liver, and spleen and the latter exists mainly as hemoglobin in red blood cells, and to a lesser degree in myoglobin and various tissue enzymes (Cook & Skikne, 1989). Transferrin is an iron transport protein that supplies tissues with iron. Transferrin receptors are expressed on the surface of cells that require iron and are responsible for internalisation of transferrin-bound iron and its intracellular release. The number of membrane receptors is proportionate to the number of receptors found in the plasma. Measurements of circulating soluble transferrin receptors can therefore be used as an indicator of the rate of erythropoiesis and as an indicator of iron deficiency. Ferritin, another iron-binding protein, transports iron from iron stores to functional sites. When iron depletion begins; that is, when stores start to decrease due to inadequate supply, there is no dysfunction. However, when iron stores are completely empty, or possibly merely considerably depleted (iron depletion), this continual imbalance will result in an inadequate iron supply to the red cell producing marrow and to tissues for normal biochemistry and function. At this point, abnormalities in physiological functions can occur. Small functional deficits have been related to reduced memory/attention, increased fatigue, and possibly causing excess menstrual bleeding in women (Bruner, Joffe, Duggan, Casella, & Brandt, 1996); Verdon et al., 2003; Taymor, Sturgis, & Yahia, 1964). Iron-deficiency is defined as the point at which iron stores are completely depleted and is most commonly reflected by measuring serum ferritin concentration. The greater the severity and duration of iron deficiency, the higher the probability that the iron deficiency may significantly change

other laboratory measures such as hemoglobin, transferrin saturation, red cell protoporphyrin (RCP), and red cell indices - mean cell volume (MCV) and red cell distribution width (RDW). Iron deficient erythropoiesis therefore starts as soon as iron stores are depleted, but our ability to detect it depends on methods and cut-offs selected. As a result, this classification is vague and the boundary line between erythropoiesis and iron deficiency is not stable (Hallberg & Rossander-Hulten, 1991). Iron deficiency anemia occurs when there is inadequate iron available to the extent that the amount of functional hemoglobin is decreased below a defining level. In the present study, we will define the different levels of iron status using serum ferritin concentration, red cell indices, and hemoglobin. However, the distinction between depleted iron stores without anemia and depleted iron stores with anemia is of primary interest, and the other iron status sublevels should be used only as a conceptual guide. In the absence of a definitive quantitative relationship between serum ferritin measures and clinical symptoms in mild cases of iron depletion, iron depletion will be treated as a grey-zone with various cut-off values (Table 1). An overview of the literature with respect to the utility of using serum ferritin as the sole measure of iron status (without MCV, RDW, etc.), the various serum ferritin cut-off values, and the differences between the cut-off values for men and women are discussed in the methods chapter.

Table 1.1: Laboratory definitions of sub-optimal iron status for females and males

Sub-optimal Iron Status	Sex	Laboratory Definition			
		Serum Ferritin ($\mu\text{g/L}$)	MCV(fL)	RDW (Units)	Hemoglobin (g/L)
Iron depletion	F	<50, 40, or 20*	Normal	Normal	Normal
	M	<50, 40, or 30			
Iron Deficiency	F	<11	Normal	Normal	Normal
	M	<24			
Iron Deficient Erythropoiesis	F	<11	<80	>14.5	Normal
	M	<24			
Iron Deficiency Anemia	F	<11	<80	>14.5	<120
	M	<24			<140

* Iron depletion is not well defined and will be treated as a grey-zone with various cut-off values

2.3.2 Prevalence of Iron Deficiency

Iron deficiency is far less prevalent in developed countries than in undeveloped countries. However, while significant progress has been made over the last century in combating iron deficiency in many industrialized countries, iron deficiency still remains a public health concern for selected subgroups. Women of reproductive age with excess menstrual blood losses and pregnant women who cannot meet requirements from their diet alone are at risk and iron deficiency still remains the most common nutritional deficiency among 15-49 year old women in the western world (Ramakrishnan, & Yip, 2002; Ferguson et al., 2001).

Depending on the laboratory criteria, methodology, epidemiologic factors (co-morbid conditions, age, contraception), and nutrition factors (heme and non-heme iron intake, vitamin C, iron supplementation), the prevalence of sub-optimal iron status will vary. Prevalence measures primarily using serum ferritin will be noted because it shows the least overlap between iron replete and iron deficient states (Guyatt et al., 1992; Hallberg, Bengtsson, Lapidus, Lindstedt, Lundberg, & Hultén, 1993). From a random sample of adults aged 25-65 years in the capital area of Finland, iron status was evaluated via serum ferritin in 137 non-pregnant women. In women aged 25-50 years, 20% had serum ferritin results $<12\mu\text{g/L}$ and 32% $<16\mu\text{g/L}$ (mean \pm SD = $32 \pm 12\mu\text{g/L}$; n=84). In contrast, women aged 51-65 years had corresponding serum ferritin results of 11% and 13% (mean \pm SD = $62 \pm 59\mu\text{g/L}$; n=53) (Lahti-Koski, Valsta, Alfthan, Tapanainen, & Aro, 2003). In Norway, iron deficiency defined as ferritin $<17\mu\text{g/L}$ was observed in 21.8% (mean \pm SD = 34.1 ± 23.9 ; n=147) of menstruating, non-pregnant, women aged 18-48 years who were non-blood-donors, aged 18-48 years (Borch-Johnsen, Meltzer,

Stenberg, & Reinskou, 1989). In contrast, iron deficiency as defined by serum ferritin $<16\mu\text{g/L}$ was observed in 9.7% of 268 menstruating, non-pregnant Danish women 18-30 years of age (Milman, Clausen, & Byg, 1998). In the Netherlands 16% of 75 non-pregnant women aged 20-49 had low iron stores (serum ferritin $<10\mu\text{g/L}$) (Brussaard, Brants, Bouman, & Löwik, 1997).

In The Dietary and Nutritional Survey of British Adults (1990) 17% of 520 menstruating women aged 18-49 had serum ferritin scores $<13\mu\text{g/L}$ (Hallberg, 1995); in the area of Coleraine, Northern Ireland, 18.2 % of 192 women aged 18-44 had serum ferritin scores $<12\mu\text{g/L}$ (Strain, Thompson, Barker, & Carville, 1990); and in Dublin, 36% and 45% of 106 women aged 16-25 had scores $<12\mu\text{g/L}$ and $<15\mu\text{g/L}$, respectively (Wickham, Broin, O'Rourke, Condren, Scott, & Kevany, 1985). The overall prevalence of sub-optimal iron status among 15-49 year old women in New Zealand ($n=1751$) was also shown to range from 7 ($<12\mu\text{g/L}$) to 13% ($<16\mu\text{g/L}$) (Ferguson et al., 2001). In summary, for populations of non-pregnant, menstruating women, varying in age from 15-50 years, which reside in either northern / central Europe or New Zealand, the prevalence of sub-optimal iron status ranges from 7-45%, as defined by serum ferritin cut-offs ranging from 10-20 $\mu\text{g/L}$.

The National Health and Nutrition Examination Surveys (NHANES III, 1988-1994 and NHANES 1999-2000) conducted in the United States used more stringent criteria for iron deficiency; that is, the definition of iron deficiency was based on 3 laboratory tests of iron status – free erythrocyte protoporphyrin, transferrin saturation, and serum ferritin – rather than serum ferritin alone. To be considered iron deficient, individuals had to have abnormal values for two or more measures of iron status.

NHANES III showed that for non-pregnant women aged 20-49 (n=4495), 11% were found to be iron deficient with either 2 of the following 3 results: transferrin saturation <15%, serum ferritin <12µg/L, or erythrocyte protoporphyrin >1.24 µmol/L RBCs (Looker, Dallman, Carroll, Gunter, & Johnson, 1997). Similarly, using the same criteria, NHANES 1999-2000 indicated that 12% of non-pregnant women aged 20-49 (n=949) were iron deficient (CDC, 2002).

In contrast to Europe, and possibly the United States, measurements of iron status for adult Canadian women are sparse. Using data from the Nutrition Canada Survey (1973) designed to sample the population in five regions (Atlantic, Quebec, Ontario, Prairies, and British Columbia), Valberg et al. found 27% of non-pregnant women aged 20-39 (n=100) and 24% of non-pregnant women aged 40-64 (n=105) had serum ferritin results <15µg/L (Valberg, Sorbie, Ludwig, & Pelletier, 1976). To our knowledge, the only other study of iron status in Canadian women was conducted by Newhouse et al. in Thunder Bay, Ontario. Iron depletion measured by serum ferritin levels <20µg/L was identified in 39% of 111 women aged 18-40 (Newhouse, Clement, & Lai, 1993). In a 1 day recall study, Troppman et al. found that only about 15% of men and 19% of women used iron containing (multivitamin supplements while less than 2% of contacted persons used a pure iron preparation (Troppmann, Gray-Donald, Johns, 2002).

Community studies of iron status for women in Canada are somewhat lacking and are essentially non-existent for women in Newfoundland and Labrador. While there are no recent studies showing the body iron status of individuals in Newfoundland and Labrador, Nutrition Newfoundland and Labrador: The Report of a Survey of Residents of Newfoundland and Labrador, 1996, suggested that although the iron intakes of males and

senior females appeared adequate, many females less than 50 years appeared to be consuming an inadequate amount of iron. Inadequate intake was calculated using the Estimated Average Requirement (EAR) method according to the Full Probability approach. The calculation accounted for the asymmetry in iron requirements over the population as a result of menstruating women, and allowed for the consideration of some uncertainty factors in the estimation (oral contraception use and sampling distribution). The survey showed that 25.3% of females aged 19-30 and 33.3% of females aged 31-49 were consuming <EAR (Roebathan, 2003). In the present study we measured iron status, and calculated the prevalence of the different levels of suboptimal iron status, for women and men living in rural Newfoundland and Labrador.

2.3.3 Blood Loss and the Etiology of Iron Deficiency

There are numerous factors that appear to influence iron status and that may make women susceptible to iron depletion, iron deficiency, developing subsequent iron-deficient erythropoiesis, or anemia. A few potentially modifiable factors are menstrual losses, nutrition, iron absorption and BMI.

Since blood is rich in heme-iron, excessive blood loss can lead to iron-deficiency and possibly anemia. Women presenting with menorrhagia are at risk of becoming iron-deficient and may require nutritional supplementation. Signs of iron deficiency are present in women who experience more than 60 ml of blood loss and occur in 67% of women whose menstrual losses exceed 80 ml (Hallberg et al., 1966). It has also been suggested that iron deficiency itself can lead to or aggravate the menstrual blood loss; that is, the coagulative capacity of the uterus may be reduced, creating a cycle of more

blood loss, and consequently more iron loss. A double-blind placebo controlled trial found that supplementing with iron decreases excess menstrual blood loss in iron-deficient women who had no underlying cause for their condition (Taymor et al., 1964). However the validity of these results are in question, considering that the methodology used, by present day standards, was weak; that is, no objective assessment of menstrual blood loss was used and serum iron levels, which may not always accurately reflect the status of tissue iron in the body, were used to measure iron status.

Iron status has been shown to be significantly less in pre-menopausal than in post-menopausal women. Milman et al. compared iron status in relation to menstruation in pre and post-menopausal Danish women aged 35-65. Menopausal status was determined from the detailed medical history given by the participants. Pre-menopausal women (n=676) had lower serum ferritin scores, median 42 $\mu\text{g/L}$, than post-menopausal women (n=207), median 80 $\mu\text{g/L}$ ($p < 0.0001$). Of the pre-menopausal women, 12.1% had serum ferritin scores $< 20 \mu\text{g/L}$ and 47.6% had scores $< 40 \mu\text{g/L}$. The corresponding percentages in post-menopausal were 0.5% and 13.5%. Serum ferritin levels in pre-menopausal women were strongly dependent on the duration of menstrual bleeding ($p < 0.0001$), which was in turn related to the method of contraception (Milman, Rosdahl, Lyhne, Jørgensen, & Graudal, 1993). A national sample of adults aged 35-60 years living in France also showed that post-menopausal women (n=1706) had a higher mean serum ferritin level than pre-menopausal women (n=4942) (71.3 $\mu\text{g/L}$ vs. 33.6 $\mu\text{g/L}$, $p < 0.001$) and pre-menopausal women had a higher prevalence of iron deficiency (serum ferritin $< 15 \mu\text{g/L}$) than post-menopausal women and men (n=2337) (22.7 vs. 5.3% vs. 1.8%, $p < 0.001$). (Galan et al., 1998).

Blood loss and diet play a role in the etiology of iron depletion. In a cross-sectional study of a volunteer sample of 384 pre-menopausal women, women aged 18-40 years were chosen to maximise the likelihood that all reached menarche and had finished growing and to minimise the likelihood that they had reached menopause. The 87 (23%) women with iron depletion (serum ferritin $<20 \mu\text{g/L}$; hemoglobin $>120 \text{g/L}$), termed as mild iron deficiency by the authors, had significantly lower BMI (21.8; SD = 20.4, interquartile range = 24.5) than the 297 women without iron depletion (23.0; SD = 21.1, interquartile range = 25.1) ($p=0.01$) and significantly lower intake of meat/fish/poultry (86g vs. 111g) ($p<0.01$). Menstrual blood loss, as determined by a menstrual recall method, was not significantly different between the two groups (Heath, Skeaff, Williams, & Gibson, 2001).

Logistic regression analysis suggested that meat/fish/poultry intake and menstrual blood loss were important predictors of mild iron deficiency, when adjusting for other independent risk factors. Consuming medium or high intakes of meat/fish/poultry was associated with a decreased risk of iron depletion in women who experienced low menstrual losses (Odds Ratio (OR) = 0.22, 95% CI = 0.09-0.56 and OR = 0.26, 95% CI = 0.10-0.67, respectively) and experiencing low menstrual losses was also associated with a decreased risk in women consuming a medium amount of meat/fish/poultry (OR = 0.13, 95% CI = 0.03-0.59). Independent risk factors that were significantly important predictors included: duration of menstrual period <4 days (OR = 0.35, 95% CI = 0.16-0.77), <4 months since last blood donation (OR = 7.27, 95% CI = 2.79-18.92), nose bleeds (yes) (OR = 2.15, 95% CI = 1.12-4.11), BMI <20 (OR = 2.50, 95% CI = 1.14-5.50), and multivitamin-mineral supplementation in the past year (yes) (OR = 0.48, 95%

CI = 0.24-0.98). The authors demonstrated that the risk of iron depletion in premenopausal New Zealand women is associated with blood loss; namely, menstrual blood loss, recent blood donation and nosebleeds, and is also associated with intake of meat/fish/poultry. Women with low menstrual losses may be able to lower their risk for iron depletion by increasing their intake of heme-iron and those with a medium intake of meat/fish/poultry may benefit from taking steps to decrease their menstrual losses. They also suggest that women with a low BMI should strive to reach the healthy range (Heath et al., 2001).

Moderate daily doses of ferrous iron supplementation appear to influence the iron status of women with inadequate iron stores. Iron deficiency, as defined by serum ferritin $<16\mu\text{g/L}$, was observed in 9.7% of 268 menstruating, non-pregnant Danish women 18-30 years of age, whereas the prevalence of iron deficiency was 4.3% in users ($n=94$) vs. 12.6% in non-users ($n=174$) when consumption of iron supplements was considered ($p=0.05$) (Milman et al., 1998). Milman et al. did not measure menstrual blood loss, but they did show that in non-iron-supplemented women, serum ferritin levels were inversely correlated with the duration of menstrual bleeding ($r = -0.25$, $p<0.001$) and with self-assessment of intensity of menstrual bleeding ($r = -0.27$, $p<0.001$). No correlation was observed in iron supplemented women.

While it is well accepted that excessive menstrual blood loss leads to iron deficiency, the amount of menstrual losses experienced by women can also be highly variable. Consequently, variables that influence menstrual losses may influence iron status. Contraception, age and parity, and baby weight have all been suggested to be associated with menstrual losses (Cole, Bilewicz, & Thomson, 1971). Cole et al. (1971)

measured menstrual blood losses, via the most accurate and reliable alkaline hematin method, in 348 women aged 17 to 45 years in a Northumbrian mining village. Menstrual loss was found to be related to whether oral contraception (mean losses = 12.7 ml, sd = 20.4, n=23), an intrauterine device (mean losses = 56.3 ml, sd = 35.2, n=25) or no contraception (mean losses = 37.9, n=280) was used ($p<0.001$); parity independent of contraception ($r = 0.19$, $p<0.01$, $n=280$) and within parity groups, to the average birth weight of previous children ($r = 0.28$, $p<0.01$), but not age. The authors also suggested that menstrual losses, excluding the influence of parity, may be associated with height ($r_{\text{partial}} = 0.19$, $p<0.01$), although when birth weight of previous child is taken into account the correlation fails to reach significance. Endometrial area or vascularity may explain this relationship as taller and heavier women tend to have heavier babies and may have larger uteri. Weight was also correlated with menstrual losses, but not independent of parity and age as body weight tends to change with these variables (Cole et al., 1971). In another study designed to isolate epidemiological risk factors for menorrhagia, menstrual blood loss during one bleeding episode for each of 182 women was measured with the alkaline hematin method. Menstrual blood loss increased significantly with age ($p<0.03$) and the percentage of women with menorrhagia was significantly higher above 40 years of age ($p<0.05$). BMI was found not to be significantly related to menorrhagia nor was parity after adjustment for age (Janseen, Scholten, & Heintz, 1997). Similarly, Deeny et al measured menstrual blood loss in 54 women, referred for endometrial ablation on account of dysfunctional uterine bleeding. They also found that obesity did not appear to be related to menstrual blood loss. However obesity was a factor in the referral of some of these women for endometrial ablation as an alternative to hysterectomy for treatment

of excessive menstrual bleeding and consequently, the mean BMI and the mean menstrual blood loss was higher than normal in their study population (Deeny & Davis, 1994).

Nutrition also plays an important role in the development of iron deficiency. Consumption of iron-rich foods, supplements, and enhancers of iron absorption could all potentially increase iron stores in individuals with sub-optimal iron status. In a 16 week, randomized (stratified block) placebo-controlled trial, Heath et al. (2001) investigated the efficiency of a dietary regime involving increased consumption of these factors, as well as decreased consumption of inhibitors of iron absorption, for increasing iron stores in 75 adult New Zealand women aged 18 to 40 years with iron depletion or mild iron deficiency as classified by the authors (serum ferritin <20 $\mu\text{g/L}$, hemoglobin ≥ 120 g/L). Women were excluded if they were pregnant or lactating, had irregular menstruation, health problems or were consuming medication likely to effect iron status, or were vegans; and participants were asked not to take iron, vitamin C, or calcium supplements during the study or to donate blood. The participants were assigned to receive either placebo, supplement (50 mg iron/day as amino acid chelate) or a diet regime consisting of individual dietary counselling to increase the intake and bioavailability of dietary iron. A fasting, morning venipuncture blood sample was taken each month to assess iron status and dietary iron intake was assessed at weeks 4, 8 and 16 via a validated computer-administered Iron Food Frequency Questionnaire. Serum ferritin increased in women receiving supplement (n=16) and diet (n=22) by 59% (p=0.001) and 26% (p=0.068) in comparison to the placebo group (n=19), respectively. The authors suggest that the improvement achieved in response to dietary change was considerably less than that

achieved with supplementation due to the difficulty associated with embarking on and sustaining a dietary regime that primarily requires an increased consumption of the more easily absorbed, and nonheme absorption enhancing, heme iron from meat/fish/poultry (Heath, Skeaff, O'Brien, Williams, & Gibson, 2001).

We collected data on bleeding and gynecological history, the amount of menstrual losses and BMI. The association of these variables with iron status was analyzed, keeping in mind that a complete picture of what caused low iron stores could not be made largely because data on dietary intake or iron absorption were not collected in the present study.

2.3.4 Cognition and Fatigue

An abundance of research has been produced on the affects of iron deficiency anemia for some time, particular in the growing infant or child, but only recently have groups focused on the clinical impact of iron deficiency without anemia in women of reproductive age. Research on the impact of iron deficiency without anemia in this type of population, similar to our study cohort, has focused primarily on three intersecting areas: (i) cognition and memory, (ii) exercise capacity and physical performance, and (iii) general health and fatigue.

(i) Cognition and memory

Most of the work on the neurophysiological underpinnings of cognitive impairment associated with iron deficiency has been performed using animal models. However, the results of one clinical study suggested that body iron stores are relevant to specific neurophysiological processes supporting attention. An association between

ferritin concentrations and electroencephalographic (EEG) asymmetry was shown in 69 normal male (n=17) and female (n=52) university students studying introductory psychology. Consistent with these EEG asymmetries, iron status was also significantly related to performance on two cognitive tasks (Tucker, Sandstead, Penland, Dawson, & Milne, 1984).

Other studies have shown that iron deficiency can affect cognition as assessed by test performance. A recent blinded placebo trial of 113 women (30 iron-sufficient, 53 iron deficient, 30 iron-deficient anemic), showed that iron status alters cognitive functioning in women during reproductive years. Murray-Kolb et al. (2004) administered eight computerized cognitive performance tasks from the Detterman's Cognitive Abilities Test at baseline and again after 16 weeks of consuming iron supplements or placebo. While the investigators have yet to publish the study in full, in an abstract they state that women who started the study iron-deficient without anemia performed with significantly less accuracy than women who were iron sufficient. Repeated measures analysis of covariance (ANCOVA) also revealed an improvement in performance as well as time to complete attention, memory, and learning tasks in women who improved their iron status over time (Murray-Kolb, Whitfield, & Beard, 2004).

A relationship between iron status, and mean performance on a measure of sustained attention (Bakan Vigilance Task) was also observed in 14 obese (BMI >31.5) dieting women. Hemoglobin ($r = 0.72$, $p < 0.01$) and transferrin saturation ($r = 0.86$, $p < 0.01$), for those subjects whose hemoglobin declined, were both positively correlated to sustained attention task performance at 15 weeks. Based on these results the authors

suggest that an inability to sustain attention may be an early sign of developing iron deficiency in obese dieting women (Kretsch, Fong, Green, & Johnson, 1998).

The iron status of adolescents and young women also appears to impact cognitive performance. In a double-blind placebo trial, non-anemic (hemoglobin >11.5 g/dL for African Americans, hemoglobin >12.0 g/dL for white) iron deficient (serum ferritin <12.0 µg/L) adolescent girls also demonstrated improved verbal learning and memory with iron supplementation. Seventy-three girls aged 13-18 enrolled at four different high schools, randomized to receive either a ferrous sulphate preparation (n=37) or a placebo (n=36) twice daily for eight weeks, had their cognitive functioning assessed at baseline and eight weeks via three measures of attention and one multi-component test of verbal learning and memory – The Hopkins Verbal Learning Test (HVLT). Multiple regression analysis revealed that iron treatment had no significant effect after eight weeks on any of the three measures of attention. However, on the total recall score of the HVLT, iron supplemented girls showed a significant improvement compared to controls ($p < 0.02$) with a change in serum ferritin significantly correlating with change HVLT ($r = 0.21$, $p < 0.04$). The authors suggest that a ceiling effect may have limited the ability of some of the measures of attention to detect changes between groups (Bruner et al., 1996). Groner et al. (1986) also assessed the effects of oral iron therapy on psychometric test scores and the association between iron status and test scores using a double-blind randomized clinical trial. Thirty-eight inner-city pregnant females aged 14-24 were chosen as subjects because of their high risk for being iron deficient due to both their socioeconomic status and extra iron demand during pregnancy and because pregnancy required them to attend a health facility on a regular basis. Only subjects who were ≤ 16 weeks gestation (i.e., not in

a period where the demand for iron increases greatly) and who had hematocrits > 30% (i.e., not severely iron-deficient) were enrolled in the study with 25 completing the study. The experimental group (n=16) received an iron supplement, the equivalent of 60 mg of elemental iron daily, along with prenatal vitamins and the control group (n=9) received only prenatal vitamins. While the authors do not provide an explanation for the uneven distribution of dropouts (10 from control, 3 from experimental group), they point out that the principle investigator was not aware of the uneven distribution nor did any of the dropouts state that she was aware that she was taking vitamins without iron. The dropouts also did not change the composition of the two groups in terms of demographic, hematological, or psychometric variables. Hematological status (Hemoglobin, Hemocrit, MCV, Mean Corpuscular Hemoglobin (MCH), and ferritin) and performance on six psychometric tests were assessed at baseline and after one month of treatment: five focusing on short-term memory and attention span and one vocabulary test that was expected to remain relatively constant. The experimental group showed significant improvement on the most sensitive measure of short-term memory (Digit Symbol score, $p < 0.005$) and three subtests (one subsection of Consonant Trigrams, two subsections of the Rey Auditory Verbal Learning Test), while the control group showed no significant change, as revealed by one-tailed paired t-tests. When the change between baseline and post-treatment scores were compared between the groups, the experimental group showed significant or borderline greater improvement than controls on three tests (Arithmetic score, $p < 0.02$; subsection of Consonant Trigrams, $p < 0.05$; Digit Symbol score, $p < 0.08$). No correlation was found between the raw or change scores of any hematological

measure and any of the psychometric test scores (Groner, Holtzman, Charney, & Mellits, 1986).

(ii) Exercise capacity and physical performance

Women's iron status has also been associated with exercise capacity and physical performance. Factors related to reduced body iron storage, but unrelated to decreased oxygen-transport capacity of the blood, have been suggested as a putative cause. When physical activity level and fat-free mass were controlled for, iron depleted (serum ferritin $<12 \mu\text{g/L}$) non-anemic (hemoglobin $> 120 \text{ g/L}$) women ($n=15$) aged 19-36 were shown to have a significantly lower VO_2max than women with normal iron status ($n=15$), with the difference in VO_2max significantly associated with serum ferritin concentration, but not hemoglobin concentration (Zhu & Haas, 1997). Furthermore, iron supplementing non-anemic, iron-deficient women improves progressive fatigue resistance during dynamic knee extensor exercises and endurance times during treadmill running (Brutsaert, Hernandez-Cordero, Rivera, Viola, Hughes, & Haas, 2003; Rowland, Deisroth, Green, & Kelleher, 1988). In a randomized clinical trial, 20 non-anemic (as defined by a rather lax cut-off of hemoglobin $>110 \text{ g/L}$) iron-deficient (serum ferritin $<20 \mu\text{g/L}$) women aged 18-45 received either iron ($n=10$) or placebo ($n=10$) and were assessed on progressive fatigue resistance for 6 weeks, via 2-3 second maximal voluntary static contractions (MVC) with dynamic knee extensions. Iron status increased significantly, the rate of decrease of MVC was reduced in the iron group compared to placebo ($p = 0.01$), and MVC was significantly higher after treatment at some assessment times (Brutsaert et al., 2003). Similarly, 14 non-anemic (hemoglobin $>120 \text{ g/L}$) iron-deficient (serum ferritin

<20 µg/L) high-school adolescent runners received either iron (n=7) or placebo (n=7) for one month, but were assessed on treadmill endurance time. Iron status improved in patients taking iron, decreased in patients taking placebo, and treadmill endurance times improved significantly in the iron-treated runners compared with controls, with the latter showing a decline (Rowland et al., 1988).

(iii) General health and fatigue

Clinical studies of non-anemic iron deficient women have also focused on fatigue. Anecdotal clinical reports documenting the occurrence of symptoms of chlorosis without anemia (then determined via qualitatively and quantitatively normal red blood cells and later via hemoglobinometry) have been reported since the 19th century and the first half of the 20th century. Chlorosis, known as the “green sickness”, was considered to be a common malady characterized by a hypochromic anemia that responded to iron therapy. It is now accepted that the basic pathogenesis was iron deficiency. It was not until the late 1950s that a group undertook the first controlled clinical trial, comparing response to oral iron and a bismuth subcarbonate placebo in chronically fatigued, non-anemic women (Beutler, 2002; Beutler, Larsh, & Gurney, 1960). In a double-blind crossover clinical trial, 30 otherwise healthy non-anemic (hemoglobin > 120 g/L) women complaining of chronic fatigue (with or without symptoms such as headaches, dizziness, and nervousness) received iron tablets and placebo tablets each for three months with an inter-treatment interval of one month. An initial one month baseline observation period was used to exclude patients who improved spontaneously or who were non-compliant. Beutler et al. (1960) reported that women who showed no signs of iron on bone marrow

examination taken at baseline had a significantly better symptomatic response to iron than placebo, but the same did not occur in women in whom marrow iron was present (Beutler, Larsh, & Gurney, 1960).

More recently, Verdon et al. (2003) confirmed the results of Beutler et al. (1960). They showed that iron supplementation may benefit women of reproductive age with unexplained fatigue in the absence of anemia, in whom baseline serum ferritin concentrations were $\leq 50\mu\text{g/L}$. They measured the level of fatigue in 144 women aged 18 to 55 presenting with unexplained fatigue via a 10 point visual analogue scale in a double blind randomised placebo trial. Women were excluded if they had hemoglobin concentrations $< 117\text{ g/L}$, another obvious physical or psychiatric cause for fatigue, or chronic fatigue syndrome. One hundred thirty-six women completed the study with 71 receiving ferrous sulphate equivalent to 80 mg/day of elemental iron daily and 65 receiving placebo. Fifty-one percent had serum ferritin concentrations $\leq 20\mu\text{g/L}$ and 85% $\leq 50\mu\text{g/L}$. Mean age, serum ferritin concentration, hemoglobin concentration, level of fatigue, depression, and anxiety were similar in both groups. The level of fatigue after one month decreased by 29% in the group receiving oral iron, 13% in the control group and the difference in the decrease between both groups was significant as revealed by two tailed t-test ($p = 0.004$). A sub-group analysis also showed that women with ferritin concentrations $< 50\mu\text{g/L}$ improved with oral iron supplementation. A greater decrease in anxiety was also observed in the iron treated group (-1.7 , $SD = 6$) than in the placebo group (1.3 , $SD = 6$) ($p=0.003$) (Verdon et al., 2003).

In addition to experimental studies using an iron supplement intervention, a large non-experimental cross-sectional cohort study, using baseline and follow-up data from

the first two years of the Australian Longitudinal Study on Women's Health (ALSWH), found an association between self-reported iron deficiency and general health and well-being, vitality and fatigue in Australian Women. Twenty-four percent of 14,762 young (18-23 years) and 31% of 14,072 middle-aged (45-50 years) women reported yes to ever having been told by their doctor that they had low iron. Both age-groups' mean scores for the vitality subscale and for the physical and mental component summary scores of the MOS short-form survey (SF-36) were significantly lower than the mean scores of women who reported never having been told by their doctor that they had low iron ($p < 0.0001$) (Patterson, Brown, Powers, & Roberts, 2000).

In follow-up to the associations observed in the ALSWH study, the effects of iron deficiency without anemia on general health, well-being, and tiredness was systemically investigated in a randomized controlled clinical trial of Australian women aged 18 years or older. Fifty-two iron-deficient and 24 iron-replete menstruating women matched for age and parity, were recruited for the study, with the former randomly allocated within their age and parity category to receive dietary treatment or supplements. Participants allocated to the supplement group were asked to take 350 mg ferrous sulphate daily for 12 weeks and participants allocated to the diet group were asked to follow a high iron diet (providing approx. the recommended 2.25 mg per day intake of absorbed iron) for the same period. Treatment of iron deficiency (serum ferritin $< 15 \mu\text{g/L}$ or serum ferritin 15-20 $\mu\text{g/L}$ with two other hematological parameters indicative of iron deficiency) with either supplementation or a high iron diet resulted in improved mental health and decreased fatigue among menstruating women ($P < 0.01$) (Patterson, Brown, & Roberts, 2001).

We looked at the association of iron status with various quality of life parameters, as measured via the SF-36, in a population of men and women including individuals who are symptomatic and asymptomatic obligate carriers of the Val2016Ala allele mutation that underlies mild hemophilia A.

2.4 Summary

In Newfoundland & Labrador there are an unusually high proportion of mild cases of hemophilia A and although mild hemophilia has been reported in the literature since 1953, there is a paucity of studies with adequate power addressing bleeding in mild hemophiliacs. Furthermore, while the literature that has evaluated iron status in male hemophiliacs - with only a few of the hemophiliacs studied being of mild severity - suggests that iron deficiency is likely not a complication of the disease, the literature on this topic is scarce.

It is also not known whether female carriers of the gene that underlies mild hemophilia A tend to be more iron-deficient than non-carrier women. Using the PBAC to assess menstrual blood loss in women with bleeding disorders, menorrhagia (PBAC score > 100) was putatively confirmed in 57% hemophilia carriers, and in only 29% of control women. And using the accurate and reliable alkaline hematin method to measure menstrual losses, and various parameters reflecting the iron state of the body, Hallberg et al. showed that signs of iron deficiency are present in women who experience more than 60 ml of blood loss and occurs in 67% of women whose menstrual exceeds 80 ml. Therefore, it is worth investigating whether carrier women have greater menstrual losses and as a result, tend to have lower iron stores.

In general, there is also a need to document the iron status of individuals residing in Newfoundland and Labrador. Studies of iron status among adult women in Canada are greatly lacking and are essentially non-existent for women in Newfoundland and Labrador. Finally, literature also suggests that iron depletion can possibly have a significant effect on cognition and fatigue and may more generally affect quality of life.

Chapter 3
Research Question

3.0 Research Questions

Primary Question:

What is the prevalence of iron depletion (female serum ferritin < 50, 40, or 20 µg/L; male < 50, 40, or 30 µg/L) and iron deficiency (female serum ferritin < 11 µg/L; male < µg/L 24) in a population residing in Newfoundland and Labrador, sub-grouped by gender, Val2016Ala allele mutation status, and menstrual status?

Secondary Questions:

Does iron depletion and iron deficiency impact the quality of life, as measured by the SF-36 questionnaire, of a population residing in Newfoundland and Labrador?

Is iron status associated with menstrual blood loss (PBAC score), bleeding history, Factor VIII level, BMI, and age in a population residing in Newfoundland and Labrador, sub-grouped by gender, Val2016Ala allele mutation status, and menstrual status?

What is the sensitivity and specificity of MCV, RDW, and hemoglobin for diagnosing suboptimal iron status in women as defined by various serum ferritin concentrations?

3.1 Outcome Measures

3.1.1 Primary Measure

- The prevalence of suboptimal iron status (iron depletion or iron deficiency) in (i) women vs. men, (ii) menstruating women vs. non-menstruating women, (iii)

menstruating carrier women vs. menstruating control women, and (iv) affected men vs. control men.

3.1.2 Secondary Measures

- Median serum ferritin concentration
- Prevalence of menorrhagia (PBAC >100)
- Mean PBAC score
- Bleeding history
- Age
- BMI
- Factor VIII level
- Mean scores of the 8 domains and 2 summary component scores of the SF-36
- The sensitivity and specificity of MCV, RDW, and hemoglobin for diagnosing suboptimal iron status

Chapter 4
Materials & Methods

4.0 Study Design

This is a two-year cross-sectional cohort clinical study. The study has several components. The first is an objective clinical description of a cohort of patients carrying the Factor VIII mutation. This component served to determine the burden of bleeding illness, Factor VIII replacement therapy, prevalence of inhibitor formation and potential blood borne pathogen associated disease in this kindred with mild Hemophilia A. Information from this clinical assessment was also used to seek potential environmental factors associated with the severity of clinical expression. Comparison of musculoskeletal and quality of life scores with siblings matched for age was used to further objectively delineate the burden of illness and potential psychological impact of carrier status in this population.

In recognition of the need to document the prevalence of iron deficiency in rural Newfoundland and Labrador, an additional component was later created; namely, an objective description of iron status in a cohort of patients, including those carrying the Factor VIII mutation. This component is the focus of this thesis and it has served in documenting the prevalence of iron deficiency and iron depletion, its association with blood loss and other potentially influential variables, and the potential impact iron deficiency and/or iron depletion may have on various quality of life parameters.

4.1 Study Population

The study involved affected patients with mild hemophilia A, carrier females who have been shown by molecular analysis to have the same causative Factor VIII mutation (val2016ala), unaffected control males, and non-carrier control females. Due to a founder effect, the majority of patients belonged to one very large kindred comprising over 1900

individuals. Another 5 smaller families known to have this mutation were included although their precise ancestral connection to the larger kindred is unclear. Most of the participants resided in Newfoundland and many had settled in roughly the same rural area in the central region of the province.

4.1.1 Inclusion Criteria

1. All individuals ≥ 18 years of age and meeting the requirements for the following cohorts were considered eligible:

Cohort A: Affected males and homozygous females who test positive for the val2016ala mutation.

Cohort B: Unaffected males who test negative for the val2016ala mutation. Normal male siblings of the primary patient population will be used preferentially followed by age and sex-matched controls from the community.

Cohort C: Carrier females who are heterozygous for the val2016ala mutation, regardless of whether they are manifesting symptoms.

Cohort D: Non-carrier females who test negative for the val2016ala mutation. Normal female siblings of the primary population will be used preferentially followed by age and sex-matched controls from the community.

Women were also classified as menstruating or non-menstruating. Non-menstruating women were either post-menopausal or had a hysterectomy.

2. Individuals must be able to give informed consent to participate.

4.1.2 Exclusion Criteria

1. Individuals living out-of-province who are unable to comply with the assessments required of the study.
2. Pregnant individuals
3. Individual(s) judged to be non-compliant by the treating physician.

4.2 Patient Recruitment

4.2.1 Patient Identification

Patients eligible for the study were identified through the hemophilia program registry and family histories on file in the genetics department. Based upon pedigree information, eligible individuals were approached to participate by the genetic counsellor. Surviving members from the 6 known mutation positive kindreds born since 1915 were contacted by the genetic counsellor to discuss study participation. For patients or relatives unknown to either the hemophilia program or genetics, contact was only made via a known family contact. It was emphasized that participation was voluntary and in no way would interrupt the care offered through the hemophilia comprehensive care team.

4.2.2 Patient Enrollment

Patients already known to the hemophilia clinic or to the genetics department were contacted directly to discuss study participation. Patients unknown to the hemophilia clinic or to the genetics clinic were approached only via a known family contact. Health professionals in the area were asked to make families aware of the study and to provide contact numbers for the investigators. Community forums were also used to introduce the

study objectives and encourage participation. Physician or self-referral was considered acceptable.

4.2.3 Sample Size

This study was undertaken as an ad hoc component of a 2 year cross-sectional study aimed at defining the clinical impact of mild hemophilia A. The clinical impact of mild hemophilia A study attempted to recruit about 80 affected males, 100 female carriers and 200 unaffected family controls from the Twillingate area kindred. It seemed improbable to recruit any more than that number due to inherent family size and availability of data on members of the kindred. Eighty-four carrier women, 47 affected men, and 95 adult controls were recruited, and studied for the purpose of this thesis, including 46 menstruating carriers and 35 menstruating control women. Based on the results of a study by Kadir et al. (1999) that documented the prevalence of menorrhagia in control (29%) and carrier women (57%) for the gene that underlies mild hemophilia A, and given that iron deficiency is generally present in 67% of women whose menstrual losses exceed 80 ml (Hallberg et al., 1966), it is estimated that 19% (95%CI 6-32%) of menstruating control females and 38% (95%CI 24-52%) of menstruating carrier females will be iron deficient or have serum ferritin concentrations $<11 \mu\text{L}$.

4.3 Assessment

4.3.1 Laboratory evaluation of iron status

4.3.1.1 Overview

While bone marrow biopsy is widely considered to be the gold standard for clinical assessment of iron status, the semi-quantitative nature of the examination and

other limitations has caused some debate. Some of the limitations of this method include, abstraction of bone marrow is invasive, the methods subjective nature since the result depends on the skill of the investigator, sufficient amount of available marrow stroma, and a meticulous staining technique. The semi-quantitative method also produces results that may reflect a wide range of actual available iron (Barron, Hoyer, & Tefferi, 2001; Ganti, Moazzam, Laroia, Tendulkar, Potti, & Mehdi, 2003). Serum ferritin is widely accepted to be the most useful single measure of iron status due to its accuracy in reflecting body stores, its early detection of iron deficiency, and its high feasibility, but it also has limitations. In addition to the direct measurement standard of bone marrow biopsy, indirect clinical methods for measuring iron status either alone or in combination, include, serum ferritin, complete blood count (CBC), transferrin saturation, red cell protoporphyrin (RCP), serum iron, and serum transferrin receptor.

4.3.1.2 Serum Ferritin Concentration

Serum ferritin concentration is a sensitive quantitative measure reflecting the amount of stored iron in the body and is the most widely used method to discriminate between subjects who are and are not iron deficient. Addison et al. (1972) first described an immunoradiometric assay for detecting ferritin in the serum and this method was used to report the concentration of serum ferritin in normal, iron-deficient anemic patients and patients with iron overload. In their study, ferritin was shown to be present in normal serum in concentrations between 10 and 100 $\mu\text{g/L}$, for the first time establishing it as a normal constituent of serum (Addison, Beamish, Hales, Hodgkins, Jacobs, & Llewellyn, 1972). Using the same technique, Jacobs et al. (1972) showed that ferritin is normally

present in the circulation in concentrations between 10 and 200 $\mu\text{g/L}$. Less than 10 $\mu\text{g/L}$ was associated with transferrin saturations less than 16% and it was presumed that this reflected impaired iron delivery to the plasma pool. Therefore, a serum ferritin concentration of 10 $\mu\text{g/L}$ was considered a reflection of the lower limit of storage iron adequate to meet the demand of erythropoiesis (Jacobs, Miller, Worwood, Beamish, & Wardrop, 1972). The relationship between serum ferritin concentration and the amount of storage iron in normal subjects was more strongly established by Walters et al. (1973). The authors showed a strong correlation ($r = 0.83$; $p < 0.001$) between serum ferritin concentration and storage iron as measured by the accurate method of quantitative phlebotomy. Serial phlebotomies were performed at weekly intervals until hemoglobin concentration fell below 110 g/L and was maintained at this level and the transferrin saturation was consistently below 15% (Walters, Miller, & Worwood, 1973).

The diagnostic performance of serum ferritin concentration as a measure of iron stores has been consistently validated against bone marrow examinations (Ali, Luxton, & Walker, 1978; Mazza, Barr, McDonald, & Valberg, 1978; Hallberg et al., 1993). Taking a serum ferritin concentration of $\leq 12 \mu\text{g/L}$ as a diagnosis of iron deficiency as determined by no appearance of iron upon examination of bone marrow fragments, it could be determined from the data presented by Ali et al. (1978) that the test had a sensitivity of 71% and a specificity of 100% for use in a mixed hospital population, with many of the false negative cases demonstrating the presence of hepatic, malignant, or inflammatory conditions.

Serum ferritin has also been shown to be diagnostically superior to other measures of iron status. One hundred patients referred for bone marrow aspiration on account of

their condition had blood samples taken within 48 hours of aspiration so as to measure hemoglobin concentration, serum iron concentration, percent transferrin saturation, and serum ferritin concentration. These indirect methods of iron status assessment were independently validated against bone marrow examination. Using a serum ferritin cut-off of $< 18 \mu\text{g/L}$, a serum iron cut-off of $< 65 \mu\text{g/dL}$, and a transferrin saturation of $< 20\%$, the tests had a sensitivity of 79%, 84%, 84% and a specificity of 96%, 43%, and 63%, respectively. Using any of the two tests together in combination did not improve diagnostic performance (Mazza et al., 1978). The iron status of 203 women, classified according to the presence or absence of stainable bone marrow iron, were also examined via a radioactive iron absorption test, serum ferritin, hemoglobin, MCH, MCV and transferrin saturation. The diagnostic efficiency to correctly classify subjects as iron replete and iron deficient was superior for serum ferritin compared to all the other tests examined. A serum ferritin concentration $< 16 \mu\text{g/l}$ resulted in a specificity of 98% and a sensitivity of 75%. Most of the 25% of iron deficient subjects that were falsely classified as iron replete had serum ferritin concentrations in the range of 16-30 $\mu\text{g/L}$ (Hallberg et al., 1993).

The most extensive review of the diagnostic value of laboratory testing for the diagnosis of iron deficiency anemia was conducted by a research team from McMaster University. In a systematic overview of the relevant literature, Guyatt et al. (1992) identified 55 studies containing the results of laboratory tests and histological examination of bone marrow for at least 50% of an identifiable patient group. The target population was patients over 18 years old with low levels of hemoglobin ($< 130 \text{ g/L}$ for men, $< 110 \text{ g/L}$ for women) and a study was included if 10% or more of the patients met

this criteria. Studies also had to quantify MCV, transferrin saturation (TS), serum ferritin, RCP, RDW, or red cell ferritin (RCF) and at least test sensitivity had to be calculable based on bone marrow examination where at least 50% of an identifiable subgroup of patients were assessed with the gold standard. It was concluded that serum ferritin radioimmunoassay is an extremely powerful test for the diagnosis of iron-deficiency anemia. The areas under the Receiver Operating Characteristics (ROC) curves (y-axis representing sensitivity, x-axis representing 1-specificity) for all the tests were calculated with a greater area denoting a more powerful test. Serum ferritin had a mean area under ROC curve of 0.95 (95% CI: 0.94-0.96; n=2579) and was significantly different from all other tests ($p < 0.001$). The extreme likelihood ratios of the test observed at intervals of 15-25 $\mu\text{g/L}$ (LR=8.83; 95%CI: 7.22-10.44) and $\leq 15 \mu\text{g/L}$ (LR=51.85; 95%CI: 41.53-62.27) also reflect the power of the test. MCV determination proved significantly more powerful than all the other tests ($p < 0.001$) except serum ferritin, but the difference between RCP, TS, and MCV results all could have occurred by chance ($p > 0.05$). Using a cut-off of $< 70 \mu\text{m}^3$, MCV had a likelihood ratio of 12.47 (95%CI: 6.13-18.81). Based on these results, the authors conclude that serum ferritin should be the only measure used to determine iron deficiency. They also suggest that the traditional cut-off values between 12 and 20 $\mu\text{g/L}$ use by laboratories is not optimal, since the likelihood of iron deficiency in a general population does not start to drop until values are higher than approximately 40 $\mu\text{g/L}$ (Guyatt et al., 1992).

A wide range of cut-off values for serum ferritin have been set via comparing the diagnostic performance of serum ferritin measures with bone marrow examinations. Hallberg et al. (1993) summarize cut-off values used by various researchers, illustrating

the wide range (15-27 $\mu\text{g/L}$) of cut-offs used and point out that these values were determined without using the international standard proposed in 1985 (ICSH, 1985) In their study they showed that iron deficiency could be defined by serum ferritin $< 16 \mu\text{g/L}$, as signs of iron deficient erythropoiesis were already present at this level. To determine when the first signs of iron deficient erythropoiesis can be observed, a regression analysis was performed on serum ferritin and on all of the hematological parameters included in the study. As serum ferritin levels decreased, a significant reduction occurred in all of the hematological parameters studied (Hb: $r=0.86$, $p<0.01$; MCH: $r=0.93$, $p<0.01$; MCV: $r=0.92$, $p=0.04$; and TS: $r=0.84$, $p<0.05$). Iron deficient erythropoiesis was already present at serum ferritin $<16 \mu\text{g/L}$. A graphical least squares analysis describing the relationship between serum ferritin and the hematological data in all subjects, suggested that the decrease in hematological measurements starts very early during the development of iron deficiency (serum ferritin = 25-40 $\mu\text{g/L}$) and hemoglobin concentrations decrease as soon as iron stores are empty (Hallberg et al., 1993).

Physiological variables such as inflammation of chronic disease, liver disease, and malignancy must be controlled for when measuring serum ferritin concentration. An approximate three-fold rise in serum ferritin, independent of iron stores is seen in patients with infection or inflammation, and nearly five-fold in patients with liver disease (Cook et al., 1990). Serum ferritin may be elevated as a part of an acute phase reaction. The measure is not reliable for ruling out iron deficiency in women of reproductive age for more than a month after febrile illness (Eskeland, Baerheim, Ulvik, & Hunskaa, 2002). In a study of 252 hospitalized subjects with anemia or disorders in iron metabolism, Lipschitz et al. (1974) showed that inflammation, liver disease and increased red-cell

turnover elevated the serum ferritin concentration independent of iron stores. When patients with inflammation and liver disease were compared with a control group with a variety of disorders exclusive of the former, serum ferritin values progressively increased in the groups with greater amounts of marrow hemosiderin, but at each grade of marrow, iron values in patients with inflammation and liver disease were much higher ($p < 0.05$) (Lipschitz, Cook, & Finch, 1974).

In the present study serum ferritin concentration was measured using the Access analyzer. The lower limit of the normal physiological serum ferritin range (observed central 95% interval of serum ferritin), established from reference values taken from the “normal” population was 11 $\mu\text{g/L}$ for women and 24 $\mu\text{g/L}$ for men. Values below these levels defined iron deficiency. Mild iron deficiency is not well defined. Cut-off values of 20, 40, and 50 $\mu\text{g/L}$ and cut-off values of 30, 40, and 50 $\mu\text{g/L}$ were used to measure the frequency of mild iron deficiency in women and men, respectively.

4.3.1.3 Complete Blood Count: Mean Cell Volume, Red Cell Distribution Width, & Hemoglobin

The primary role of a complete blood count (CBC) with respect to iron status is to rule out the hematological effects of low iron status, rather than iron deficiency per se. Measures included in a CBC that are parameters of iron status include hemoglobin concentration and red blood cell indices; namely, MCV and red blood cell distribution width RDW.

Hemoglobin has been used longer than any other measure for iron status. Hemoglobin concentration only provides a quantitative measure of the severity of iron

deficiency upon the development of anemia; that is, a normal blood film does not rule out low iron status, only its hematological effects. Hemoglobin is a relatively insensitive index of iron deficiency, falling only in the later stages. The measure lacks sensitivity; that is, there is an overlap between values of normal and iron deficient populations (Garby, Imnell, & Werner, 1969), and has a low specificity, since other nutritional deficiencies, genetic disorders, and chronic infections can limit erythropoiesis.

Red cell indices are the basis for classifying anemias and in various combinations are used to gauge the severity of iron deficiency. MCV is derived from other direct CBC measures; that is, it is calculated by dividing the hematocrit by the red blood cell count. MCV is reduced when iron deficiency becomes severe or at the same time when anemia starts to develop. Slight microcytosis (70-80 fl) often occurs in the anemia of chronic disease, but more pronounced microcytosis greatly increases the specificity once thalassemia has been excluded (Cook & Skikne, 1989). RDW is a quantitative measurement of the variation in red blood cell volume and is equivalent to the microscopic assessment of the degree of anisocytosis. It is expressed as either the standard deviation (SD) or as the coefficient of variation (ratio of SD and MCV) of the red cell volume distribution.

In the present study, CBC was analyzed using a Coulter STKS. The MCV lower limit for both women and men was set at 80 fL and the RDW upper limit was set at 14.5 units. The hemoglobin concentration lower limit was 120 g/L for women and 140 g/L for men.

4.3.2 Factor VIII Level

Chromogenic (COAMATIC) Factor VIII coagulant (FVIII:C) assays were performed on all study participants using a Futura Beckman Coulter. According to the International Society on Thrombosis and Haemostasis (ISTH) standards, mild hemophilia was defined by factor levels between 5 and 40% (0.05 to 0.40 U/mL) (White et al., 2001).

4.3.3 Questionnaires

4.3.3.1 MOS short-form survey (SF-36)

The SF-36 was constructed to represent two major dimensions of health – physical and mental – and is composed of 36 items which are scored as eight multi-item scales, a health transition scale, and two overall summary scores: the physical component summary score (PCS) and the mental component summary score (MCS). The eight scales are scored from 0 to 100, with a higher score denoting a better health state. The summary scores are compared with norms from a reference population, such that the population average is set at 50. Thus, a PCS or MCS score below 50 indicates worse physical or mental health, while a score above 50 indicates better health than the reference population (Ware, Kosinski, & Gandek, 2000; Ware, Kosinski, & Dewey, 2000). Reported estimates of score reliability for the SF-36 scales in females exceeded accepted standards for measures used in group comparisons; for each scale, the median of the reliability coefficients across studies equals or exceeds .80, with the exception of the General Health scale (the median for this scale is .79). Validation of the SF-36 via content, construct, or criterion validation has shown that the scales generally measure their intended health concept and do not measure other concepts. Data on all eight scales,

the health transition scale and the two summary scores were collected using the self-reported SF-36 (4-week recall) questionnaire (see Appendix A).

4.3.3.2 Assessing Menstrual Blood Loss: the Pictorial Blood Loss Assessment Chart

The Pictorial Blood Loss Assessment Chart (PBAC) has been increasingly used as a method for measuring menstrual blood loss in clinical studies (Crosignani, Vercellini, Mosconi, Oldani, Cortesti, & Georgi, 1997; Kittleson & Istre, 1998, Kadir et al., 1999), however a number of other methods have been described in the literature. Recognizing the need to optimally quantify and document the prevalence of abnormal blood loss, a literature review was conducted to determine how valid, reliable, applicable, and feasible existing measurement methods are likely to be in assessing menstrual blood loss in women with hereditary bleeding disorders. Methods for measuring menstrual blood loss, as described in the literature, include (i) the Alkaline Hematin method (AHM), (ii) the PBAC, (iii) a Menstrual Pictogram, (iv) Gynaeseal, (v) a Weighed Menstrual Loss method (WML), (vi) a Menstrual Recall questionnaire and a Menstrual Record questionnaire, and (vii) menstrual / gynaecological history (Kawaja, Scully, Barrett, & Walsh, 2003).

(i) The Alkaline Hematin Method

The most widely used objective laboratory method is the alkaline hematin method, although radioisotope measurement of Fe-59 labelled hemoglobin or Cr-51 labelled red cells has also been devised (Shaw, 1977). The alkaline hematin method was first described by Hallberg and Nilsson in 1964, but has since undergone modification (Newton, Barnard, & Collins, 1977; Eijerkeren et al., 1986). Five percent sodium

hydroxide is used to extract hemoglobin from collected sanitary wear and towels, and to convert it to hematin. Originally, towels and tampons were squeezed and rubbed until all coloured spots had disappeared. The optical density of hematin was then measured with a spectrophotometer and compared to that of venous blood to determine the volume of menstrual blood loss (Hallberg et al., 1964).

The method was improved by reducing the extraction time through the use of a Stomacher Lab Blender for homogenization of the sanitary pads (Newton et al., 1977) and later modified by recognizing important changes that would improve its accuracy (Eijkeren et al., 1986); that is, blank absorption values of tampons and towels were subtracted from that of menstrual blood and the volume of added blood was factored into the determination of menstrual blood loss if towels and tampons were not allowed to dry. As part of a combined laboratory and diary method for objective assessment of menstrual blood loss, Hurskainen et al. attempted to further simplify the method by correlating hemoglobin values with the absorption of venous blood, in order to validate the replacement of the latter procedure. The diary was used to estimate the amount of blood loss during collection (extraneous blood loss) (Hurskainen et al., 1998). A spectrophotometric alternative to the alkaline hematin method involving separate extraction of blood, using a detergent solution and color development with sodium carbonate, has also been developed (Vasilenko et al., 1988). Gannon et al. later used this two-stage laboratory technique, but used a Smallboy benchtop washer, instead of 500-ml plastic jars, to permit rapid extraction of a more complete collection of sanitary material (Gannon, Day, Hammadich, & Johnson, 1996).

Due to its extremely high validity and reliability, the alkaline hematin method is presently considered to be the gold standard for measuring menstrual blood loss. However, due to the specialized nature of the test and the time required to perform it, it is not available for routine clinical use (Higham, O'Brien, & Shaw, 1990). The optical density of hematin extracted from sanitary wear, when compared to that of a known volume of venous blood, has been consistently shown to be an accurate means of determining the volume of menstrual blood loss (Hallberg et al., 1962; Newton et al., 1977; Eijkeren et al., 1986; Hurskainen et al., 1998), although as mentioned, slight modifications have improved its accuracy. The mean recovery of blood from experiments that tested known amounts of blood (10 ml) added to sanitary protection, and subsequently converted into a stable elute, was 96.3% (SE = 0.5, n = 10) (Hallberg et al., 1962) and 98% (range 91-106) (Eijkeren et al., 1986). Equal and greater volumes of added blood have similarly yielded highly accurate results (Newton et al., 1977; Eijkeren et al., 1986; Hurskainen et al., 1998). However, the most significant variable contributing to the loss of accuracy when using the Alkaline hematin method is from extraneous blood loss. For this reason, Hurskainen et al. applied the standard method in combination with a diary method to assess menstrual blood loss in 156 patients, enrolled in a randomized trial for menorrhagia, from the gynecology departments of 5 Finland universities. A clot and a spot the size of a 27 mm diameter Finish coin was estimated as 2 ml and 0.5 ml of blood, respectively. The amount of blood per one uncollected pad or tampon reported was determined from the average amount of blood collected per pad or tampon. 12 % (range 0-57%) of menstrual blood loss was lost during collection.

As illustrated via reported statistics that reflect variation, the alkaline hematin method is precise. Potential measurement errors contributing to random error include: errors resulting from hematin extraction, spectrophotometric analysis, and patients' collection of sanitary material.

The alkaline hematin method would be expected to have the same accuracy among members of a pedigree, who are currently menstruating, but not for those patients who no longer menstruate. That is, menstrual blood loss of patients who no longer menstruate cannot be retrospectively assessed with this method. The gold standard is therefore only highly applicable for menstruating women.

Although the method is highly applicable for menstruating women, it is not a feasible method for routine clinical use. The method requires trained laboratory technicians, equipment, significant time, and subjects who are motivated enough to collect a complete collection of soiled sanitary wear. The method also generates large volumes of caustic solutions and an unpleasant odour. Attempts that have been made to increase its feasibility include: the introduction of the Stomacher Lab-Blender which reduced the extraction time to a few minutes (Newton et al., 1977); the replacement of venous hematin absorbance with venous hemoglobin concentration (Hurskainen et al., 1998) which made the procedure somewhat easier and cheaper; and a modification of the procedure involving the separate extraction of blood using a detergent solution and color development of smaller aliquot samples (Vasilenko et al., 1988). Since the latter modification requires extra equipment (washer) and the safe disposal of the washing liquid, it is not a feasible alternative. Despite these attempts, the alkaline hematin method's feasibility for use in a cross-sectional study remains extremely low.

(ii) Pictorial Blood Loss Assessment Chart

The most widely used non-laboratory method is the pictorial blood loss assessment chart (PBAC). The pictorial method has been shown to be superior to other subjective methods that use weighing techniques or visual analogue scores. Devised by Higham et al. (1990), the PBAC takes into account the degree to which each item of sanitary protection was soiled with blood as well as the total number of pads or tampons used (Higham et al., 1990), it does not, however, pictorially account for extraneous blood loss (Wyatt, Dimmock, Walker, & O'Brien, 2001). The chart consists of a series of diagrams representing light, moderately and heavily soiled tampons or pads. A mark is made in the appropriate box at the time of soiling of each sanitary item and, if a sufficient number were used, counted in groups of five. In the initial study by Higham et al., clots of a diameter equal to or less than 1 cm, diameter greater than 1 cm, and episodes of flooding were similarly recorded. Patients were required to complete the chart after being given an explanation of how it was to be used and an instruction leaflet. Proportional scores are assigned for each degree of soiling (1, 5, and 10 for tampons; and 1, 5, and 20 for pads) and the passage of clots (1 for small clots and 5 for large clots) (Higham et al., 1990).

Higham et al. devised the PBAC in an attempt to create a more accurate non-laboratory method of menstrual blood loss assessment. In their study, 30 patients were provided with specific sanitary protection (Tampax and Kotex Fems super plus tampons and Kortex Simplicity size 2 towels), which were to be collected in plastic bags after their use. Twenty-eight patients submitted the chart that monitored between one and three

cycles each; resulting in a total of 55 assessed menstrual cycles. Following the patients' submission of their PBAC charts, a similar assessment, based on the collected sanitary wear, was made by a gynecologist. The gynecologist was blinded from the patients' assessments. Subsequently, independent of the PBAC results, the blood content of all collected sanitary material was quantified using the alkaline hematin method. In addition, 67 menstrual collections were assessed solely by the gynecologist using the PBAC and the alkaline hematin method (122 cycles in total), so as to strengthen the comparison of the PBAC with the gold standard (Higham et al., 1990).

Taking a PBAC of > 100 as diagnosis of menorrhagia (blood loss > 80 ml as determined by the alkaline hematin method), Higham et al. reported that the method had a sensitivity of 86% and a specificity of 89% when used by patients, and a sensitivity of 86% and a specificity of 81% when used by the gynecologist. The corresponding likelihood ratios (positive test) are 7.8 and 4.5, respectively. There was also a strong correlation between both the patient's ($r=0.847$) and the gynecologist's ($r=0.872$) assessment of blood loss and that of the alkaline hematin method. Reasonable agreement was shown between the patients' and the gynecologist's scores when scores were less than 150 (Higham et al., 1990).

Fifty-three patients referred for endometrial ablation on account of dysfunctional uterine bleeding were also assessed with the PBAC (Deeny & Davis, 1994). Specific brands of tampons and pads were not supplied to the patients; that is, women used their customary sanitary material. In addition, visual analogue scores were also used to subjectively assess blood loss. Each patient was asked to score a 10-cm line to demonstrate how much trouble her periods caused her. The subjective methods were

independently validated against the alkaline hematin method (Deeny & Davis, 1994). Deeny et al., used the same PBAC cut-off score > 100 as set by Higham et al. to predict menstrual blood loss in excess of 80 ml (menorrhagia). The PBAC had a sensitivity of 88%, a specificity of 52%, and a corresponding likelihood ratio of 1.8. Multiple regression showed that menstrual blood loss, as determined via the gold standard, was closely related to the PBAC ($p = 0.001$), but not to the visual analogue score ($p = 0.724$) (Deeny & Davis, 1994).

Janssen et al. analyzed 489 bleeding episodes from 288 women complaining of heavy menstrual blood loss or suffering from an unexplained anemia. To obtain a well-balanced distribution of menstrual blood loss, women requesting contraceptives and healthy volunteers were also asked to participate. Again, menstrual blood loss was assessed with the PBAC method and compared to the gold standard. Subjects collected their sanitary wear during one ($n=87$) or two ($n=201$) consecutive bleeding episodes. Like the Higham et al. study, sanitary wear was provided (Kortex Maxi Long pads and Tampax Super tampons). Only the first 57 women enrolled also assessed the size of blood clots, in comparison to Dutch coins. The former subgroup's collection of sanitary wear was also independently assessed by two investigators (Janssen, Scholten, & Heintz, 1995). The authors calculated the sensitivity, specificity, positive predictive value, and negative predictive value for several PBAC cut-off scores for patients' first menstrual cycles. The ideal cut-off point (that with the highest sensitivity and specificity) was determined to be 130 (sensitivity 91%, specificity 81.9%) with a corresponding likelihood ratio of 5.0. However, the cut-off point at which the predictive values (PV) were maximized was 185 (Positive PV 85.9%, Negative PV 84.8%). The correlation

between the patients' subjective assessment of their first menstrual cycle and the alkaline hematin method was moderate ($r = 0.56$). The mean difference between the investigator scores and the patients' scores was +29.0 (SD= 103.2, SE=13.8, 95%CI=1.2-56.7) in favour of the subjects. Calculation of the agreement of scores under 150 yielded a mean difference +10.7 (SD=31.0, SE=6.1, 95%CI=-1.9-23.3) in favour of the subjects (Janssen et al., 1995).

In an attempt to assess the accuracy of a PBAC as a method for estimating menstrual blood loss in women complaining of heavy periods, Reid et al. prospectively analyzed one menstrual cycle of 103 women complaining of excessive menstrual blood loss. Women were given the same tampons and sanitary towels as in the original study by Higham et al. As in the former studies, the PBAC was validated against the alkaline hematin method and an investigator objectively assessed women's menstrual blood loss independently. The correlation between the patients' subjective assessment of their menstrual blood loss and that determined by the alkaline hematin method was 0.46 (95%CI=0.3-0.6). As a diagnostic test for menorrhagia, using a cut-off score of > 100 as being positive, the sensitivity was 97% (95% CI=93-100), the specificity was 7.5% (95% CI = 0-15.5), and the corresponding likelihood ratio 1.0. The Positive Predictive Value (PPV) was 62% (95% CI = 54-74) and the Negative Predictive Value (NPV) was 60% (95% CI = 52-72) (Reid, Coker, & Coltart, 2000).

Since the PBAC method relies on both discrete (number of pads and tampons) and ordinal (degree of soiling) variables, it is more apt to require human judgement and to be susceptible to bias than the standard alkaline hematin method. The patient's accurate assessment of the degree of soiling of sanitary wear could potentially be affected by

subject bias. In all the studies mentioned, blood loss assessment by patients using the PBAC, investigators using the PBAC and investigators using the standard method, were done independently, thereby potentially eliminating any observer bias.

Although a score of 100, as initially reported by Higham et al., has not been consistently determined to be the optimal cut-off point for diagnosing menorrhagia and the correlation between patient PBAC scores and those of the alkaline hematin method vary ($r = 0.466$, $n = 103$ (Reid et al., 2000); $r = 0.847$, $n = 55$ (Higham et al., 1990)), the method demonstrates moderate validity. Two studies made an attempt to make comparisons among repeated PBAC measures and to analyze the results. Independent scores of the patients and the investigators showed reasonable agreement, particularly under scores of 150 (Higham et al., 1990; Janssen et al., 1995) and demonstrated low inter-observer variation. The reliability of the measurement method is moderate.

As with the alkaline hematin method, the PBAC method is highly applicable for menstruating women, but is not applicable for women who no longer menstruate. The most significant advantage of using the PBAC method for assessing menstrual blood loss is that it is highly feasible for clinical use. The method does not require the collection of soiled sanitary wear, but rather only the simple completion of the chart by the subjects. Only two women out of all the subjects who participated in the studies discussed found the chart too difficult to fill in. The providing of pads and tampons may also be required to control for the varying absorption capacity of the wide variety of available sanitary protection (Higham et al., 1990; Deeny et al., 1994).

(iii) Menstrual Pictogram

The Menstrual Pictogram has been developed as an accurate alternative to the PBAC and was validated against the alkaline hematin method (Wyatt et al., 2001). The method has an increased range of diagrams from slight to severely stained sanitary pads and tampons and makes the distinction between the differing levels of absorbency of blood into different pads (daytime or nighttime) and tampons (regular, super, or super plus). In addition, extraneous blood loss is estimated using three pictogram representations when changing feminine hygiene products. Patients are instructed to mark down the loss each time they changed their pad or tampon. A scoring system for the feminine hygiene products was devised such that each grade of staining of each brand was given a score in ml, although it is not clear from the methods section what was the rationale for each particular score assignment (Wyatt et al., 2001). Similarly, conservative scores of 1, 3, and 5 ml were devised for each clot size.

The study by Wyatt et al., (2001) was the only study found in the literature that validated the method, although it was later incorporated into a computer-aided method used to quantify menstrual cycle disorders (Wyatt, Dimmock, Hayes-Gill, Crowe, & O'Brien, 2002). Sixty-two women complaining of menorrhagia and 59 women who considered their blood loss to be normal were recruited for the study (Wyatt et al., 2001). Patients were provided with different types of Kotex Maxi napkins, Tampax tampons and airtight containers for their collection. Sixteen of the women did not complete the study because they either used other brands of sanitary wear than those provided (n =1) or they failed to collect all of the soiled products (n =12). Although comparisons were made against the standard method, it is not clear whether blood loss assessment using the standard method was assessed independently of menstrual pictogram scores. The

menstrual pictogram (excluding the scores for extraneous blood loss) had a sensitivity of 86%, a specificity of 88% and a corresponding likelihood ratio of 7.2, in diagnosing menorrhagia. In addition, very good agreement between the menstrual pictogram method and the alkaline hematin method was demonstrated using the technique of measure of agreement described by Bland and Altman ($k = 0.8$). There was no correlation between the amount of blood collected on the sanitary products (as assessed by either method) and extraneous blood loss ($r = 0.58$).

Although the menstrual pictogram method appears to be more accurate than the original PBAC, more studies are required to verify its validity. As mentioned, the rationale for the score assignments, and more generally, a verification of independent assessment is required to rule out potential observer bias. The authors suggested that the increased range of icons as well as making the distinction between the different levels of absorption between pads and tampons, may have explained the increased accuracy.

The reliability of the method appears comparable to the PBAC, as demonstrated via a plot of the difference between menstrual pictogram and alkaline hematin blood loss measurement against their mean score, although inter-observer or intra-observer variation was not directly assessed in the study. The methods reliability is moderate.

As with the PBAC, the menstrual pictogram method is highly applicable for menstruating women, but is not applicable for women who no longer menstruate. The method is also highly feasible for clinical use. The method does not require the collection of soiled sanitary wear, but rather only the simple completion of the chart by subjects. The provision of the specific pads and tampons scored in the study would also be

required to control for the varying absorption capacity of the wide variety of available sanitary protection (Wyatt et al., 2001).

(iv) Gynaeseal

Gleeson et al. investigated the use of a vaginally placed latex menstrual seal with regard to its suitability for the measurement of menstrual blood loss and efficacy as alternative sanitary protection. The Gynaeseal collection device consists of an inner cup and an outer collection pouch. Menstrual loss of two cycles was measured with the gold standard, prior to the use of Gynaeseal. Twelve women with menorrhagia and 10 with normal blood loss used Gynaeseal during 1 menstrual cycle. Of the 12 menorrhagia women asked to measure their losses at home by aspirating the fluid from the collection chamber, only five patients submitted a record of menstrual volume. Only 16-47% of the losses measured by the alkaline hematin method were collected. All of the women who attempted to record the blood volume reported spillage (Gleeson, Devitt, Buggy, & Bonnar, 1993).

The method clearly has extremely low validity and reliability. Although the method is potentially applicable for menstruating women, it seems that only sufficiently motivated patients would be willing to use the cup. Gynaeseal also has an extremely low feasibility. Due to the unpleasant spillage, all the women with menorrhagia said they would prefer the standard method of menstrual blood collection. The cup was also messy to remove and the odour of the latex was offensive (Gleeson et al., 1993).

(v) Weighed Menstrual Loss

The weighing of collected sanitary wear has been reported under the assumption that the entire menstrual loss could be accounted for by whole blood (Pendergrass, Scott, & Ream, 1984). Fraser et al. weighed the collected sanitary wear of 53 women (14 currently complaining of menorrhagia, 34 without complaint of menorrhagia, and five who formally complained of menorrhagia) and compared the fluid volume to the blood volume determined by the alkaline hematin method. It was confirmed that a high proportion of fluid in menstrual discharge cannot be accounted for by blood content. Patients were supplied with “regular” or “super” sanitary pads (without “wings”), tampons, and labelled self-sealing polythene bags. After weighing the collected sanitary wear (soiled weight – original weight) hemoglobin content of each sanitary item was measured and the blood volume determined. For the whole study group, there was a strong linear correlation between total fluid volume and blood loss ($r = 0.93$; $df = 104$; $p < .001$). For using total fluid volume to diagnose menorrhagia, the sensitivity was 89%, the specificity 98%, and the corresponding likelihood ratio was 44.5 (Fraser, Warner, & Marantos, 2001).

The authors contend that the simple method of estimation using weighed total fluid volume appears to be more accurate than the PBAC. They suggest the superiority is evident in the estimate it provides of actual blood loss and also in its diagnostic value. The present estimator, like the alkaline hematin method, is not subject to perception bias. The proposed weighing technique has a high validity and reliability.

The weighing technique is applicable for members of a pedigree who are menstruating. Total fluid volume is easier to calculate than blood loss, since it only requires weighing sanitary products prior to, and following, their use (Fraser et al., 2001). The method also has an advantage in that a variety of sanitary products could be used since the subject's perception of the degree of soiling is not required. The method may therefore be potentially much cheaper. However, women must still be able to completely collect their menstrual flow. Therefore, the method is more feasible than the gold standard, but not more feasible than the PBAC or the menstrual pictogram.

(vi) Menstrual Record and Menstrual Recall Questionnaires

Heath et al. attempted to validate two indirect methods (a menstrual recall and a menstrual record questionnaire) for estimating the extent of menstrual blood loss. The methods were not validated against the alkaline hematin method, but against weighed menstrual loss. Young adult women (18-30 years old) were recruited by poster to take part in a study designed to find out about women's iron losses (Heath, Skeaff, & Gibson, 1999). Subjects were given the Menstrual Recall questionnaire and then subsequently required to attend an interview where they were given instructions on how to complete the Menstrual Record questionnaire and how to collect their sanitary wear. The Menstrual Recall questionnaire required subjects to state how many "heavy" and "light" days they usually have during a period, how many pads and/or tampons they usually used on each type of day, the usual brand they used, and what was the absorbency stated on the product's packet (i.e. "super", "regular", etc.). Largely according to manufacturers reported absorbency levels (g of fluid per product), products were assigned a relative

absorbency score (1, 2, or 3). The former variables, factor into the calculation of the Menstrual Recall score (g) (Heath et al., 1999). Like the PBAC and the Menstrual Pictogram, the Menstrual Record requires each woman to record the proportion of which each sanitary item is soiled (in eighths for tampons and in ninths for pads). The estimated blood loss (g) was calculated by multiplying the reported proportion of soiling by the absorbency of the product (Heath et al., 1999).

Spearman rank correlation coefficients between blood loss assessed by weighed menstrual blood loss and Menstrual Record and between Weighed Menstrual Loss and Menstrual Recall were $r = 0.47$ ($p = 0.012$) and $r = 0.61$ ($p = 0.001$), respectively. Individual subject results were classified into tertiles for each of the three measurement methods. The Record method correctly classified 66% of participants into the same tertile, grossly (by two tertiles) misclassified 14%, and the Recall method correctly classified 59% of participants into the same tertile, grossly misclassified 7% (Heath et al., 1999).

The Validity of both the Menstrual Recall and the Menstrual Record questionnaires are compromised by the fact that they were not compared against the standard alkaline hematin method. Both questionnaires discriminated between low and high levels of menstrual blood loss fairly well. Although the latter questionnaire is seemingly slightly more accurate and reliable than the former, both generally have a low validity and reliability. While the Menstrual Record questionnaire is only applicable for women who can menstruate, the Menstrual Recall questionnaire may be potentially used for assessing women who no longer menstruate. A more remote recall of menstruating will likely reduce its accuracy and precision, but a modification of the method may be

useful. The Menstrual Recall questionnaire has therefore an extremely high applicability, while the Menstrual Records applicability is high.

The Record questionnaire, like the pictorial methods, is highly feasible, since it requires approximately the same level of participation on the subject's, and level of analysis on the investigator's behalf. The Recall questionnaire is quick to complete and analyze, and has a lower participation burden (Health et al., 1999). Each questionnaire has a high and extremely high feasibility, respectively.

(vii) Summary

Of all the methods discussed, the pictorial methods appeared to be the optimal measurement method for use in a pedigree. All the measurement methods are approximately equally applicable for menstruating women. The Menstrual Recall questionnaire was rated slightly higher because it has potential for use in assessing a more remote recall of losses; that is, it may also be applied to women who no longer menstruate. It is more likely, however, that for a more remote recall of losses, subjective methods such as menstrual/gynecological histories will be appropriate. There is no standard method for assessing menstrual blood loss in women in a clinical setting and all available tools have certain limitations. Comparing the alkaline hematin method and several diagnostic tests in a single study of individuals, more similar to the patients enrolled in our study, would more clearly determine which questionnaire is optimal. For example, many of the studies described evaluated the assessment tools in patients referred for menorrhagia or with known anemia, making the results less applicable to the group of

patients in this study. However, based on the available literature, the PBAC was used to assess menstrual blood loss in menstruating women.

Pre-menopausal women enrolled in the study who were menstruating were given a PBAC and were asked to document their menstrual flow and return the questionnaire (see Appendix B). In the absence of a consistent PBAC cut-off for defining menorrhagia, a PBAC score ≥ 100 was used. This cut-off was used by Kadir et al. (1999) in their assessment of menstrual blood loss and gynecological problems in patients with inherited bleeding disorders and was the cut-off originally suggested by Higham et al. (1990).

4.3.3.3 Bleeding History

Bleeding histories for both women and men were also taken as part of the Personal Interview Questionnaire. If participants answered “yes”, “a lot”, or “very much” to three of the first five questions under the Bleeding History section of the Personal Interview Questionnaire, they were considered to have a history of severe bleeding. This questionnaire was not pre-tested or validated (see Appendix C).

4.3.3.4 Body Mass Index

BMI was measured as part of the Health Status section of the self-administered Health Assessment Questionnaire. Participants were asked to record their height and weight.

4.4 Statistical Analysis

SPSS for Windows Version 11.0 was used to carry out all statistical analyses. Evidence for a difference between select groups was tested using independent Student t-

tests for continuous variables that were normally distributed, the Mann-Whitney U test for those that were not normally distributed. These variables were: serum ferritin concentrations, hemoglobin concentration, PBAC scores, Factor VIII levels, and domain and component summary scores of the SF-36. Chi-square tests were used for categorical variables. These variables were: the prevalence of sub-optimal iron status and the prevalence of menorrhagia (PBAC>100). Regression analysis was used to examine the relationship between PBAC scores, factor VIII level, age, BMI, and serum ferritin concentration. Lastly, the sensitivity and specificity of MCV, RDW, and hemoglobin for diagnosing suboptimal iron status as defined by various serum ferritin cut-offs was determined.

4.5 Data Management

A Microsoft Access database was used for entering the data and checked for accuracy. When all the data was entered and reviewed, it was stored under lock and key.

4.6 Ethical Considerations

The study design and implementation met all the guidelines of the Tri-Council's policy statement and was approved by the Human Investigations Committee of Memorial University of Newfoundland. Participant data were stored in a separate computer database from individual identifiers and were linked only by patient identification numbers. All participants gave informed consent and were free to discontinue participation in the study at any time.

Chapter 5

Results

5.0 Characteristics of Women and Men

The prevalence of co-morbid disease, iron status, and SF-36 quality of life scores for females and males are shown in Table 5.0. Women in general self-reported a clinically, lower mean (95% Confidence Interval) General Health Scale score (63.9, 59.9-67.9 vs. 70.6, 69.5-71.7) and a higher mean Role Emotional Scale score (89.3, 85.8-92.8 vs. 79.5, 77.7-81.3) than norms for the general U.S. female population.

5.1 Prevalence of Iron Deficiency

Iron deficiency was prevalent in women residing in rural Newfoundland, particularly in menstruating women. The prevalence of iron depletion for women and men as defined by serum ferritin concentrations of less than 50, 40, and 20 $\mu\text{g/L}$ and the prevalence of iron deficiency as defined by serum ferritin concentrations less than 11 $\mu\text{g/L}$ are shown in Table 5.1. Women are sub-grouped by mutation status and menstrual status and men are just sub-grouped by mutation status. The prevalence of both iron depletion and iron deficiency is significantly higher in women than in men and the prevalence of mild iron deficiency is significantly higher in menstruating women than in non-menstruating (post-menopausal or who had undergone hysterectomy) women. Mutation status did not influence iron status in either women or men (Table 5.1). Six women had hemoglobin concentrations less than 120 g/L and 11 men had concentrations less than 140 g/L , with anemia comparably observed in patients with or without the mutation (data not shown).

5.2 Serum Ferritin Concentration and Associations

As expected, serum ferritin concentrations for females were significantly lower than males. Men with a history of severe bleeding had significantly lower ferritin levels than men without a history of severe bleeding (123.4 $\mu\text{g/L}$, 63.6-186.3 vs. 189.8 $\mu\text{g/L}$, 105.9-301.5; $p < 0.05$). The serum ferritin concentrations of affected males and control males were not significantly different (Table 5.2).

Women's age appeared to be associated with ferritin ($r = 0.325$, $p < 0.0001$) and the median (range) serum ferritin concentration for women less than 40 years of age (26.0 $\mu\text{g/L}$, 14.4-44.7) was significantly lower than for women 40 years or older (38.8 $\mu\text{g/L}$, 15.6-74.4), two-tailed $p < 0.05$. However, age and menstrual status were understandably highly correlated ($r = 0.711$) and univariate analyses suggested that menstrual status ($R^2 = 0.208$, $p < 0.0001$) explained more of the variation in ferritin than age ($R^2 = 0.105$, $p < 0.0001$). Multiple regression also revealed that with menstrual status considered *a priori*, the proportion of the variance in serum ferritin explained by regression remained the same after adding age to the analysis ($R^2 = 0.208$, $p < 0.0001$). BMI was associated with ferritin ($r = 0.235$), $p < 0.01$ (Figure 5.0), but history of severe bleeding did not appear to influence women's serum ferritin concentrations (data not shown).

Menstruating women showed significantly lower serum ferritin levels than postmenopausal women or women who had undergone hysterectomy ($p < 0.0001$) (Table. 5.2). However, there was no association between the amount of menstrual losses, as measured by the PBAC, and serum ferritin concentration.

Mutation status did not influence iron status or menstrual blood loss as measured by the PBAC. Ferritin levels in menstruating control women were not significantly

different from levels observed in menstruating women who were carriers of the gene that underlies mild hemophilia (Table 5.2). The PBAC scores of both groups also did not differ and there appeared to be no association between women's factor VIII level and PBAC score or iron status (data known shown). Multiple regression analysis utilizing PBAC score ($\beta = -0.046$), age ($\beta = 0.063$), BMI ($\beta = 0.267$), and Factor VIII ($\beta = -0.193$) did not appear to have any value with respect to explaining the variation in menstruating women's serum ferritin concentrations given the non-significant statistical testing ($R^2 = 0.107$, $p > 0.05$), although it is likely that other variables that were not considered, such as diet and supplement use, could be important in explaining the variation.

5.3 SF-36 Scores

Neither the SF-36 domains', health transition scale, nor the two component summary measures' mean scores were significantly lower for mildly iron-deficient or iron-deficient women. The mean scores for some domains and the physical component summary score at select cut-offs were significantly higher for iron-deficient women (Table 5.3), however when BMI or age are treated as covariates these differences did not reach statistical significance (data not shown).

5.4 Diagnostic Performance

The greater the severity of iron deficiency as defined by serum ferritin concentration, the higher the probability that the iron deficiency may significantly change other laboratory measures such as MCV, RDW and hemoglobin. Table 5.4 shows improvement in the sensitivities and the corresponding likelihood ratios of a positive test

result at lower serum ferritin cut-offs. As expected, when iron stores are considered fully depleted (serum ferritin $<11 \mu\text{g/L}$) the percentage of women who test positive with MCV, RDW and hemoglobin is substantially greater than at higher serum ferritin cut-offs (Table 5.4).

Table 5.0 Characteristics of female and male study participants.

Characteristic	Females (n = 146)	Males (n = 80)
Cohort		
Val2016Ala Mutation	84 (57.5%)	47 (58.8%)
Control	62 (42.5%)	33 (41.3%)
Menstruating	84 (57.5%)	-
Post-menopausal or hysterectomy	59 (40.4%)	-
Age (years) / Range (years)	45.7 (43.5-48.0)* / 70	46.1 (43.0-49.2)* / 61
BMI	28.0 (25.0-32.0) [†]	29.0 (27.0-32.0) [†]
BMI ≥30	54.8% (n=68)	45.1% (n=32)
Hepatitis C	0% (n=0)	17.5% (n=14)
Diabetes	10.4% (n=15)	16.7% (n=13)
Hypertension	27.1% (n=39)	24.4% (n=19)
Heart Disease	6.3% (n=9)	14.1% (n=11)
Arthritis	19.4% (n=28)	19.2% (n=15)
Hysterectomy	23.8% (n=34)	-
Hemoglobin (g/L)	135.5 (131.0-143.0) [†]	153.0 (147.0-162.0) [†]
Serum Ferritin (µg/L)	34.8 (15.6-64.3) [†]	156.9 (97.8-262.2) [†]
SF-36 Domains / Components		
General Health Scale	63.9 (59.9-67.9)*	63.7 (57.6-69.8)*
Reported Health Transition Scale	2.96 (2.85-3.08)*	3.02 (2.86-3.20)*
Physical Functioning Scale	80.1 (75.7-84.6)*	81.9 (76.3-87.6)*
Role Physical Scale	80.4 (75.6-85.2)*	78.0 (71.2-84.8)*
Role Emotional Scale	89.3 (85.8-92.8)*	93.0 (89.3-96.6)*
Social Functioning Scale	85.7 (82.0-89.5)*	86.9 (82.3-91.5)*
Bodily Pain Scale	69.2 (64.7-73.6)*	66.5 (60.5-72.5)*
Vitality Scale	59.1 (55.8-62.3)*	65.0 (59.3-70.7)*
Mental Health Scale	75.6 (72.8-78.3)*	79.3 (75.3-83.3)*
Physical Component Summary Scale	47.7 (45.9-49.4)*	47.0 (44.3-49.7)*
Mental Component Summary Scale	51.1 (49.7-52.5)*	53.5 (51.5-55.5)*

Values are expressed as number, mean (95% confidence interval) *, median (interquartile range)[†], or percentage.

Table 5.1 Prevalence (%) of suboptimal iron status for women and men sub-grouped by menstrual status and mutation status.

Cohort	Serum Ferritin Cut-off ($\mu\text{g/L}$)			
	<50	<40	<20	<11
Females (n=141)	62.4 **	55.3 **	32.6 **	12.1 *
Males (n=77)	6.5	6.5	2.6	1.3
Menstruating Women (n=81)	80.2 **	71.6 **	46.9 **	15.0
Non-menstruating Women (n=58)	36.2	31.0	13.8	8.6
Menstruating Carriers (n=46)	73.9	65.2	57.1	8.7
Menstruating Controls (n=35)	88.6	80.0	39.1	23.5
Affected Males (n=46)	6.5	6.5	2.2	0.0
Control Males (n=31)	6.5	6.5	3.2	3.2

No ferritin data for 5 females and 3 males; menstrual status unknown for 2 women.

** $P_{\text{Chi-Square}} < 0.0001$

* $P_{\text{Chi-Square}} < 0.01$

Table 5.2 Median serum ferritin concentrations for females and males grouped by menstrual status and cohort

Cohort	Median (Interquartile Range) Serum Ferritin Concentration ($\mu\text{g/L}$)	Two-tailed p-value
Females (n=141)	34.8 (15.6-64.3)	P<0.001
Males (n=77)	156.9 (97.8-262.2)	
Menstruating Women (n=81)	22.1 (13.8-44.1)	p <0.001
Non-menstruating Women (n=58)	62.6 (36.8-112.6)	
Menstruating Carriers (n=46)	24.2 (14.7-54.4)	p >0.05
Menstruating Controls (n=35)	18.7 (11.3-32.5)	
Affected Males (n=46)	135.3 (94.5-237.1)	p >0.05
Control Males (n=31)	238.2 (104.3-301.5)	

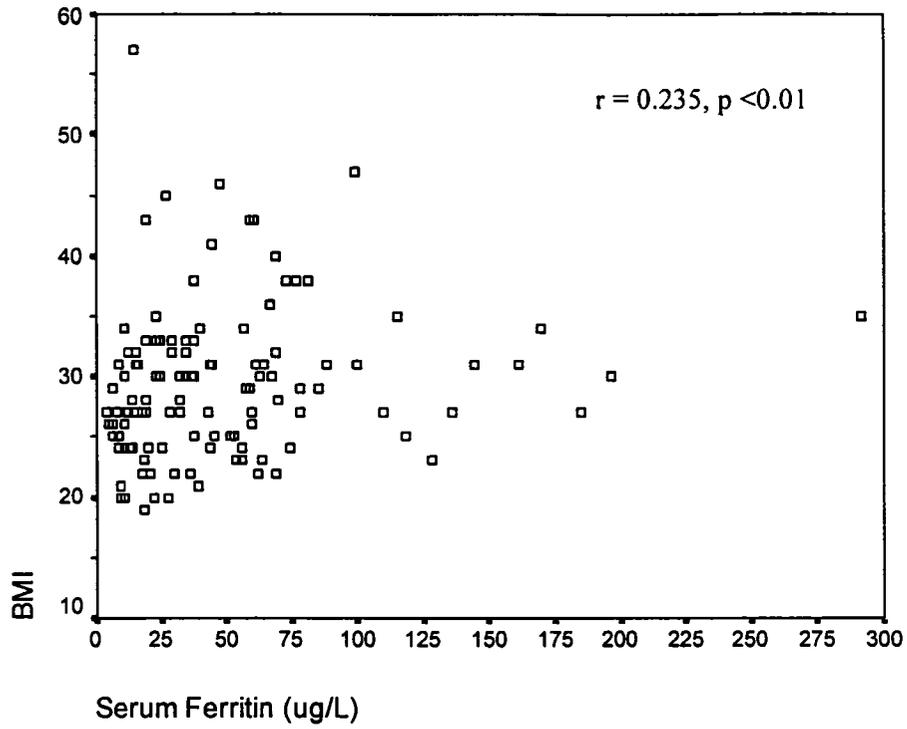


Figure 5.0 Scatter plot of serum ferritin concentration ($\mu\text{g/L}$) vs. BMI.

Table 5.3 Mean (95% confidence interval) scores on the eight domains, health transition scale and two component summary scores of the SF-36 for women of different iron status as defined by various serum ferritin cut-offs.

Iron status: serum ferritin conc. ($\mu\text{g/L}$)	Women's mean (95% confidence interval) scores on the eight domains and two component summary scores of the SF-36										
	GHS	HTS	PFS	RPS	RES	SFS	BPS	VTS	MHS	PCS	MCS
<50	68.0 (63.7-72.4) *	2.91 (2.78-3.05)	83.9 (78.4-89.3)	79.9 (73.4-86.5)	91.6 (87.3-95.8)	85.6 (80.8-90.5)	72.4 (66.8-78.1)	61.3 (57.8-64.9)	76.5 (73.1-79.9)	48.9 (46.7-51.2)	51.5 (49.8-53.3)
\geq 50	57.8 (50.1-65.5)	3.00 (2.79-3.21)	75.7 (67.9-83.5)	82.0 (74.4-89.6)	87.2 (81.0-93.4)	87.3 (81.2-93.3)	64.2 (56.7-64.8)	56.3 (49.9-62.6)	75.3 (70.7-79.9)	45.8 (42.7-48.8)	51.1 (48.5-53.7)
<40	68.5 (63.9-73.0) *	2.92 (2.77-3.07)	84.9 (79.3-90.6) *	79.7 (72.5-87.0)	91.4 (86.7-96.2)	85.7 (80.3-91.1)	73.9 (68.3-79.6) *	60.6 (56.7-64.4)	76.3 (72.7-79.9)	49.5 (47.2-51.8) *	51.3 (49.4-53.2)
\geq 40	59.1 (52.4-65.9)	2.98 (2.80-3.17)	75.8 (68.7-83.0)	81.9 (75.1-88.6)	88.1 (82.8-93.4)	86.9 (81.6-92.2)	63.8 (56.7-70.9)	58.0 (52.4-63.6)	75.8 (71.5-80.0)	45.7 (42.9-48.6)	51.5 (49.3-53.7)
<20	68.4 (62.8-74.0)	2.96 (2.80-3.11)	85.8 (78.6-93.0)	85.1 (77.6-92.5)	91.3 (85.7-96.9)	85.5 (78.4-92.6)	75.6 (68.9-82.2) *	61.8 (56.9-66.7)	74.1 (69.3-78.9)	50.7 (48.2-53.2) *	50.4 (47.7-53.0)
\geq 20	61.9 (56.5-67.3)	2.94 (2.78-3.10)	78.1 (72.4-83.8)	78.5 (72.1-85.0)	89.1 (84.6-93.7)	86.6 (82.2-91.1)	66.0 (60.2-71.9)	58.1 (53.9-62.4)	77.0 (73.7-80.4)	46.2 (43.8-48.6)	51.9 (50.2-53.6)
<11	68.9 (58.8-78.9)	2.94 (2.60-3.28)	77.5 (61.2-93.8)	79.7 (63.5-95.9)	89.6 (79.1-100.0)	80.5 (65.3-95.7)	68.4 (55.8-81.0)	57.4 (50.2-64.5)	70.9 (62.6-79.2)	48.1 (43.0-53.1)	49.6 (44.2-54.9)
\geq 11	63.3 (58.9-67.7)	2.95 (2.82-3.07)	81.0 (76.3-85.7)	80.7 (75.4-86.0)	89.8 (86.0-93.6)	86.9 (83.1-90.8)	69.2 (64.3-74.1)	59.5 (55.8-63.1)	76.7 (73.8-79.6)	47.6 (45.7-49.6)	51.4 (50.1-53.0)

* $p < 0.05$, two-tailed

Table 5.4 Sensitivities, specificities, and likelihood ratios for mean cell volume (MCV) <80 fL, red cell distribution width (RDW) >14.5, and hemoglobin (Hb) <120 g/L as diagnostic tests of sub-optimal iron status defined by women's serum ferritin concentrations.

Serum Ferritin Cut-off	Test	Sensitivity (%)	Specificity (%)	Likelihood Ratio	
				Positive Test Result	Negative Test Result
<50 µg/L	MCV	4.5	98.1	2.37	0.97
	RDW	12.5	86.8	0.95	1.01
	Hb	5.7	98.1	3.00	0.96
<40 µg/L	MCV	5.1	98.4	3.19	0.96
	RDW	12.8	87.3	1.01	1.00
	Hb	6.4	98.4	4.00	0.95
<20 µg/L	MCV	8.7	98.9	7.91	0.92
	RDW	17.4	89.5	1.66	0.92
	Hb	10.9	98.9	9.91	0.90
<11 µg/L	MCV	23.5	99.2	29.4	0.77
	RDW	23.5	88.6	2.06	0.86
	Hb	29.4	99.2	36.8	0.71

Chapter 6

Discussion

6.0 Iron Deficiency in Women

Iron deficiency is prevalent in women residing in rural Newfoundland, particularly in menstruating women. While the population studied is not representative of the general population because of the high prevalence of carrier status for mild hemophilia A, the prevalence of iron deficiency in the women studied is similar to that of two other Canadian studies. Valberg et al. found 27% of non-pregnant women aged 20-39 (n=100) and 24% of non-pregnant women aged 40-64 (n=105) had serum ferritin results $<15\mu\text{g/L}$ (Valberg, Sorbie, Ludwig, & Pelletier, 1976). Using a cut-off of $<15\mu\text{g/L}$, we observed a prevalence of 26% in women less than 40 years of age and a prevalence of 21% in women 40 years and older. Newhouse et al. observed iron deficiency, measured by serum ferritin levels $<20\mu\text{g/L}$, in 39% of 111 women aged 18-40 residing in Thunder Bay Ontario (Newhouse, Clement, & Lai, 1993). Using a cut-off of $<20\mu\text{g/L}$, we observed a prevalence of 41% in women less than 40 years of age. We used a range of cut-off values for diagnosing sub-optimal iron status in both women and men. The validity of using a lower, gender-specific serum ferritin value for women has been questioned (Rushton, Dover, Sainsbury, Norris, Gilkes, & Ramsay, 2001). Laboratories, with different methods of measuring serum ferritin, establish their own reference range based on reference values from a healthy sample of the population. These reference values may in fact be taken from women who have a suboptimal level of storage iron; that is, their iron stores are not necessarily empty, but are mildly depleted.

Evidence suggests that the effects of mild iron deficiency on cognition and fatigue may be evident before anemia or even before iron store depletion. Indeed, Verdon et al. showed a significant improvement in fatigue after iron supplementation in women with a

baseline serum ferritin concentration $< 50 \mu\text{g/L}$ (Verdon et al., 2003). Several well-designed studies, of women and adolescent girls, have shown that there iron deficiency without anemia is related to impaired cognition, fatigue and physical activity (Bruner et al., 1996; Rowland et al., 1988; Verdon et al., 2003). Possible casual mechanisms by which iron may effect behavioural and cognitive changes that have been observed in iron deficiency are believed to be associated with a decreased activity of iron-containing enzymes in the brain and possibly independent of hemoglobin synthesis. Animal models have suggested that the neurophysiological underpinnings of the behavioural and cognitive changes observed during iron deficiency may be the impairment of iron-dependent enzymes activity necessary for the synthesis, function, and degradation of neurotransmitters, such as dopamine, serotonin and, norepinephrine (Dallman, 1986). Similarly, one clinical study suggested that body iron stores are relevant to specific neurophysiological processes supporting attention (Tucker et al., 1984).

Deficits observed in mildly iron-deficient individuals and the debate surrounding what constitutes a “normal” physiological reference range in women suggest that lower values used by laboratories to establish reference ranges for women may actually be abnormally low. Using a more moderate cut-off of serum ferritin $< 40 \mu\text{g/L}$, the prevalence of iron deficiency in our population was 71.6% for menstruating women and 55.3% in all women. Further studies on the clinical impact of iron depletion are required to determine whether such high percentages call for an investigation into identifying areas of intervention, necessary for the development and implementation of strategies aimed at repleting this cohort. There is growing evidence that in some settings lower iron stores may have physiological advantages – namely, decreased risk of atherosclerosis,

myocardial infarction, and ischemic stroke (Tumainen, Salonen, Nyysönen, & Salonen, 2003; Kiechi, Willeit, Egger, Poewe, & Oberhollenzer, 1997; Sullivan, 1989). A potential for iron overload in some people also exists if iron supplementation is broadly applied, as the population will include patients carrying one or more copies of mutated genes responsible for hemochromatosis, a disorder of iron overload due to over-absorption of iron from the diet. The gene mutations responsible for hereditary hemochromatosis are most prevalent among persons of European origin and descent, with a reported C282Y heterozygosity of 9.2% and a H63D carrier frequency of 22%. In populations with European origin or descent, the prevalence estimate for C282Y homozygosity is 0.4% and it is estimated that 40-70% of persons with this homozygous genotype will develop clinical evidence of iron overload (Hanson, Imperatore, and Burke, 2001).

Likewise, a comprehensive literature search on the prevalence of celiac disease in western European populations listed the prevalence of celiac disease in pre-menopausal women with iron deficiency anemia to be 8.5% after biopsy examination confirmation and the prevalence in general Western populations as being close to 1% (Dube et al., 2005). Celiac disease is a chronic nutritional disturbance caused by the inability to metabolize gluten, which may result in inadequate absorption and malnutrition. These considerations create uncertainty as to whether an intervention to iron replete a group such as this cohort will do more good than harm. Large simple clinical trials or population-based cohort studies would be warranted before a policy of routinely increasing the iron intake of a population could be justified.

Studies of iron repletion and/or food fortification have supported increasing the iron intake of non-anemic menstruating women with ferritin levels ranging from <12 to <50 $\mu\text{g/L}$. Experimental studies using iron supplementation demonstrated improved verbal learning and memory in non-anemic adolescent girls (Bruner et al., 1996), showed improved exercise capacity and physical performance in menstruating adult women (Brutsaert et al., 2003; Rowland et al., 1988), and benefited women of reproductive age with unexplained fatigue in the absence of anemia (Verdon et al., 2003). A large non-experimental cross-sectional cohort study, using baseline and follow-up data from the first two years of the Australian Longitudinal Study on Women's Health (ALSWH), also found an association between self-reported iron deficiency and general health and well-being, vitality and fatigue in Australian Women. (Patterson et al., 2000). In follow-up to the associations observed in the ALSWH study, the effects of iron deficiency without anemia on general health, well-being, and tiredness was systemically investigated in a randomized controlled clinical trial. Treatment of iron deficiency with either supplementation or a high iron diet resulted in improved mental health and decreased fatigue among menstruating women (Patterson et al., 2001).

Menstruation has been consistently identified as the main cause of iron loss in women and evidence suggests that women may not be adequately repleting their iron stores. The Report of a Survey of Residents of Newfoundland and Labrador, 1996, suggested that although the iron intakes of males and senior females appeared adequate, many females less than 50 years appeared to be consuming an inadequate amount of iron. The survey showed that 25.3% of females aged 19-30 and 33.3% of females aged 31-49 were consuming less than the Estimated Average Requirement (EAR) (Roebathan, 2003).

Most of the variation in serum ferritin concentrations in the present study was due to other factors that were not considered, such as supplement use, inadequate dietary intake of iron and insufficient iron absorption. Further research is required to determine whether the low ferritin levels observed in women could possibly be due to these factors and whether such levels have a negative clinical impact.

6.1 Iron Deficiency and Quality of Life

Neither the means of the eight SF-36 domains, health transition scale, nor the two component summary measures were significantly lower for mildly iron-depleted or iron-deficient women. The sample sizes in our groups were large enough to detect the 5-point difference required for a clinically and socially relevant difference between the group means. In fact positive correlates of iron depletion were shown for the General Health Scale, Physical Functioning Scale, Bodily Pain Scale, and the Physical Component Summary Score. However, treating BMI and Age as covariates eliminated these differences, suggesting that they were likely the result of the confounding by correlations observed between serum ferritin concentration with both BMI and age. It is also possible that these differences may reflect physiological advantages of having low body iron stores, but this would have to be tested with further study.

The SF-36 is also a general measure of various quality of life domains and may not have been sensitive enough to measure the effects iron deficiency potentially had on women's cognition and fatigue. This study was undertaken as an ad hoc component of a 2 year cross-sectional study aimed at defining the clinical impact of mild hemophilia A and therefore, existing instruments that directly measure cognition and fatigue were not

utilized. A study using a disease specific instrument, more sensitive to these effects, would better investigate the impact of iron deficiency or iron depletion.

6.2 Iron Deficiency and Mild Hemophilia A

Mutation status did not influence iron status in either sex. Mutation status also had no effect on menstrual blood loss as measured by the PBAC, nor was factor VIII level associated with PBAC score. It appears that the factor VIII levels observed in the women carriers of mild hemophilia A did not put them at risk for experiencing menorrhagia or iron deficiency.

PBAC score also did not appear to be associated with iron status. The PBAC may not have been sensitive enough to detect the difference in blood loss between women with low and normal ferritin levels. Pads and tampons were not provided for the study participants. Providing specific sanitary protection may have better controlled for the varying absorptive capacity of the wide variety of products that are available. Even if specific sanitary protection is provided, there may not be an accurate and reliable method for assessing menstrual blood loss other than the alkaline hematin method. There is generally poor correlation between menstrual blood loss and patients' subjective assessment of blood loss and other parameters applicable to the menstrual cycle (number of days of bleeding, number of sanitary pads and tampons used) (Chimbira, Anderson, & Turnbull, 1980) and it appears that this study may have shown the same result despite the usage of a pictorial guide. However, to be certain we would have had to measure the PBAC against the alkaline hematin method.

The literature validating the PBAC against the alkaline hematin method is not consistent. Reid et al. showed poor correlation between observed PBAC score and menstrual blood loss as measured by the alkaline hematin method and dismiss the PBAC method as unable to accurately assess menstrual blood loss (Reid et al., 2000). Studies also suggest different cut-offs for diagnosing menorrhagia. We choose a PBAC score of ≤ 100 to diagnose menorrhagia as this was the cut-off used by Kadir et al. (1999) to demonstrate menorrhagia in 57% of hemophilia A/B carriers, in comparison to only 29% for control women ($p=0.001$) (Kadir et al., 1999).

As previously mentioned, we found no association between mutation status and menstrual blood loss as measured by the PBAC. Rather than the PBAC simply being an invalid measure, it is possible that mild hemophilia carriers are not at risk for excessive menstrual bleeding and that the differences observed by Kadir et al. were an overestimate resulting from a poor response rate (26%) from these carriers. The low rate possibly being due to the fact that they suffer fewer problems compared to the women with other mild bleeding disorders (vWD response rate, 69%; FXI, 47%; Control, 60%) (Kadir et al., 1999).

6.3 Future Directions

The prevalence of iron depletion in rural Newfoundland is high. Further research is required to determine whether the low ferritin levels observed in women could possibly be a result of inadequate dietary intake of iron or insufficient iron absorption. We were unable to show that carriers of the gene responsible for mild hemophilia A in our study population experience more menstrual blood loss than controls. Nor could we show that

the former have lower iron stores. Using the alkaline hematin method to measure menstrual blood loss and measuring iron consumption and absorption could make more accurate and reliable comparisons on these fields. Furthermore, whether such levels have a clinical effect harmful enough to suggest that a repletion intervention would do more good than harm also warrants further study. Neither iron depletion nor iron deficiency appeared to negatively affect quality of life as measured by the SF-36 questionnaire. A study using measures to investigate the potential effects iron depletion and iron deficiency have on cognition and fatigue could more specifically explore its negative impact.

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Appendix A: MOS short-form survey (SF-36)

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. *Thank you for completing this survey!*

For each of the following questions, please mark an in the one box that best describes your answer.

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

Excellent	Very good	Good	Fair	Poor
▼	▼	▼	▼	▼
<input type="checkbox"/>				

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

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
▼	▼	▼	▼	▼
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3. The following questions are about activities you might do during 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
1. <u>Vigorous activities</u> , such as running, lifting heavy objects, participating in strenuous sports.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2. <u>Moderate activities</u> , such as moving a table, pushing a vacuum cleaner, bowling, or playing golf.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3. Lifting or carrying groceries.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4. Climbing <u>several</u> flights of stairs.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5. Climbing <u>one</u> flight of stairs.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6. Bending, kneeling, or stooping.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7. Walking <u>more than a kilometre</u>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8. Walking <u>several hundred metres</u>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9. Walking <u>one hundred metres</u>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10. Bathing or dressing yourself.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4. 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
1. Cut down on the <u>amount of time</u> you spent on work or other activities.....	<input type="checkbox"/>				
2. <u>Accomplished less</u> than you would like.....	<input type="checkbox"/>				
3. Were limited in the <u>kind</u> of work or other activities.....	<input type="checkbox"/>				
4. Had <u>difficulty</u> performing the work or other activities (for example, it took extra effort).....	<input type="checkbox"/>				

5. 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
▼	▼	▼	▼	▼

- Cut down on the amount of time you spent on work or other activities..... 1 2 3 4 5
- Accomplished less than you would like..... 1 2 3 4 5
- Did work or other activities less carefully than usual..... 1 2 3 4 5

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

Not at all	Slightly	Moderately	Quite a bit	Extremely
▼	▼	▼	▼	▼
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5

7. How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very severe
▼	▼	▼	▼	▼	▼
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5	<input type="checkbox"/> 6

8. 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
▼	▼	▼	▼	▼
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one 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
	▼	▼	▼	▼	▼

1.	Did you feel full of life?	<input type="checkbox"/> 1	:	<input type="checkbox"/> 2	:	<input type="checkbox"/> 3	:	<input type="checkbox"/> 4	:	<input type="checkbox"/> 5
2.	Have you been very nervous?	<input type="checkbox"/> 1	:	<input type="checkbox"/> 2	:	<input type="checkbox"/> 3	:	<input type="checkbox"/> 4	:	<input type="checkbox"/> 5
3.	Have you felt so down in the dumps that nothing could cheer you up?	<input type="checkbox"/> 1	:	<input type="checkbox"/> 2	:	<input type="checkbox"/> 3	:	<input type="checkbox"/> 4	:	<input type="checkbox"/> 5
4.	Have you felt calm and peaceful?	<input type="checkbox"/> 1	:	<input type="checkbox"/> 2	:	<input type="checkbox"/> 3	:	<input type="checkbox"/> 4	:	<input type="checkbox"/> 5
5.	Did you have a lot of energy?	<input type="checkbox"/> 1	:	<input type="checkbox"/> 2	:	<input type="checkbox"/> 3	:	<input type="checkbox"/> 4	:	<input type="checkbox"/> 5
6.	Have you felt downhearted and depressed?	<input type="checkbox"/> 1	:	<input type="checkbox"/> 2	:	<input type="checkbox"/> 3	:	<input type="checkbox"/> 4	:	<input type="checkbox"/> 5
7.	Did you feel worn out?	<input type="checkbox"/> 1	:	<input type="checkbox"/> 2	:	<input type="checkbox"/> 3	:	<input type="checkbox"/> 4	:	<input type="checkbox"/> 5
8.	Have you been happy?	<input type="checkbox"/> 1	:	<input type="checkbox"/> 2	:	<input type="checkbox"/> 3	:	<input type="checkbox"/> 4	:	<input type="checkbox"/> 5
9.	Did you feel tired?	<input type="checkbox"/> 1	:	<input type="checkbox"/> 2	:	<input type="checkbox"/> 3	:	<input type="checkbox"/> 4	:	<input type="checkbox"/> 5

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

All of the time	Most of the time	Some of the time	A little of the time	None of the time
▼	▼	▼	▼	▼
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5

11. How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
	▼	▼	▼	▼	▼
1. I seem to get sick a little easier than other people	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
2. I am as healthy as anybody I know	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
3. I expect my health to get worse.....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
4. My health is excellent.....	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5

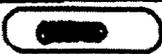
Thank you for completing these questions!

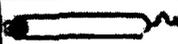
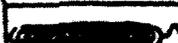
Appendix B: Pictorial Blood Loss Assessment Chart

How to Use the Pictorial Blood Assessment Chart

Menorrhagia is defined as heavy, prolonged bleeding throughout the menstrual cycle. This can impact a woman's daily life causing disruptions in work and daily activities. We are using this assessment chart to gain a better understanding and rate of menorrhagia. Please complete the following questionnaire during your next menstrual period. In order to assure reliable results, we would ask you to track the information concerning your menstrual period on a daily basis in the tables that follow, for each tampon or pad used.

How to: Each / represents one pad or tampon used. For each day your period lasts check off a (/) indicating the number and description of the tampon or pad used. For example, if on day 1 you have used 2 lightly soaked pads and nothing else you would write // in the appropriate box or on day 5 you used 2 lightly soaked pads and one lightly soaked tampon you mark // in the corresponding box for pads and / in the corresponding box for tampons. *See illustration below.

DATE								
TOWEL	#1	2	3	4	#5	6	7	8
	//				//			
		+++						
								

TAMPON	#1	2	3	4	#5	6	7	8
								
								
		+++						

Please indicate which brand of pads you use: _____

Please check the word that best describes the type of pad:

Light Regular Heavy Extra heavy

Please indicate which brand of tampons you use: _____

Please check the word that best describes the type of pad:

Light Regular Heavy Extra heavy

Once you have completed this questionnaire please mail it back to us in the self-addressed envelope supplied. You may be contacted by study personnel as a friendly reminder. Please do not hesitate to contact us with any questions. You can reach Meghan at 777-8536 or David at 777-7530. *Thank you again for participating in our study!!*

DAYS	1	2	3	4	5	6	7	8	9	10	11	12
DATES												
PADS												
Lightly soaked 												
Moderately soaked 												
Heavily soaked 												
TAMPONS												
Lightly soaked 												
Moderately soaked 												
Heavily soaked 												
Clots 1cm or less												
Clots More than 1cm												
Blood flowing outside the pad or tampon												

(Table inspired by J.M. Higham et al. Br. J. Obstet. Gynaecol. 1990;97:734-9)

Total number of days of menstrual period: _____

Appendix C: Personal Interview Questionnaire

Personal Interview Questionnaire

Thank you for participating in our study. The purpose of this questionnaire is to get a general picture of our participants. Personal identifiers such as you name and phone number is requested only to confirm some questions we may have following your participation or to fill-in missing information. Once this information is gathered **these identifiers will be removed before being entered in our database as outlined in the consent form.**

General Information

Name: _____ Date (yy/mm/dd): _____

Mailing Address: _____

Date of birth (yy/mm/dd): _____ Age: _____

Family Physician: _____

Personal Health History

- Have you ever been diagnosed with a bleeding disorder or had an abnormal loss of blood making it necessary to consult with a doctor following an injury?

Yes No If yes:

How old were you? _____

What was the problem? _____

What was the treatment? _____

- Are you presently receiving care for a bleeding disorder?

Yes No If yes:

What treatment are you receiving? _____

- Have you been diagnosed with any other medical disorders or problems?

Yes No If yes:

Please check off any of the following that apply:

Diabetes

Hypertension (high blood pressure)

Heart disease

Arthritis

Other: _____

- Do you take medication on a regular basis?

Yes No If yes:

List the names of the medication you have taken in the last month:

Bleeding History

- Do you have a tendency to bruise easily?

Not at all Very little A bit A lot Very much

- Do you bleed from your gums when you brush your teeth?

Not at all Very little A bit A lot Very much

- Do you have a tendency to have nosebleeds for no apparent reason?

Not at all Very little A bit A lot Very much

- Have you ever had an abnormal loss of blood from an injury, dental work or surgery?

Yes No If yes:

How old were you? _____

What was the problem? _____

What was the treatment? _____

- Did you ever receive a blood transfusion or blood product due to an abnormal loss of blood?

Yes No If yes:

How old were you? _____

Was it more than once? Yes No

What was the treatment? _____

Gynecological History

- How old were you when your menstrual periods began? _____
Typically, how many days do they last? _____
In how many days does your menstrual cycle reoccur? _____

- Do you bleed between your menstrual periods?

Yes No If yes:

Very rarely Rarely Often Very Often Always

- Do you have clots during your menstruation?

Yes No If yes:

Very rarely Rarely Often Very Often Always

- Do you stain your clothing during your menstruation?
 Yes No If yes:
 Very rarely Rarely Often Very Often Always

- Do you stain your bed during your menstruation?
 Very rarely Rarely Often Very Often Always

- Do you have pain during your menstruation?
 Not at all Very little A bit A lot Very much

- Do you take medicine to ease your menstrual pain?
 Never Rarely Often Very Often Always

- Have you ever had to consult a doctor because of abnormal menstrual periods?
 Yes No If yes:
 What was the treatment? _____

- Do you sometimes miss school or work because of your menstruation?
 Yes No If yes: How many days? _____

- Does your menstrual period interfere with your social life?
 Not at all Very little A bit A lot Very much

- Does your menstrual period interfere with your sex life?
 Not at all Very little A bit A lot Very much

- Have you ever taken contraceptives (the Pill)?
 Yes No If yes:
 For contraception
 To regularize your cycle
 To diminish your bleeding

- Did the contraceptives:
 - Increase your menstrual period
 - Diminish your menstrual period
 - Change nothing in your menstrual period

Obstetrical History

- Have you ever tried to get pregnant?
Yes No

- How many times have you been pregnant? _____

- In general, for the majority of your pregnancies, how much time passed, on average, between the time you decided o get pregnant and the moment you became pregnant?
 - Less than 3 months
 - Between 3 and 6 months
 - Between 6 and 12 months
 - More than 12 months

- Have you had any miscarriages or stillbirths?
Yes No If yes:
How many miscarriages? _____ Stillbirths? _____

- How many times have you given birth? _____
Vaginal birth: _____
Caesarean section: _____

- Did your doctor tell you that you bled abnormally after one or more of your childbirths?
Yes No If yes, how many?
Vaginal births _____ Cesarean section _____

- Have you needed a blood transfusion for a hemorrhage associated with childbirth?
Yes No
- Have you had a hysterectomy? Yes No If yes, at what age? _____
What was the reason? _____
Were you treated for excessive bleeding after the procedure? _____

Family History

- Do you have brothers? Yes No
Do you have sisters? Yes No If yes:
How many living brothers do you have? _____
How many living sister do you have? _____
Have you ever lost a brother or sister because of bleeding?
Yes No
Details: _____
- Do you have sisters with abnormal periods?
Yes No Don't know If yes:
How many? _____
Is there an illness known to explain their abnormal menstrual periods? _____

- Does/Did your mother have a history of abnormal menstrual periods?
Yes No Don't know
Is/Was there an illness known to explain their abnormal menstrual periods? _____

- Do you know of other women in you family who have had hysterectomy due to heavy bleeding? Yes No If yes, please list relationship to you. _____

- Do you have someone in your immediate or close family who is known to have a hereditary (from birth) hemorrhagic problem?

Yes No Don't know If yes:

What is your relation to this person? _____

What is the name of the hereditary hemorrhagic problem? _____

- Do you have someone in your immediate or close family who has had any of the following problems? Yes No Don't know

If yes, please check those that apply:

Nosebleeds Bleeding after surgery

Bleeding with dental work Easy bruising

Thank you for participating in our study!



