PREVALENCE AND DIETARY PREDICTORS OF IRON DEFICIENCY ANEMIA IN WOMEN 1-YEAR POSTPARTUM LIVING IN CENTRAL MONTREAL

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ABSTRACT

We estimated the prevalence of iron deficiency anemia (IDA) in women 1-year postpartum in central Montreal. Blood samples were obtained by venipuncture and questionnaires administered. Iron intake was assessed by a food frequency questionnaire. Mothers with at least two of the following laboratory values were considered to have IDA: serum ferritin (SF) < 12 μg/L, mean corpuscular volume (MCV) < 80 fL and hemoglobin (Hb) < 120 g/L. Blood samples were analysed for 201 women. The estimates of prevalence of anemia (Hb < 120 g/L), iron deficiency (SF < 12 μg/L) and IDA were 7.0% (95% confidence interval [CI] 3.8%-10.9%), 5.5% (95% CI 2.5%-8.9%) and 2.5% (95% CI 0.3%-4.7%) respectively. No significant differences were observed between level of income and anemia, iron deficiency and IDA rates. Anemia was not related to dietary iron intake. In conclusion, the prevalence of IDA is low among healthy women 1-year postpartum in central Montreal.

ABRÉGÉ

Nous avons estimé la prévalence d'anémie ferriprive chez les femmes 1-an post-partum au centre de Montréal. Des prélèvements sanguins furent obtenus par ponction veineuse et des questionnaires furent remplis par entrevue. L'apport en fer fut estimé par un questionnaire de fréquence alimentaire. Les mères ayant au moins deux des valeurs de laboratoires suivantes furent jugées atteintes d'anémie ferriprive : ferritine sérique (FS) <12 µg/L, volume globulaire moyen (VGM) < 80 fL et hémoglobine (Hgb) < 120 g/L. Les prélèvements sanguins furent analysés chez 201 femmes. Les estimés de prévalence d'anémie, de déplétion des réserves en fer et d'anémie ferriprive furent 7.0% (intervalle de confiance [IC] de 95%, 3.8%-10.9%), 5.5% (IC de 95%, 2.5%-8.9%) et 2.5% (IC de 95%, 0.3%-4.7%) respectivement. Aucune différence significative ne fut observée lorsque le niveau de revenue fut comparé avec le taux d'anémie, de déplétion des réserves en fer et d'anémie ferriprive. L'anémie ne fut pas liée à l'apport en fer. En conclusion, l'anémie ferriprive n'est pas répandue chez les femmes en santé 1-an post-partum au centre de Montréal.

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LIST OF ABBREVIATIONS

ADA	American Dietetic Association
CDC	Centers for Disease Control and Prevention
CI / IC	Confidence interval / Intervale de confiance
DC	Dietitians of Canada
DSP	Direction de la santé publique
EAR	Estimated average requirement
ESWG	Expert Scientific Working Group
FAO	Food and Agriculture Organization
FFQ	Food frequency questionnaire
Hb / Hgb	Hemoglobin / Hémoglobine
ICSH	International Committee for Standardization in Haematology
ID	Iron deficiency
IDA	Iron deficiency anemia
IOM	Institute of Medicine
MCV / VGM	Mean corpuscular volume / Volume globulaire moyen
MFP	Meat, fish and poultry
NHANES	National Health and Nutrition Examination Survey
RDA	Recommended dietary allowance
SF / FS	Serum ferritin / Ferritine sérique
TfR	Transferrin receptor
TIBC	Total iron binding capacity
UL	Upper intake level
US	United States
WHO	World Health Organization
WIC	Special Supplemental Nutrition Program for Women, Infants, and Children

CONTRIBUTION OF AUTHORS

The following findings were part of a larger study evaluating maternal and infant iron indicators. This study was a follow-up to a study of infant anemia conducted in the late 1980's (Lehmann *et al.*, 1992). The investigators of the present study had also collaborated in the 1992 study. Dr. Katherine Gray-Donald, Dr. François Lehmann and Dr. Stephen DiTommaso all contributed to the conception of the present study and collaborated in the data analysis and data interpretation. Dr. DiTommaso further acted as the research physician and was responsible for overseeing all clinical activities.

The candidate's roles in this study included obtaining data through the *Commission d'accès à l'information du Québec* and the *Régie régionale de la santé et des services sociaux Montréal Centre* for recruitment purposes, organizing the data files, preparing mail-outs of letters of invitation and thanks, supervising the research nurse and training her in dietary measurement, developing the study questionnaire, compiling the data and conducting the analyses. The candidate prepared the manuscript under the guidance of the co-authors.

The material presented in this thesis will only pertain to maternal anemia since the collection of infant data is still underway and will be the subject of another student's thesis.

CHAPTER 1. INTRODUCTION

Iron deficiency is the most frequently observed nutrient deficiency and accompanied with anemia, it is estimated to affect more than 500 million individuals across the globe (DeMaeyer & Adiels-Tegman, 1985). Individuals particularly at risk of developing this condition include young children and women during their childbearing years (World Health Organization (WHO), 2001). In North America, there has been a significant decrease in the overall prevalence of iron deficiency anemia in the past 30 years (Feightner, 1994). This is particularly true in infants where the decreased incidence has been attributed, in large part, to a rise in breastfeeding, increased and prolonged use of iron fortified infant formulas and increased use of infant cereals fortified with iron (Yip *et al.*, 1987a; Yip *et al.*, 1987b). At the present time, women of childbearing age and adolescents comprise the groups in which the prevalence of iron deficiency is highest in industrialised countries (Food and Agriculture Organization (FAO) & WHO, 2001).

The adverse consequences of iron deficiency anemia in women include diminished work capacity (Haas & Brownlie, 2001), adverse pregnancy outcomes (Ramakrishnan, 2001) and potentially, decreased immune function (Oppenheimer, 2001). Knowledge of the prevalence and dietary predictors of iron deficiency anemia among postpartum women is therefore of importance if this condition is to be prevented.

1.1. Study rationale

The present findings were part of a larger study evaluating maternal and infant anemia. In the late 1980's, Lehmann *et al.* (1992) studied the prevalence of iron deficiency anemia among one-year old infants of disadvantaged families in Montreal. They found that iron deficiency anemia was prevalent in 25% of infants. Following this study, the Quebec government implemented a policy enabling families receiving social assistance to purchase infant formula (including iron-fortified formula) at a reduced cost or to receive a bonus if the infant is breastfed. Since this program has never been evaluated and current prevalence of iron deficiency anemia among one-year old infants in Montreal was unknown, the present study was initiated.

Consequently, this study provided an opportunity to also evaluate the iron status of the mothers of these infants given that: 1) current prevalence data of iron deficiency anemia among Canadian women is lacking, 2) women during their childbearing years are especially vulnerable to this condition because of their increased requirements for iron (Ramakrishnan, 2001) and 3) previous surveys have illustrated that some Canadian women may not be meeting their dietary requirements for iron (Tessier *et al.*, 2002; Gray-Donald *et al.*, 2000). It was therefore hypothesized that iron deficiency anemia may be prevalent among postpartum women living in central Montreal. Moreover, this area of the city has people from a broad range of income groups, making it possible to survey a cross-section of Canadian women in regard to income, education and ethnic background.

1.2. Research objectives

1. To measure the iron status of women 1-year postpartum living in central Montreal.
2. To compare the diets of anemic and non-anemic women 1-year postpartum in order to identify patterns of food intake which prevent iron deficiency anemia.

CHAPTER 2. LITERATURE REVIEW

2.1. Iron compounds in the body

Iron compounds in the body can be classified in two groups: essential iron compounds and storage iron compounds (Dallman, 1993). Essential iron compounds serve physiological functions and include hemoglobin, myoglobin, the cytochromes as well as other proteins that play a role in the transport, storage and utilization of oxygen (Dallman *et al.*, 1980). Hemoglobin, found in red blood cells, carries oxygen to the tissues from the lungs and accounts for about 75% of total body iron (Dallman, 1993). Myoglobin is located in the cytoplasm of muscle cells and facilitates the diffusion of oxygen towards the mitochondria (Institute of Medicine (IOM), 2001). Other heme proteins include the cytochromes (Dalman *et al.*, 1993). They act as electron carriers and are involved in oxidative metabolism, notably in the production of adenosine triphosphate (ATP) (Dallman *et al.*, 1993). The remainder of iron is stored primarily in the liver, spleen and bone marrow in the form of ferritin and hemosiderrin (Dallman, 1993). These storage compounds serve as a reserve and mobilize iron when supply is inadequate in order to maintain the production of essential iron compounds (Dallman, 1993). They also play a role in maintaining iron homeostasis in the body by regulating the absorption of dietary iron (Dallman, 1993).

2.2. Iron absorption

Dietary iron is absorbed in the intestinal tract with the aid of two proteins: mucosal ferritin and mucosal transferrin (Whitney *et al.*, 2002). Iron is taken up by mucosal ferritin and is then stored in the mucosal cell (Whitney *et al.*, 2002). When the body requires iron, mucosal ferritin releases some to mucosal transferrin (Whitney *et al.*, 2002). Iron is then transferred to serum transferrin, which carries iron in the blood (Whitney *et al.*, 2002). If the body does not require all of the iron, a portion is eliminated through the shed intestinal cells (Whitney *et al.*, 2002). The absorption of dietary iron is influenced by many factors including body stores, the amount and form of iron in the diet as well as

the presence of dietary factors that enhance or inhibit iron absorption (Centers for Disease Control and Prevention (CDC), 1998). These factors will be discussed briefly.

The iron status of an individual is the main factor controlling iron absorption (CDC, 1998). When iron stores are diminished, iron absorption is increased (CDC, 1998). In the presence of iron deficiency anemia, the rate of absorption of dietary iron can increase to approximately 20-35% in contrast to the 2-15% absorption rate in iron sufficient individuals (Monsen *et al.*, 1978).

The composition of the meal also affects iron bio-availability (Monsen *et al.*, 1978). Dietary iron is found in two forms: heme and non-heme iron (Whitney *et al.*, 2002). The amount of iron absorbed differs between these forms. Heme iron, found in meats, fish and poultry (MFP), is much better absorbed (15-35%) compared to non-heme iron (2-20%) (Monsen *et al.*, 1978). Heme iron accounts for approximately 30-60% of iron in meats, fish and poultry and the composition of the meal does not affect its absorption (Cook & Monsen, 1976).

On the other hand, the absorption rate of non-heme iron is dependant on the composition of the meal (Monsen *et al.*, 1978). Non-heme iron is found in vegetables, fruits, grains, eggs and dairy products as well as in lower percentages in meats, fish and poultry (Monsen *et al.*, 1978). Non-heme iron absorption can be enhanced or inhibited by dietary components (Monsen *et al.*, 1978). Dietary enhancing factors include MFP (Bjorn-Rasmussen & Hallberg, 1979), ascorbic acid (vitamin C) (Hallberg *et al.*, 1986) as well as other organic acids (Gillooly *et al.*, 1983). Food cooked in iron skillets (Brittin & Nossaman, 1986), iron pots (Adish *et al.*, 1999) or stainless steel cookware (Park & Brittin, 1997) may also improve iron absorption.

In contrast, dietary components such as phytates, polyphenols, oxalates and soy protein all inhibit non-heme iron absorption (American Dietetic Association (ADA) & Dietitians of Canada (DC), 2000). The vast majority of phytates come from cereals in the North American diet but seeds, nuts, vegetables, roots and fruits also contain a certain amount

of phytates (FAO & WHO, 2001). The greater the amount of phytates consumed the greater is the inhibition of iron absorption (Hallberg *et al.*, 1989). The main iron binding polyphenols are found in tea and coffee (Disler *et al.*, 1975; Morck *et al.*, 1983; Hallberg & Rossander, 1982a). Oxalates, found in green leafy vegetables such as spinach, are also important iron absorption inhibitors (ADA & DC, 2000). Soy protein also negatively influences the absorption of iron, however, the effect is usually negligible due to its high iron content (Cook *et al.*, 1981; Hallberg & Rossander, 1982b). Lastly, calcium also seems to interfere with iron absorption. Supplemental calcium has been shown to inhibit the absorption of inorganic iron when taken together (Cook *et al.*, 1991). Dietary iron was also inhibited when both inorganic and organic forms of calcium were added to a meal (Hallberg *et al.*, 1991). In this study, the inhibition appeared to be dose-related and interestingly, heme iron absorption was also affected.

2.3. Iron balance and requirements

2.3.1. Iron balance.

Our body regulates iron absorption in order to maintain adequate iron levels (FAO & WHO, 2001). This is achieved through three mechanisms: 1) recycling iron from the breakdown of red blood cells in the body, 2) releasing storage iron when iron requirements are high and 3) regulating iron absorption at the intestinal level (increasing absorption when body iron stores decrease and vice-versa) (FAO & WHO, 2001). The amount of iron ingested and its form, the amount of iron lost by the body and the amount of iron stores in the body are therefore the primary factors influencing iron balance (FAO & WHO, 2001). The onset of an iron-deficient erythropoiesis occurs when iron requirements increase or iron bio-availability decreases to the point where losses cannot be balanced by the regulation of iron absorption (Hallberg *et al.*, 1995).

Basal iron losses amount to approximately 0.9-1.02 mg/day in healthy adults who are not bleeding (Green *et al.*, 1968). The majority of iron is lost from the desquamation of surface cells from the body's skin, gastrointestinal and urinary tracts (FAO & WHO,

2001). Women lose additional iron during menstruation, pregnancy and lactation (Bothwell & Charlton, 1981).

Menstrual blood loss varies considerably among women but is fairly consistent from one menstrual cycle to the next for the individual woman (Hallberg & Nilsson, 1964). The amount of menstrual blood lost can be affected by contraceptive practices (IOM, 2001). Oral contraceptives decrease menstrual blood loss by about half (Nilsson & Sölvell, 1967) while certain intrauterine devices approximately double the amount of blood loss (Guttorm, 1971). In most cases, iron lost from menstrual blood is counterbalanced by an increased absorption of dietary iron (Dallman *et al.*, 1993). However, the risk of developing iron deficiency is substantially increased when menstrual blood loss exceeds 80 mL per month (Hallberg *et al.*, 1966).

During pregnancy, iron needs increase substantially even though there is temporary cessation of menstruation (Bothwell & Charlton, 1981). Iron is needed to support the expansion of maternal blood volume, the growth of the fetus and blood loss during delivery (Whitney *et al.*, 2002). Iron supplementation is often required since women are seldom able to meet their dietary iron requirements from food alone (Allen & Casterline-Sabel, 2001).

Small amounts of iron are lost in breastmilk during lactation. However, menses usually do not return until about six months postpartum in mothers exclusively breastfeeding (IOM, 2001). Iron requirements are therefore lower during lactation (IOM, 2001).

2.3.2. Dietary iron requirements.

Iron requirements in non-pregnant menstruating women are estimated as the total of basal and menstrual iron losses (IOM, 2001). Dietary iron requirements further take into consideration the amount of absorbable iron (IOM, 2001). The Estimated Average Requirement (EAR) for women 19 to 50 years is 8.1 mg/day of iron while the Recommended Dietary Allowance (RDA) for the same age category is 18 mg/day of iron (IOM, 2001). By definition, the EAR is "the average daily nutrient intake level estimated

to meet the requirements of half the healthy individuals in a particular life stage and gender group (IOM, 2001)". On the other hand, the RDA is "the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life stage and gender group (IOM, 2001)." For adults, the Tolerable Upper Intake Level (UL) is 45 mg/day of iron. This level represents "the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects may increase (IOM, 2001)."

In non-menstruating women who are breastfeeding, iron requirements are estimated as the sum of iron secreted in milk and basal iron losses (IOM, 2001). For women 19-50 years of age, the EAR is 6.5 mg/day and the RDA is 9 mg/day (IOM, 2001).

2.4. Stages of iron deficiency

Iron deficiency, or diminished iron stores, occurs when an insufficient quantity of iron is provided by the diet or when iron losses are greater than the amount absorbed (Whitney *et al.*, 2002). When iron depletion is severe enough to affect the erythrocyte, iron deficiency anemia develops (IOM, 2001). There exist three main stages of iron depletion (Dallman *et al.*, 1993; Expert Scientific Working Group (ESWG), 1985):

Stage I: Iron Depletion. During this first stage, there is a substantial reduction in iron stores. Low serum ferritin values are observed.

Stage II: Iron-deficient erythropoiesis. During the second stage, iron's total binding capacity by transferrin is increased. This in turn decreases serum iron and percent of transferrin saturation. Mobilization of iron to hemoglobin precursors is diminished and free protoporphyrin levels are increased in red blood cells since it is not able to bind to iron to form hemoglobin.

Stage III: Iron Deficiency Anemia. At this stage, there is an inadequate production of functional iron compounds. Hemoglobin, hematocrit and mean corpuscular volume (MCV) values are decreased.

2.5. Laboratory assessment of iron deficiency

Certain signs and symptoms such as pallor of the skin and conjunctiva, fatigue, shortness of breath, lack of appetite and spoon shaped nails are associated with iron deficiency and anemia (Andrews, 2004). However, diagnosis relies on laboratory assessment since these signs and symptoms are non-specific and difficult to detect (DeMaeyer, 1989). Moreover, anemia is often severe when individuals seek medical attention because of these symptoms (Andrews, 2004).

Numerous laboratory tests are used to detect iron deficiency and anemia. The absence of stainable iron in bone marrow smears is often seen as a gold standard for detecting iron deficiency, however, this procedure is rarely used due to its invasive nature (Brugnara, 2002). More common and practical tests include hematological tests (i.e. hemoglobin concentration, hematocrit, mean corpuscular volume and red blood cell distribution width) and biochemical tests (i.e. transferrin saturation, serum ferritin and erythrocyte protoporphyrin) (CDC, 1998). Hematological tests look at the characteristics of red blood cells and are often more accessible and affordable, however, biochemical tests have the advantage of detecting early changes in iron status (CDC, 1998). The diagnosis of iron deficiency anemia often relies on more than one laboratory test.

2.5.1. Hemoglobin.

Hemoglobin, present in red blood cells, transports oxygen from the lungs to cells throughout the body and is formed in part by iron (Whitney *et al.*, 2002). Its synthesis is compromised during iron deficiency (Whitney *et al.*, 2002). However, low hemoglobin levels are not specific to iron deficiency and may occur in other conditions such as folate or vitamin B12 deficiency, thalassemia and other hemoglobinopathies, infection and chronic inflammation (ESWG, 1985). Misdiagnosis can therefore occur if the diagnosis

of iron deficiency anemia is based solely on the presence of low hemoglobin concentration (IOM, 2001). Age, sex, physiological state, race, smoking and altitude must also be considered when interpreting hemoglobin values (Sauberlich, 1999). Hemoglobin concentration is increased in persons who smoke (Nordenberg *et al.*, 1990) or live at high altitudes (Dirren *et al.*, 1994). Proper diagnosis of anemia in these individuals therefore requires adjustments in cutoff values (Table 1) (CDC, 1989). In contrast, hemoglobin levels have been shown to be lower in healthy African Americans (Perry *et al.*, 1992). Johnson-Spear and Yip (1994) have suggested lowering the hemoglobin cutoff by 10 g/L in African American women in order to ensure a more comparable sensitivity and specificity between blacks and non-blacks. However, others have deemed unnecessary the use of a race specific hemoglobin criterion (Perry *et al.*, 1992). In general, the laboratory definition of anemia is based on a hemoglobin (or hematocrit) value that is below the 95 percent reference range for age and sex (Dallman, 1993). In women, the standard cutoff value is 120 g/L (CDC, 1989).

Table 1. Smoking adjustments for hemoglobin

Smoking status	Hemoglobin (g/L)
10-19 cigarettes per day	+ 3
20-39 cigarettes per day	+ 5
40 or more cigarettes per day	+ 7

Based on data from Nordenberg et al., 1990.

2.5.2. Hematocrit.

Hematocrit represents the percentage of the packed red cell volume of whole blood and low values are due to inadequate hemoglobin formation (Sauberlich, 1999). Hematocrit is often used as a diagnostic for iron deficiency, however, it is limited by many of the same factors influencing hemoglobin concentration (Sauberlich, 1999). Values below 36% are considered low in women (CDC, 1989).

2.5.3. Mean corpuscular volume (MCV).

MCV is a measure of the average size of a red blood cell (Whitney *et al.*, 2002). Microcytic (smaller than average) and hypochromic (pale) red blood cells are observed in iron deficiency (Whitney *et al.*, 2002). However, reduced MCV is also found in other conditions that affect hemoglobin synthesis and is therefore not specific to iron deficiency (IOM, 2001). These conditions include thalassemia minor, hemoblogin E and in some cases, anemia of infection and chronic disease (Dallman *et al.*, 1993). In adults, a cutoff of 80 fl is accepted as the lower limit of normal and is indicative of microcytic anemia (Glader, 2004).

2.5.4. Erythrocyte protoporphyrin.

Iron and protoporphyrin make up heme, which is found in the hemoglobin molecule (Whitney *et al.*, 2002). When the formation of heme is compromised due to inadequate iron supplies, protoporphyrin accumulates in the blood (Dallman *et al.*, 1993). A rise in erythrocyte protoporphyrin is indicative of iron deficiency as it usually occurs before the development of anemia (Yip & Dallman, 1984). However, caution must be warranted when evaluating these measurements since erythrocyte protoporphyrin levels are also increased in the presence of lead poisoning (Yip & Dallman, 1984), infection and inflammatory disease (Dallman *et al.*, 1993). In individuals 5 years or older, a value greater than 70 μg/dL erythrocytes is considered elevated (ESWG, 1985; Dallman *et al.*, 1993).

2.5.5. Serum ferritin.

Serum ferritin measures provide an estimate of iron stores and low concentrations are a sensitive index of iron depletion (Valberg, 1980). In healthy individuals, serum ferritin concentration is directly proportional to total body iron stores (Glader, 2004; Walters *et al.*, 1973). In adults, 1 μg/L of serum ferritin indicates the presence of about 8 mg of storage iron (Walters *et al.*, 1973). However, serum ferritin values may falsely be in the normal range during the presence of infection, inflammatory disorders and liver disease (Dallman *et al.*, 1993; Lipschitz *et al.*, 1974). In adults, iron stores are considered

depleted when serum ferritin concentration falls below 12 μg/L (Cook *et al.*, 1974; IOM, 2001).

2.5.6. Serum iron.

Serum iron levels are indicative of the amount of iron bound to transferrin and levels are decreased in the presence of iron deficiency (Glader, 2004). Biological variations must however be considered when using this measure (Sauberlich, 1999). In normal subjects, serum iron levels are highest in the morning and tend to decrease throughout the day (Sauberlich, 1999). Intra-individual day-to-day variations also exist (Statland & Winkel, 1977). Serum iron values are considered low when they fall below 30 μg/dL (Dallman *et al.*, 1993).

2.5.7. Total iron-binding capacity (TIBC).

TIBC reflects the total amount of iron that the protein transferrin can bind (Whitney *et al.*, 2002). During iron deficiency, TIBC is increased since a decrease in serum iron increases the number of unbound iron binding sites on transferrin (Gibson, 1990). A TIBC value greater than 400 μg/dL is indicative of iron deficiency (IOM, 2001).

2.5.8. Transferrin saturation.

Transferrin saturation is an indication of the supply of iron to the tissue (IOM, 2001). The percent of transferrin saturation can be calculated by dividing the serum iron value by the TIBC value and multiplying the results by 100 (Dallman *et al.*, 1993). When levels fall below 16% in adults, normal hemoglobin synthesis is compromised due to the diminished rate of iron delivery (IOM, 2001). A decreased transferrin saturation level is not specific to iron deficiency since levels may also be low in the presence of inflammatory disease (Lipschitz, 1990).

2.5.9. Transferrin receptor (TfR).

The amount of transferrin receptors on the surface of cells is proportional to their iron requirements (IOM, 2001). Serum TfR levels therefore increase as iron deficiency progresses (IOM, 2001). Levels appear to be independent of age and sex (Kohgo *et al.*,

1986). Furthermore, serum TfR measurements can help distinguish iron deficiency anemia from anemia caused by infection and inflammation since levels are not affected by these conditions (Pettersson *et al.*, 1994). This relatively new test could therefore be of benefit, however, the test is not widely available and further investigation is needed (Kazal, 2002).

2.6. Anemia of inflammation

Anemia of inflammation can occur in the presence inflammatory infections, neoplastic diseases and chronic infections (Jurado, 1997). This type of anemia results from decreased red blood cell production due to increased activity of the inflammatory cytokines (Glader, 2004). Cytokines such as interleukin-1 and tumor necrosis factor prevent iron from being adequately mobilized from its storage sites and may inhibit erythropoietin production (Carlson, 2000). The obstruction of iron transport may be a non-specific defence mechanism in order to prevent the growth of infecting microorganisms since they require iron for metabolic function (Carlson, 2000).

2.6.1. C-reactive protein.

C-reactive protein is produced by the liver and is present during episodes of acute inflammation (Barany, 2001). As an acute phase protein, it measures and reflects the level of inflammation (International Committee for Standardization in Haematology (ICHS), 1988). An increase in C-reactive protein concentration can be detected within 6-10 hours following tissue injury (ICHS, 1988). Peak levels are reached at 48 hours and then fall with a half time of 48 hours reflecting resolution of the injury (ICHS, 1988). The normal reference range for C-reactive protein is 0.1-8.0 mg/L (ICHS, 1988). Elevations are seen during trauma, ischemia, infections and other disease (ICHS, 1988). Factors such as age, gender and anemia do not affect values (ICHS, 1988). C-reactive protein can therefore be useful to determine if anemia is due to inflammation or iron deficiency.

Studies looking at iron status in women have used various cutoffs for C-reactive protein to define infection. Wolmarans *et al.* (2003) considered C-reactive protein to be elevated when the value was greater than 6 mg/L while a value above 8 mg/L was considered elevated by Ferguson *et al.* (2001). Others have excluded participants from their analysis only when C-reactive protein levels were greater than 10 mg/L (Heath *et al.*, 2001; CDC, 2002).

2.7. Prevalence of iron deficiency

Pehrsson *et al.* (2001) assessed the iron status of non-lactating postpartum women in order to examine the benefits associated with participation in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC). WIC, a federally funded program in the United States (US), serves mainly low-income pregnant women and infants by providing them supplemental nutritious foods, nutrition education and health care referrals in order to help alleviate health problems and improve nutrition status. Capillary blood samples were obtained at baseline (within 30 days of delivery) and repeated at two, four, and six months postpartum. Fifty-seven WIC participants and 53 WIC-eligible non-participants were initially assessed and of these, 47 participants and 47 non-participants completed at least the first and last visit. Both groups were similar in terms of demographic, health and reproductive characteristics. Anemia was defined as a hemoglobin (Hb) concentration below 120 g/L (7.45 mmol/L). Iron depletion was determined by two iron measurements: serum ferritin (SF) concentration < 12 μg/L and serum transferrin receptor (TfR) levels > 8.5 mg/L. No significant differences in dietary iron intake and iron supplementation were observed between the groups at any point during the study. Dietary iron intake ranged from 9.2-11.1 mg/day across groups and time. Mean hemoglobin concentration was similar in both groups at baseline. At six months postpartum, mean hemoglobin levels were significantly higher among WIC participants (8.01 ± 0.12 mmol/L versus 7.63 ± 0.12 mmol/L; $p < 0.05$) and the prevalence of anemia was significantly lower (17% in participants versus 51% in non-participants; $p < 0.05$). The proportion of women with iron deficiency did not differ significantly between the groups at any point during the study. When iron deficiency was

defined by low serum ferritin concentration prevalence was 9% in WIC participants and 11% in non-participants at six months postpartum. When TfR values were used to define iron deficiency, 15% of WIC participants and 13% of non-participants had depleted iron stores six months postpartum. None of the three iron measurements (Hb, SF, TfR) were correlated and few subjects had low values in all three measurements at any given time point. This indicates that anemia was most likely not due to iron deficiency. Infection, other nutritional deficiencies or poor access to health care may possibly explain higher anemia rates in non-participants.

Bodnar *et al.* (2001) studied the prevalence and predictors of postpartum anemia in low-income postpartum (4-26 weeks) women in the United States. In total, data from 59,428 WIC participants in 12 states were analyzed retrospectively. Hemoglobin levels were obtained by capillary blood samples. Postpartum anemia (hemoglobin < 118 g/L in women 12-15 years of age and < 120 g/L in women 15 years or older) was found in 27.2% of the total population sampled. Prevalence was highest at 12 to 18 weeks postpartum (29.4%-30.9%). Among the lowest prevalence, were women 19-26 weeks postpartum (25.5%). Anemia during pregnancy was the strongest predictor of postpartum anemia (adjusted odds ratio, 2.7; 95% CI, 2.5-2.8). The prevalence of anemia was also higher among minority groups with non-Hispanic black women having the highest prevalence (43.4%). Other positive predictors of anemia included maternal obesity, multiple births and not breastfeeding. Since no other measure of iron status was collected, it is impossible to determine with certainty that iron deficiency was the cause of anemia among these women.

Bodnar *et al.* (2002) also estimated the prevalence of postpartum anemia, iron deficiency and iron deficiency anemia in the United States and compared the prevalence of iron deficiency between postpartum women (0-24 months) and never-pregnant women 20-40 years of age. Data from 680 postpartum women and 587 never-pregnant women were collected from the National Health and Nutrition Examination Survey, 1988-1994 (NHANES III). Women with abnormal results for at least two of three iron measurements [serum ferritin (< 12 μg/L), free erythrocyte protoporphyrin (> 1.24

mmol/L) and transferrin saturation (< 15%)] were considered to have iron deficiency. Iron deficiency anemia was defined as iron deficiency accompanied by a hemoglobin value less than 120 g/L. The estimates of prevalence of anemia among women 0-6, 7-12 and 13-24 months postpartum were 10.3%, 12.7% and 8.8%, respectively and 8.0% in never-pregnant women. Iron deficiency without anemia was found in 12.7%, 12.4% and 7.8% of women 0-6, 7-12 and 13-24 months postpartum, respectively and was 6.5% in never-pregnant women. Iron deficiency anemia was found in 4.2%, 4.4% and 2.3% of women 0-6, 7-12 and 13-24 months postpartum, respectively and was 2.8% in never-pregnant women. Factors associated with iron deficiency included women of minority, not married, multigravid, not using iron or vitamin C supplements and in the lowest third of serum vitamin A concentration. Furthermore, iron deficiency was more prevalent among low-income postpartum women than among postpartum women with higher incomes and never-pregnant women.

The prevalence of iron deficiency and iron deficiency anemia in the US population was determined by Looker *et al.* (1997). Estimates were based on data collected in the NHANES III (1988-1994). Biochemical indicators of iron status were available for 24,894 individuals over one year of age. Of these, 5195 were non-pregnant females 16-49 years of age. Iron deficiency was defined as having an abnormal value for two or more iron status indicators (transferrin saturation, serum ferritin and erythrocyte protoporphyrin). Cutoff values for these indicators in females 16 years or older were: transferrin saturation < 15%, serum ferritin < 12 μg/L and erythrocyte protoporphyrin >1.22 μmol/L RBC. When iron deficiency was accompanied by a hemoglobin value less than 120 g/L in females 16-49 years old, iron deficiency anemia was confirmed. Iron deficiency was found in 11% of females 16-49 years of age. Iron deficiency anemia was 3% in females 16-19 years old and 5% in females 20-49 years old. Women from this last group were more likely to be iron deficient if they were of racial minority, of low-income status or multiparous.

Following the NHANES 1999-2000, the Centers for Disease Control and Prevention (2002) estimated the prevalence of iron deficiency and iron deficiency anemia in the US

and compared these findings with those observed in the NHANES III (1988-1994). Venous blood samples were collected from, among others, 1,415 non-pregnant females aged 16-49 during the NHANES 1999-2000. The definition of iron deficiency and iron deficiency anemia corresponded to that of the NHANES III described previously. Iron deficiency was found in 16% and 12% of females aged 16-19 and 20-49 years respectively. The prevalence of iron deficiency anemia was 2% in females 12-19 years old and was 4% among women 20-49 years old. The prevalence of iron deficiency and iron deficiency anemia among these age groups was similar to that found in the NHANES III.

Only one study has estimated the prevalence of iron deficiency in Canadian women. Data from the Nutrition Canada national survey conducted from 1970-1972 was used to determine the iron status of Canadians (Valberg *et al.*, 1976). Iron status measurements were available for 1105 individuals, 100 of whom were non-pregnant women 20-39 years of age. Individuals were considered to have a high probability of depleted iron stores when serum ferritin levels fell below 15 μg/L. Iron deficiency anemia was defined as a hemoglobin concentration less than 120 g/L accompanied by a serum ferritin level below 15 μg/L. Iron deficiency was found in 27% of women 20-39 years of age while the prevalence of iron deficiency anemia in these same women was 3%.

2.8. Dietary iron intakes in women

In Canada, iron fortification of white flours, enriched pastas, enriched pre-cooked rice and certain substitute foods has been mandatory since 1976 (Walter *et al.*, 2001). In spite of this, some women still have difficulty meeting their dietary iron requirements.

Tessier *et al.* (2002) estimated absorbable iron intakes in 2,118 healthy Quebec adults, 18-74 years of age. Data was obtained from the Quebec Nutrition Survey. Iron intakes were below the estimated average requirement (EAR) in 18.9% of women and absorbable iron intakes, estimated using Monsen and coworker's model, were below the

recommended absorbed iron level in 66.2% of women. The mean iron intake was 11.7 ± 5.1 mg/day.

Low iron intakes were also observed in the Food Habits of Canadians survey (Gray-Donald *et al.*, 2000). Although mean iron intakes were above the recommended intake level for all age groups, iron intakes among women of childbearing age at the 25th percentile were low (< 10 mg/day).

Inadequate dietary iron intakes in some women may be related to recent trends in total and saturated fat intake. In the past thirty years, important reductions in dietary fat intake have been observed in the diet of Canadians (Gray-Donald *et al.*, 2000). Increased low-fat food choices may therefore result in decreased red meat consumption. Individuals with low-income levels may also have difficulty meeting their iron requirements given the high cost of meat and fresh fruits (Marx, 1997). Finally, vegetarian women may not easily meet their iron requirements since highly bio-available heme iron foods are limited (IOM, 2001).

2.9. Adverse consequences of iron deficiency

Functional consequences of iron deficiency include developmental delay and cognitive impairment in infants, pre-schoolers and school aged children (Grantham-McGregor & Ani, 2001); impaired immune status in all age groups (Oppenheimer, 2001); impaired physical work performance in adolescents and adults (Haas & Brownlie, 2001); and adverse pregnancy outcomes (Ramakrishnan, 2001). The following discussion will focus on the main adverse consequences affecting women.

2.9.1. Pregnancy outcomes.

Rasmussen (2001) reviewed current evidence of a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth or length of gestation. Maternal hemoglobin concentration was strongly associated with both birth weight and pre-term birth. In numerous studies, these adverse affects occurred when hemoglobin

concentrations fell below 110 g/L. In addition, high concentrations of maternal hemoglobin also increased the risk of low birth weight and/or premature birth making this a U-shaped relationship. The extent to which iron-deficiency anemia plays a role in this association remains to be determined.

The potential consequences of low birth weight include increased risk of morbidity and mortality, growth retardation, long-term adverse effects on physical and mental performance and increased risk of adult chronic disease (Martorell, 1997).

Anemia also increases the risk of maternal mortality in pregnant women particularly when the anemia is severe (Brabin *et al.*, 2001). Brabin *et al.* (2001) estimated the relative risk of maternal mortality due to anemia. In the presence of moderate anemia (hemoglobin 40-80 g/L) the relative risk was 1.35 (95% CI: 0.92-2.00) while in the case of severe anemia (hemoglobin < 47g/L) it was 3.51 (95% CI: 2.05-6.00). It should, however, be noted that these estimates were derived from observational studies which are often limited by confounding variables. Furthermore, most of these studies were undertaken in developing countries.

Direct effects of maternal anemia on infant health outcomes are not as well known (Ramakrishnan, 2001).

2.9.2. Immune function.

The relationship between iron and increased susceptibility to infection is unclear and debatable. Experimental studies in animals have shown that iron deficiency is associated with negative effects on immune function and that these effects can be reversed through iron therapy (Oppenheimer, 2001).

During iron deficiency, the body's immune system can be impaired in two ways (Walter *et al.*, 1997). The first involves the response of T lymphocytes (Walter *et al.*, 1997). Ribonucleotide reductase's function is impaired when iron supply is inadequate leading to

a reduction in DNA synthesis, which in turn affects the synthesis of T lymphocytes (FAO & WHO, 2001). The end result is reduction in cell-mediated immunologic response.

The second abnormality implicates the activity of neutrophils (Walter *et al.*, 1997). Activation of NADPH oxidase (an iron-sulphur enzyme) and most likely cytochrome b (a heme enzyme) are associated with the "respiratory burst" (an increase in oxygen consumption) and thus the formation of free hydroxyl radicals (Walter *et al.*, 1997). These radicals play an important role in bacterial killing by neutrophil leukocytes (FAO & WHO, 2001). Since iron is an essential component of this process, this defence mechanism is impaired during iron deficiency.

There does nonetheless remain a lack of evidence that the severity and the rate of infection is greater in iron deficient subjects than in control subjects (FAO & WHO, 2001). This lack of evidence *in vivo* may be explained by the presence of numerous confounding factors in these types of studies (Oppenheimer, 2001).

Conversely, iron deficiency may protect against infection (Bothwell & Charlton, 1981). When the iron-binding proteins transferrin and lactoferrin are left unsaturated, iron is unavailable to bacteria thus hindering their growth (Dallman *et al.*, 1993). Iron fortification and treatment practices do not however seem to increase susceptibility to infection under usual circumstances (Walter *et al.*, 1997; Oppenheimer, 2001).

2.9.3. Work capacity.

Iron is involved in energy production (Haas & Brownlie, 2001). Functional iron compounds such as hemoglobin, myoglobin, iron-dependant enzymes and respiratory chain proteins are implicated in the transportation and utilization of oxygen (Haas & Brownlie, 2001).

There exist two distinct mechanisms by which iron deficiency and anemia can affect work capacity (Haas & Brownlie, 2001). The first mechanism is related to iron deficiency with or without anemia and implicates tissue iron oxidative capacity, which

affects endurance and energy capacity (Haas & Brownlie, 2001). This is explained by an increased formation of lactic acid resulting from the impairment of the oxidative metabolism in the muscle due to the lack of iron-containing enzymes (Scrimshaw, 1984).

The second mechanism implicates oxygen-carrying capacity, which affects aerobic capacity, and is only influenced when iron deficiency is severe enough to cause anemia (Haas & Brownlie, 2001). In the presence of anemia, hemoglobin concentration is decreased and thus, so is the amount of oxygen transported from the lung to the tissues (Dallman *et al.*, 1993). Depending on the severity of iron deficiency anemia, VO_2 max can be reduced from 10% to 50% (Haas & Brownlie, 2001). By increasing hemoglobin concentration, aerobic capacity can be improved (Haas & Brownlie, 2001).

This impairment in work capacity is particularly important as it relates to work productivity. Edgerton *et al.* (1979) studied the effects of iron-deficiency anemia among Sri Lankan tea-pickers. Subjects were given either iron supplements or a placebo and their work productivity was assessed before and after a treatment period of one month. In subjects whose hemoglobin concentration was improved by supplementation, the amount of tea picked was significantly increased. This increase was greater in subjects with more severely depleted hemoglobin levels (6.0-9.0 g/dL). Furthermore, the physical activity level of anemic subjects was significantly greater in the treatment group than in the placebo group after three weeks.

BRIDGE

Iron deficiency is the most frequently observed nutrient deficiency in the world and young children and women of childbearing age are the most affected by this condition (WHO, 2001). In Canada, current prevalence of iron deficiency anemia among women of childbearing age is lacking and existing data illustrates that some Canadian women may not be meeting their nutritional needs for iron (Tessier *et al.*, 2002; Gray-Donald *et al.*, 2000). It is essential that health care professionals such as nutritionists know not only the extent of this problem, but also its relationship to diet since they play a key role in prevention efforts. Preventing iron deficiency anemia is important given the negative effects associated with this condition. The following manuscript was prepared with the intention of shedding light on these issues.

CHAPTER 3. MANUSCRIPT

LOW RATES OF IRON DEFICIENCY ANEMIA IN WOMEN 1-YEAR POSTPARTUM

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3.1. Abstract

Background: Current prevalence data on iron deficiency anemia (IDA) in women of childbearing age are lacking in Canada and dietary surveys have suggested that some women may not be obtaining adequate amounts of iron in their diet. This study estimated the prevalence of IDA in women 1-year postpartum living in central Montreal.

Methods: Women 10 to 15 months postpartum were identified through a list of registered births from July 2002 to August 2003 in four districts of Montreal. Home visits were conducted and socio-demographic and health information was collected through an interview-administered questionnaire. Iron intake was assessed by a food frequency questionnaire and blood samples were obtained by venipuncture. Mothers with at least two of the following laboratory values were considered to have IDA: serum ferritin < 12 μg/L, mean corpuscular volume (MCV) < 80 fL and hemoglobin < 120 g/L. Anemia was defined as having a low hemoglobin level and iron deficiency (ID) was defined as having a low serum ferritin level.

Results: Blood samples were analysed for 201 women. The estimates of prevalence of anemia, ID and IDA were 7.0% (95% confidence interval [CI] 3.8%-10.9%), 5.5% (95% CI 2.5%-8.9%) and 2.5% (95% CI 0.3%-4.7%) respectively. Rates of anemia, ID and IDA were not significantly related to income. Anemia was not related to dietary iron intake.

Interpretation: The prevalence of IDA is low among healthy women 1-year postpartum living in central Montreal.

3.2. Introduction

Iron deficiency is the most frequently observed nutrient deficiency and when accompanied with anemia, it is estimated to affect more than 500 million individuals across the globe (DeMaeyer & Adiels-Tegman, 1985). Individuals particularly at risk of developing this condition include young children and women during their childbearing years (WHO, 2001). In North America, there has been a significant decrease in the overall prevalence of iron deficiency anemia in the past 30 years (Feightner, 1994). At the present time, women of childbearing age and adolescents comprise the groups in which the prevalence of iron deficiency is highest in industrialized countries (FAO & WHO, 2001).

The adverse consequences of iron deficiency anemia in women include diminished work capacity (Haas & Brownlie, 2001), adverse pregnancy outcomes (Ramakrishnan, 2001) and potentially, decreased immune function (Oppenheimer, 2001). Given the negative consequences associated with this condition, knowledge of the prevalence and dietary predictors of iron deficiency anemia among postpartum women is of importance if this condition is to be prevented.

In Canada, iron deficiency and anemia have been extensively studied in infants, however, little is known about this condition among postpartum women. In fact, there exist no current prevalence data of iron deficiency anemia among women of childbearing age in Canada. This condition is, however, encountered in the clinical setting. Furthermore, dietary surveys undertaken in Canada have suggested that some women may not be obtaining adequate amounts of iron in their diet (Tessier *et al.*, 2002; Gray-Donald *et al.*, 2000). In the United States, the prevalence of iron deficiency and iron deficiency anemia among non-pregnant women 20-49 years of age has been reported as 12% and 4% respectively (CDC, 2002).

The objectives of this study were two-fold: 1) to measure the iron status of women 1-year postpartum living in central Montreal and 2) to compare the diets of anemic and non-

anemic women 1-year postpartum in order to identify patterns of food intake which prevent iron deficiency anemia.

3.3. Methods

This study was part of a larger study evaluating maternal and infant iron indicators. This paper describes the results for the mothers.

3.3.1. Participants.

A total of 1,420 postpartum women were identified as having given birth between July 01, 2002 and August 01, 2003 through the *Système d'avis de naissance* provided by the *Régie régionale de la santé et des services sociaux Montréal-Centre* in four districts of central Montreal. A letter of invitation was sent to 745 randomly selected eligible postpartum women (10-15 months). A bilingual research nurse followed-up by telephone to determine eligibility and invited subjects to participate. A minimum of seven telephone attempts were made to reach study participants, including at least two evening telephone calls. The research nurse visited each household, drew blood and conducted an interview.

A home visit was also attempted if telephone numbers were erroneous. If the door was not answered during this visit, a second letter was left asking the potential participant to contact the research nurse. Home visits were attempted for 31 participants but this strategy only yielded two potential participants, both of whom refused. Given the poor success rate, this strategy was abandoned.

3.3.2. Eligibility.

Mothers were eligible providing they spoke either French or English, or had an available family member translate; they were between 18-45 years of age; they had given birth to a singleton infant in the past 10-15 months; they were in good health and they were not currently pregnant. Mothers were also not included in the study if they were currently ill with an infection or if they had given birth to a second baby following the birth of their

10-15 month old child. Lastly, mothers who had given birth to a very low birth weight infant (< 1500 g) were not contacted since we did not want to risk calling mothers whose infant had not survived.

3.3.3. Data collection.

After obtaining informed consent, mothers completed an interviewer-administered questionnaire. Mothers were asked about their food intake as well as questions pertaining to their health and socio-demographic background. The mother's dietary iron intake was assessed by a qualitative food frequency questionnaire. The questionnaires were pre-tested prior to the study.

The food frequency questionnaire (FFQ) method was chosen because 11 days of repeat recall would be needed to adequately characterize "usual" iron intake (Willet, 1998). The questionnaire was based on an iron food frequency questionnaire developed in New Zealand (Heath *et al.*, 2000). The FFQ for this study was modified to best reflect the eating habits of Montrealers and was shortened to decrease subject burden. It included foods containing high contents of iron (heme and non-heme).

Venous blood was collected following the guideline procedures from *l'Ordre professionnel des technologistes médicaux du Québec* (1999). Hemoglobin, mean corpuscular volume (MCV) and serum ferritin were evaluated as iron indicators and C-reactive protein was evaluated to determine the presence of inflammation as anemia may be caused by an inflammatory condition rather than by poor iron status (Yip & Dallman, 1988). Hemoglobin and MCV were analysed with the use of Coulter LH-750 (Beckman Coulter Canada Inc., Ville Saint Laurent, Quebec) while serum ferritin levels were determined by using the ECLIA procedure on the Elecsys (Roche Diagnostics, Laval, Quebec). C-reactive protein was analysed using kinetic nephelometry on IMMAGE (Beckman Coulter Canada Inc., Ville Saint Laurent, Quebec). All samples were transported to the hospital for analysis with a maximum delay of four hours.

3.3.4. Definition of anemia, iron deficiency and iron deficiency anemia.

Hemoglobin (Hb), mean corpuscular volume (MCV) and serum ferritin (SF) were used to define iron deficiency anemia. When substandard values were observed for two or more indicators, the participant was considered to have iron deficiency anemia. Iron defiency was defined as having a low serum ferritin value (< 12 μg/L) and anemia was defined as having a low hemoglobin level (< 120 g/L). Cutoff values used for hemoglobin and mean corpuscular volume were based on reference values derived from the Second National Health and Nutrition Examination Survey, 1976-1980 (NHANES II) (CDC, 1989). Values below the 90^{th} percentile and 95^{th} percentile in the reference group of women aged 25-44 of this study were used to define low hemoglobin and mean corpuscular volume respectively. The cutoff value for serum ferritin (< 12 μg/L) was based on the findings of Cook *et al.* (1974).

3.3.5. Ethics.

Ethics approval was received from the Faculty of Agriculture and Environmental Sciences of McGill University. Authorisation to access birth information was obtained from the *Commission d'accès à l'information du Québec*. This information was then provided by the *Régie régionale de la santé et des services sociaux Montréal Centre*. A letter was sent to all mothers to notify them of the laboratory results. In cases where moderate to severe iron deficiency anemia was found, the research nurse or research physician telephoned the participant to explain the results and to discuss follow-up treatment.

3.3.6. Analysis.

The prevalence of iron deficiency anemia was calculated with a 95% confidence interval. The sample size (200) was based on an estimated confidence interval width of 10% on the value for iron deficiency anemia. Differences in continuous variables between anemic and non-anemic mothers were determined by student's independent t-test. Categorical data were analysed using chi-square except in cases where the expected number was less than five in which case Fisher's exact test was used. All tests were two-sided. The food frequency questionnaire was used to estimate total iron, heme iron and non-heme iron

intake. A few categories in the food frequency questionnaire included mixed foods containing both heme and non-heme iron. Heme iron was calculated as 25% of the total iron value for the category of convenience foods (i.e. pizza, hot dogs, hamburgers) while the baked beans with pork food item was considered a non-heme iron food. Meat, fish and poultry items were assigned both a heme and non-heme iron percentage. Percentages were based on those used by Tessier *et al.* (2002). The remaining food categories contained foods that were purely non-heme iron sources. Heme, non-heme and total iron intake were then separately correlated with both hemoglobin and ferritin values. A correlation of $r = 0.25$, if present, had 80% power of being detected with an alpha error of 0.05 using a sample size of 200. All analyses were performed using SAS for Windows, Version 8.

3.4. Results

A total of 1,420 births were registered from July 2002 to August 2003 in the four districts studied. From this list, 30 subjects were excluded, as they did not meet our entry criteria (23 did not meet our age criteria and 7 had given birth to a very low birth weight infant). After randomly selecting 745 participants, letters were sent and telephone calls were attempted to invite mothers to participate in the study. Figure 3.1 illustrates the subject recruitment process. Of those reached and eligible, 57% accepted to participate. Table 3.1 describes the socio-demographic characteristics of the participants. The majority of mothers were of European descent, born in Canada and French speaking. The family income level was variable with 53% of mothers living below the low-income cutoff established by the federal government (Paquet, 2002). Fifty-one percent of mothers did not work and overall, 23% of mothers were on social assistance.

Although home visits were conducted with 212 subjects, 11 of these were subsequently excluded from our analyses. Blood samples were not available for seven subjects and four subjects were excluded on the basis of thalassemia. Since our interest was to study iron deficiency related anemia, persons with known (3) or suspected (1) hemoglobinopathies were excluded from our sample. Complete blood count results were

missing for two further subjects and C-reactive protein was not analysed in one subject due to laboratory error. Results of the blood analyses are described in Table 3.2. In total, 7.0% (95% confidence interval [CI] 3.8%-10.9%) of mothers were anemic (Hb < 120 g/L) and 5.5% (95% CI 2.5%-8.9%) were iron deficient (SF < 12 μg/L). Iron deficiency anemia (at least 2 of the following: Hb < 120 g/L, MVC < 80 fl, SF < 12 μg/L) was found in 2.5% (95% CI 0.3%-4.7%) of mothers. Figure 3.2 illustrates the distribution of hemoglobin levels as well as the proportion of mothers with low serum ferritin levels. The presence of inflammation, reflected by C-reactive protein levels, was low in anemic mothers. No anemic mother had a C-reactive protein level greater than 8 mg/L and only one had a level greater than 5 mg/L. The normal reference range for C-reactive protein is 0.1-8 mg/L (ICSH, 1988).

Hemoglobin cutoff was adjusted in mothers who smoked according to recommendations, as individuals who smoke have higher hemoglobin levels compared to non-smokers (Nordenber *et al.*, 1990). The proportion of mothers who were identified as anemic did not differ following adjustments since no new cases of anemia were identified.

In contrast, hemoglobin levels have been shown to be lower in healthy African Americans (Perry *et al.*, 1992). Although the use of a race specific hemoglobin criterion is debatable, Johnson-Spear and Yip (1994) have suggested that lowering the hemoglobin cutoff by 10 g/L in African American women would ensure a more comparable sensitivity and specificity between blacks and non-blacks during the detection iron deficiency. When this lower hemoglobin cutoff was applied to our data, the proportion of anemic mothers did not differ.

A closer examination of the five cases (2.5%) of iron deficiency anemia showed a mean daily iron intake of 14.1 ± 2.7 mg, none of these intakes were below the Estimated Average Requirement (8.1 mg/day) for women 18-50 years (IOM, 2001) and most were well above this level. Four of these women were not born in Canada and four had low-income levels. When we compared prevalence by place of birth, 5.9% of mothers not

born in Canada had iron deficiency anemia but this was not statistically different from those born in Canada.

The prevalence of anemia, iron deficiency and iron deficiency anemia tended to be higher in individuals whose income levels were below the low-income cutoff compared to those with higher income levels, however, this difference was not statistically significant and the percentage with iron deficiency anemia was very low in both groups. Mean hemoglobin levels were similar among mothers below (131.8 ± 10.2 g/L) and above (132.6 ± 7.4 g/L) the low-income cutoff ($p = 0.52$). It should however be noted that borderline levels of anemia (116-119 g/L) were observed in the majority (10/14) of the women classified with anemia.

The mean total iron intake of all mothers (N=201) was 14.2 ± 5.2 mg/day. The mean heme iron intake was 1.2 ± 0.6 mg/day and the mean non-heme iron intake was 13.0 ± 5.0 mg/day. Heme, non-heme and total iron intake were not different in anemic mothers (Hb < 120 g/L) compared to non-anemic mothers (Table 3.4). Heme, non-heme and total iron intake were similar between iron deficient (SF < 12 μg/L) and iron sufficient mothers (data not shown). Heme, non-heme and total iron intake were not significantly correlated to hemoglobin levels (correlation coefficients were 0.009, -0.006 and -0.005 respectively). Similarly, iron intakes were not significantly correlated with serum ferritin levels. Correlation coefficients were 0.227 for heme iron, -0.043 for non-heme iron and -0.015 for total iron.

Mean heme, non-heme and total iron intake were also compared with respect to the mother's income level (Table 3.5). Iron intakes were significantly lower in mothers with incomes above the low-income cutoff compared to those with incomes below the cutoff ($p < 0.05$). Mothers with lower income levels ate poultry, liver, and convenience foods more frequently than mothers with higher income levels ($p < 0.05$). They also consumed muffins, pancakes, rice, peas, green peppers and eggs more frequently than mothers with higher income levels ($p < 0.05$), however, they did not consume any food item less frequently than mothers with higher income levels.

When we compared income level with place of birth, we found that the majority of mothers (70%) with incomes below the low-income cutoff were not born in Canada. This difference was significant ($p < 0.001$). Furthermore, mothers not born in Canada had significantly higher heme ($p < 0.001$) and total iron intake ($p = 0.0465$) than those born in Canada.

The majority of mothers (72%) reported having regularly taken a multi-vitamin supplement during their pregnancy whereas a small proportion (13%) reported regularly taking this type of vitamin at the present time. Seven women (4%) were currently taking an iron supplement on a regular basis. Two of these women were presumably being treated for anemia as their hemoglobin levels were below 120 g/L, however, both these women had adequate ferritin levels (> 12 μg/L).

3.5. Discussion

To our knowledge, only one other study has estimated the prevalence of iron deficiency anemia in a group of adult women in Canada. This study was undertaken a generation ago using data collected during the Nutrition Canada national survey (Valberg *et al.*, 1976). Iron deficiency anemia was defined as having a hemoglobin level below 120 g/L accompanied by a serum ferritin level less than 15 μg/L. Using this criterion a prevalence of 3% was found among non-pregnant women 20-39 years of age. These findings are comparable to the 2.5% prevalence we found in our sample of women 1-year postpartum living in central Montreal.

Our findings are also similar to those of Bodnar *et al.* (2002) who studied the prevalence of iron deficiency among postpartum women in the United States. They found 4% and 2% prevalence of iron deficiency anemia among women 7-12 and 13-24 months postpartum respectively, using a criteria of serum ferritin < 12 μg/L, free erythrocyte protoporphyrin > 1.24 mmol/L and transferrin saturation < 15% (at least 2 abnormal) accompanied with a hemoglobin level < 120 g/L. Our findings are also comparable to those found in the NHANES 1999-2000 in the United States (CDC, 2002). In non-

pregnant women 20-49 years of age, the prevalence of iron deficiency anemia was 4%. This survey used the same criteria as Bodnar *et al.* (2002) to define iron deficiency anemia.

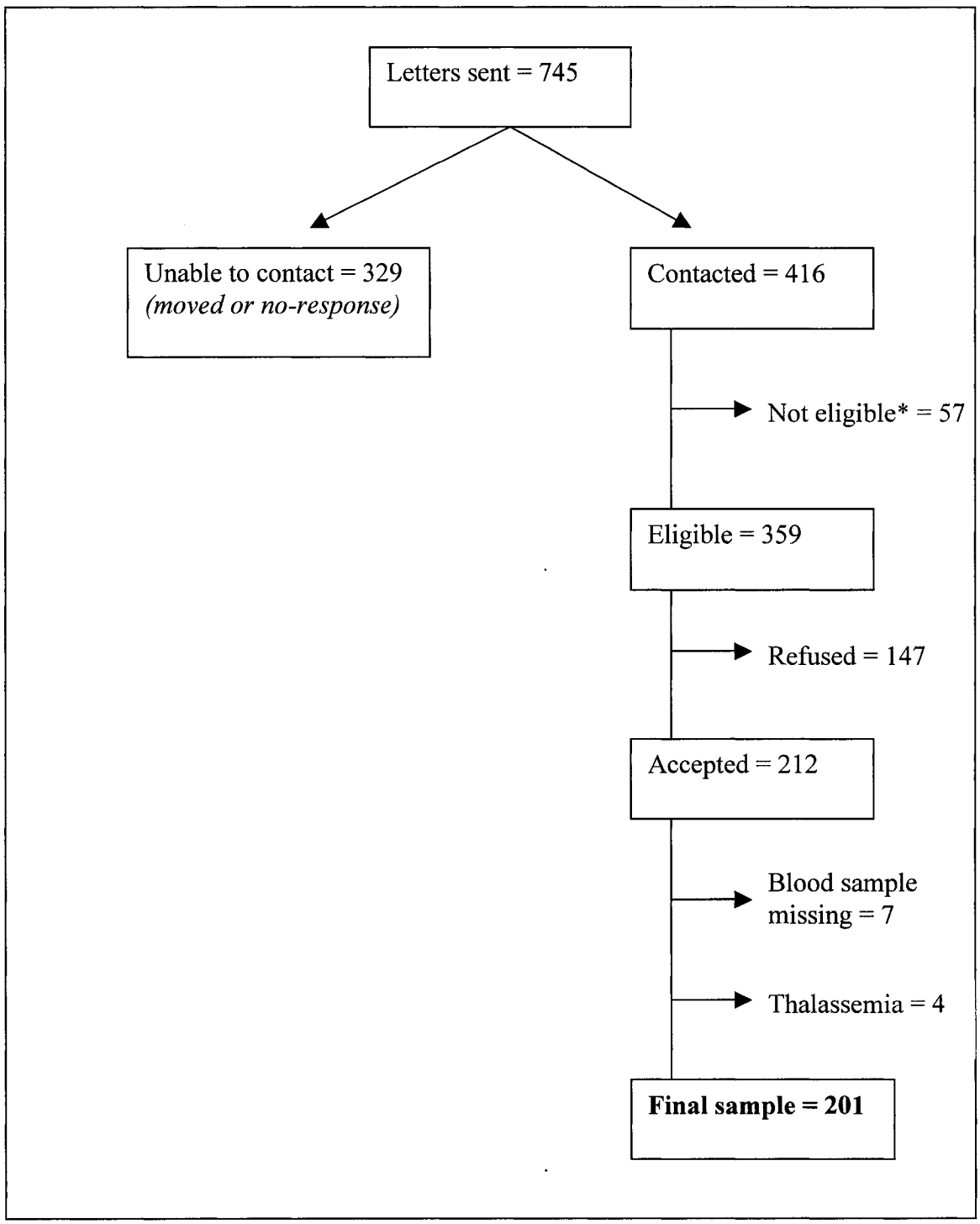
The limitation of the present study relates to the extent to which this sample is representative of postpartum women. The individuals we were unable to reach and those who refused to participate in the study may have differed from our final sample. According to demographic data of the four districts studied, the proportion of individuals living under the poverty line ranges from 46%-49% (*Direction de la santé publique* (DSP), 2004) and the proportion of newborns whose mother is born outside of Canada ranges from 21%-42% (DSP, 2003). In our sample, 53% of mothers lived below the poverty line and 34% were not born in Canada. Our sample therefore seems to be reflective of the population studied. A very small number of high-risk women were excluded: those who had given birth to a very low birth weight infant (7/1420 of the original list, 0.49%) and those who had two pregnancies within the year (26/416 of the women contacted, 6.25%). The majority of mothers sampled regularly ate heme iron sources such as meats, fish and poultry. The risk of iron deficiency may be different in vegetarian mothers and in Canada approximately 4% of adults follow a vegetarian diet (National Institute of Nutrition, 2002).

Although the presence of inflammation may confound certain iron status indicators (Yip & Dalman, 1988), this does not seem to have been a factor in our study. Inflammation levels were low in anemic mothers as indicated by C-reactive protein levels. C-reactive protein is an acute phase protein, which measures and reflects inflammation levels and values greater than 8 mg/L are considered elevated (ICSH, 1988). Our findings showed that no anemic mother had a C-reactive protein level greater than 8 mg/L and only one had a level greater than 5 mg/L. Furthermore, no anemic mother indicated to us that she had been sick two weeks prior to the blood sample collection. Chronic inflammatory conditions, however, cannot be ruled out since this indicator is less sensitive for detecting these conditions (ICSH, 1988).

We noted that dietary iron intakes were significantly higher in mothers with lower income levels. On the other hand, we also observed that the majority of mothers with low-income levels were not born in Canada. The higher iron intakes may therefore be reflective of the better eating habits of new Canadians.

The present study shows a clear picture of the state of iron deficiency anemia among women 1-year postpartum living in central Montreal. Although pockets of iron deficiency anemia may exist, this condition does not seem to be prevalent among women. Our sample included a large proportion of economically disadvantaged women and yet the occurrence of iron deficiency anemia was very low.

Figure 3.1 Flow chart of subject recruitment



* Reasons for non-eligibility: 23 women were pregnant, 18 spoke neither French nor English, 3 had given birth to twins, 3 had given birth to a second baby following the birth of their 10-15 month old child and 10 were not eligible for other reasons.

Table 3.1 Socio-demographic characteristics of 201 women 1-year postpartum living in central Montreal.

Characteristic	Value
Mean age (SD), yr	29.6 (5.38)
Racial origin [1]	
European %	71
African %	9
South-East Asian %	7
Other %	14
Born in Canada %	66
Maternal language	
French %	66
English %	7
Other %	27
Income source	
Full time work %	35
Part time work %	14
Unemployment insurance %	8
Social assistance %	23
Other support %	19
Unknown %	1
Low income cutoff [1]	
Above %	46
Below %	53
Unknown %	2
Mean number of years of schooling (SD)	13.9 (4.03)
Mean number of children (SD)	1.7 (1.14)

[1] Column does not add to 100% due to rounding.

Table 3.2 Results of blood analyses.

Measure	No. of mothers	Value		
		Mean (SD)	Median	Extremes
Hemoglobin, g/L	199	132.3 (9.1)	132.0	89.0, 161.0
Serum ferritin, μg/L	201	40.3 (29.7)	34.0	3.0, 209.0
Hematocrit, %	199	39.2 (2.5)	39.2	27.7, 46.9
Mean corpuscular volume, fL	199	88.7 (4.2)	89.1	73.0, 101.3
C-reactive protein, mg/L	200	3.8 (6.1)	1.8	< 1.0, 66.0

Figure 3.2 Distribution of hemoglobin levels and proportion of iron deficiency.

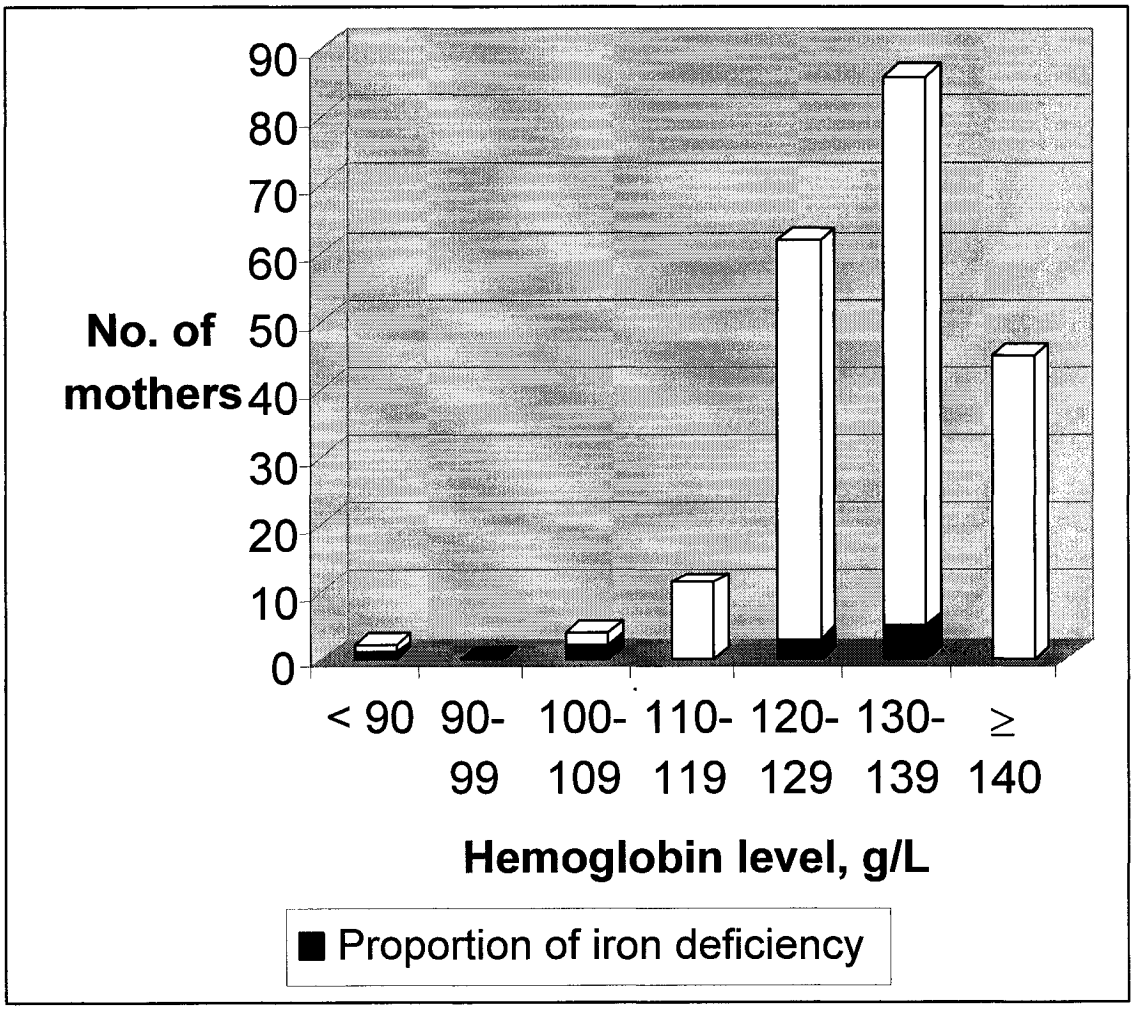


Table 3.3 Comparison of low hemoglobin concentration, iron deficiency and iron deficiency anemia rates by income level

	N[1]	% Low hemoglobin[2] (< 120g/L)	% Iron deficiency (SF < 12 µg/L)	% Iron deficiency anemia[2] (at least 2 of 3: Hb < 120g/L, MCV < 80fl, SF < 12µg/L)
Income below LIC[3]	106	10.48	8.49	3.81
Income above LIC[3]	92	3.30	2.17	1.10
P-value		0.06	0.07	0.38

[1] Three subjects were excluded from the analysis as income was unknown
[2] Hemoglobin value was missing for one subject in each of the income groups
[3] LIC=low income cutoff

Table 3.4 Mean heme, non-heme and total iron intake of mothers with hemoglobin (Hb) values below 120 g/L and equal or greater than 120 g/L.

Measure	Hb < 120 g/L	Hb ≥ 120 g/L	P-value
N[1]	14	185	
Heme iron[2], mg/day	1.02 (0.37)	1.22 (0.61)	0.08
Non-heme iron[2], mg/day	12.22 (4.12)	13.11 (5.12)	0.53
Total iron[2], mg/day	13.23 (4.28)	14.32 (5.29)	0.45

[1] Two subjects were excluded from the analysis as Hb value was missing
[2] Mean (Standard Deviation)

Table 3.5 Mean heme, non-heme and total iron intake of mothers with incomes below and above the low-income cutoff (LIC).

Measure	Income below LIC	Income above LIC	P-value
N[1]	106	92	
Heme iron[2], mg/day	1.23 (0.68)	1.11 (0.47)	0.031
Non-heme iron[2], mg/day	14.06 (5.87)	11.82 (3.53)	0.001
Total iron[2], mg/day	15.35 (6.09)	12.92 (3.57)	0.001

[1] Three subjects were excluded from the analysis as income was unknown
[2] Mean (Standard Deviation)

CHAPTER 4. SUMMARY

4.1. Main findings

The results of our study showed that the prevalence of iron deficiency anemia among women 1-year postpartum living in central Montreal was 2.5%. This prevalence is comparable to that found by Valberg *et al.* (1976) a generation ago with a sample of individuals that had participated in the Nutrition Canada national survey conducted between 1970-1972. Iron deficiency anemia, defined as a hemoglobin level below 120 g/L accompanied by a serum ferritin level less than 15 μg/L, was observed in 3% of non-pregnant women 20-39 years of age (N=100).

Our sample consisted of 201 postpartum women 19 to 45 years of age with 53% having an income level below the low-income cutoff established by the federal government. Our sample was therefore larger and included a greater proportion of economically disadvantaged women than the study by Valberg *et al.* (1976). The inclusion of a large proportion of women with low-incomes is noteworthy since such individuals may be at increased risk of iron deficiency (Bodnar *et al.*, 2002).

Similar results have also been observed in the United States. Bodnar *et al.* (2002) studied the prevalence of iron deficiency anemia among postpartum women and found a prevalence of 4% and 2% among women 7-12 and 13-24 months postpartum respectively. They defined iron deficiency anemia as a hemoglobin level less than 120 g/L accompanied by at least two of the following: serum ferritin level < 12 μg/L, free erythrocyte protoporphyrin > 1.24 mmol/L and transferrin saturation < 15%. Furthermore, iron deficiency anemia was found in 4% of non-pregnant women 20-49 years of age following the NHANES 1999-2000 (CDC, 2002). The definition criteria for the NHANES 1999-2000 study were the same as described above.

Caution should, however, be taken when comparing previous findings to our results since certain survey procedures and definition criteria differ from the present study.

The potential limitations of this study should be highlighted. First, the cross-sectional nature of this study limits our ability to determine cause and effect. Our sample consisted of women 1-year postpartum. Health care professionals would therefore have followed these women during their pregnancy and the importance of preventing anemia by having an adequate iron intake may have been discussed with them. Women who had suffered from anemia or iron deficiency in the past may also have been consciously consuming higher amounts of iron. The mother's diet may therefore have influenced the presence of anemia or, the reverse may also be true, anemia may have influenced the diet of the mother.

We must also consider the possibility of non-response bias in our sample. We were successful in recruiting more than 100 mothers who we considered to be economically disadvantaged and thus perhaps at greater risk of being iron deficient. Furthermore, the proportion of mothers living under the poverty line (53%) was similar to the proportion reported for the population in the four districts studied (46%-49%) (DSP, 2003). We do not, however, have any information on the mothers we were unable to reach or who refused to participate. We can therefore not establish with certainty that these individuals did not differ significantly from our final sample and that the prevalence of iron deficiency anemia would have been the same.

Finally, the majority of women in our sample regularly ate heme iron sources such as meats, fish and poultry. Heme iron is the most readily absorbable form of iron in the diet (Monsen *et al.*, 1978). The prevalence of iron deficiency and anemia may therefore differ in women following vegetarian diets or any other diet that limits the amount of highly bio-available iron sources from the diet. Prevalence may also differ in women with very close pregnancies and in those who have given birth to a very low birth weight infant since our sample excluded these higher-risk women.

4.2. Additional observations

Further observations were also noted but not previously discussed in the manuscript. When asked about supplement use, only 20% of mothers reported currently taking a multi-vitamin supplement and only 13% of these women took the supplement on a regular basis. This number is quite low considering that 72% of mothers indicated having regularly taken a multi-vitamin supplement during their pregnancy and another 10% reported having taken such supplement occasionally. In addition, 23 of the women we contacted to participate in our study had become pregnant again within 15 months of giving birth.

While most women seem to be aware of the importance of multivitamin supplementation during pregnancy, its importance during pre-pregnancy does not seem to be as well known. Public health messages focussed on this issue may therefore be warranted to further prevent neural tube defects.

4.3. Conclusion

The prevalence of iron deficiency anemia is low among women 1-year postpartum living in central Montreal. Iron deficiency is not of concern in these postpartum women and efforts should not be directed to this problem. The low rate of anemia among women is consistently seen in North-American surveys. We can therefore conclude that in a well-nourished group, anemia is not related to diet since the body has the capacity to regulate iron absorption. Although pockets of iron deficiency anemia may exist, this condition does not seem to be prevalent among most Canadian women.

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APPENDICES

McGill

Ms «FirstName» «LastName»
«Address1»
Montreal, PQ «PostalCode»

Dear Ms «LastName»:

Ten years ago, we studied anemia (which means low iron in the blood) in your area of Montreal. We found that a large number of infants were anemic. Programs were subsequently put in place to help reduce the number of anemic infants. We would now like to know if these programs have been successful, and if not, we would like to find ways to help reduce anemia. Anemia, or lack of iron in the blood, can affect the mental and physical development of children and may reduce mom's energy level. For these reasons, we are conducting this study and we hope very much that you will agree to participate.

Your child's name was obtained from a list of registered births. Diane Major, a registered nurse, will contact you and ask if she may interview you in person at your home or any other location, at a convenient time, and this will approximately take 30 minutes. Diane will ask about your child's food intake as well as your own food intake. Finally, the nurse will prick your child's finger and draw a drop of blood to find out if he/she is anemic. She will also draw blood from you to determine if you are anemic. We will inform you of the results of the tests, and if the results indicate anemia we will contact you for follow-up. All information collected will be kept confidential. To show our appreciation, a small gift will be given to you upon completion of the interview.

We thank you very much for considering this important request.

Sincerely,

Stephen DiTommaso, MD CMFC(F)
Assistant Professor
Head-Family Medicine Unit
CLSC des Faubourgs
Messages : 514-527-2361

(français au verso)

INFORMED CONSENT

Prevalence & dietary predictors of anemia in mothers and one-year old infants living in inner city Montreal

Principal Investigator:
Stephen DiTommaso, MD CMFC (F), CLSC des Faubourgs, 1705 Visitation, Montreal, QC, H2L 3C3, Tel.: (514) 527-2361

Research Nurse:
Diane Major, RN, CLSC des Faubourgs, 1705 Visitation, Montreal, QC, H2L 3C3, Tel.: (514) 527-9565

This study is funded in part by the Beef Information Centre.

Introduction

The purpose of this study is to look at prevalence and dietary predictors of anemia in mothers and one-year old infants. One of iron's many functions in the body is to transport the oxygen we need to carry out daily activities. If a person does not have enough iron in his or her blood, **iron deficiency anemia** can develop. This can reduce their ability to work and can affect the mental and physical development of children. Young children and women are particularly vulnerable because of their increased requirements for iron.

Study Procedure

You are being asked to take part in a study that will evaluate your and your baby's iron level. If you agree to take part in this study, a nurse will visit you and you will be asked to answer a questionnaire about your child's health as well as his/her feeding practices since birth. The nurse will also ask you about your child's food intake and your own food intake. You will be asked to answer a food frequency questionnaire that will help us assess iron intake. Finally, the nurse will draw blood from you and your child. A capillary blood sample by finger prick will be obtained from your child and a venous blood sample will be obtained from yourself.

Benefits and Risks

Drawing blood may include temporary discomfort from the needle prick, bruising, and rarely, infection. The blood sample will allow us to determine whether you or your child is anemic. A letter will be sent to you with the results of this test. If values indicate anemia, we will contact you for follow-up. Your and your baby's name will only be known to the research nurse and will be deleted from the database once all data have been collected and verified.

Withdrawal from Study

Should you wish to discontinue your and your baby's participation at any time, you are completely free to do so without any consequences to yourself or your baby. This research is completely voluntary and will help us find ways of preventing iron deficiency anemia among mothers and young children.

Compensation

To compensate you for your commitment and to show our appreciation, you will receive a small gift.

Signature

The study has been explained to me and my questions have been answered to my satisfaction. I agree to participate and have my baby participate in this study. I also agree to have a blood sample drawn from myself and from my child. I may contact the research nurse or the principal investigator at any time for questions or concerns.

_____

Signature

Witness _____ Date _____

Infant ID # _____

Mothers ID # _____

Home Visit

Identification No. _____

Interview Date (dd/mm/yy) _____

Sex of the child M/F_____

If no interview took place – indicate the reason.

I would like to ask you some questions about your child's eating habits.

What type(s) of milk does *(name)*_____ drink at present?_____

Did you breast-feed *(name)*_____? 1. Yes ____ 2. No ____

If yes, for how long? (up to what age?) From birth to _____ months

Since birth, has he/she had other types of milk? 1. Yes ____ 2. No _____

Start with the most recent, indicating the type of milk (iron fortified or not) and the reason for discontinuing this type of milk.

Milk	**Age (months)**		**Reason(s) for Stopping**
	Start	**End**	
Present		_____	_____

Are you aware of the program in which you can receive infant formula at reduced cost or extra money if you are breast-feeding your child?
1.Yes____ 2. No ____ 3. Not sure ____

Did you get infant formula at a lower cost?
1. Yes ____ 2. Yes, for a short while ____ 3. No ____

If yes, did you have any problems getting milk from this program?
1. Yes, regularly ____ 2. Yes, sometimes ____ 3. No ____

If yes, explain _____

Identification No. _____

Was your child sick yesterday? 1. Yes ____ 2. No ____

If yes, ask about usual milk consumption

How much milk did he/she drink in the last 24 hours?
(Ask to see the bottle or glass to measure the quantity of milk. Indicate quantity of diluted milk)

Hour	Quantity/Breastfed	Hour	Quantity/Breastfed

Total quantity (in 24 hours): _____ ounces _____ ml _____

At what age did you start giving your baby:

A) Infant cereals: _____ months

What type:
1. Heinz _____
2. Pablum _____
3. Nestlé _____
4. Milupa _____
5. Other _____ Brand _____

Does your child still eat infant cereals (including Nutrios)?
1. Yes _____ 2. No _____

If continues to eat infant cereal ask:
How many **days per week** does your child eat infant cereals at present?

Frequency : _____

How many **times per day** does your child eat infant cereals at present?

Frequency : _____

If no longer feeding infant cereal ask:
At what age did your child stop eating infant cereal? _____ months

Identification No. _____

At what age did you start giving your baby:

B) Meat: _____ months

How many times a **week** does your baby eat:

Meat, chicken or fish _____ frequency

Eggs _____ frequency

Beans, lentils _____ frequency

Peanut butter _____ frequency

Cheese _____ frequency

Does your child ever drink tea or coffee? 1. Yes ____ 2. No _____

If yes, how many times a week? _____ frequency

Type: 1. Tea ____ 2. Coffee ____

Since what age? _____ months

Is your child taking or has your child taken vitamins or iron supplements (in drops or pills)?

(If so, note parameters or iron drops)

Supplement	**Quantity**	**Age (months)**		**Reason for stopping**
		Start	**End**	
Present			_____	_____

Identification No. _____

Can I ask you a few questions about yourself?

Are you currently pregnant? 1. Yes ____ 2. No ____
(if yes exclude from study)

During your pregnancy did you take an iron supplement?
1.Yes (regularly) ____ 2. Yes (sometimes) _____ 3. No _____

Are you now taking an iron supplement?
1.Yes (regularly) ____ 2. Yes (sometimes) _____ 3. No _____

During your pregnancy did you take a multi-vitamin supplement?
1.Yes (regularly) ____ 2. Yes (sometimes) _____ 3. No _____

Are you now taking a multi-vitamin supplement?
1.Yes (regularly) ____ 2. Yes (sometimes) _____ 3. No _____

During your pregnancy did you take a vitamin C supplement?
1.Yes (regularly) ____ 2. Yes (sometimes) _____ 3. No _____

Are you now taking a vitamin C supplement?
1.Yes (regularly) ____ 2. Yes (sometimes) _____ 3. No _____

Did you deliver your baby by cesarean section? 1. Yes ____ 2. No ____

Did you experience complications after delivery such postpartum hemorrhaging?
1. Yes ____ 2. No _____

Have you been using birth control since giving birth? 1. Yes ____ 2. No ____

If yes, what type?_____

How long after giving birth did you begin menstruating again?
_____ months _____ menses have not yet returned

Do you currently smoke cigarettes? 1. Yes ____ 2. No ____

On average, how many cigarettes do you smoke each:
Day _____
Week _____
Month _____

I would now like to ask you a few questions about your food intake.
(Food Frequency Questionnaire)

Identification No. _____

Iron food frequency questionnaire

Please think about the foods you have eaten during the last month including foods and beverages that were part of meals and snacks, at home and away from home.

Food Frequency List

Describe how often you eat each of the following foods during a **typical** week (7-day period).

	Almost never	**1-2 per week**	**3-4 per week**	**5-6 per week**	**Daily**	**More than once daily (How often?)**
Cold cereals*(Cheerios, Cornflakes,Fruit loops, Rice Krispies,Special K, Mini Wheats)*						
Cold cereals *(All Bran, Bran Flakes, Frosted Flakes, Lucky Charms, Raisin Bran, Shreddies, Weetabix, Just Right)*						
Granola with raisins						
Cream of wheat or oatmeal, instant						
Cream of wheat or oatmeal, regular						
Pancakes, muffins						
Bread *(white, whole wheat or raisin)*, rolls, pita bread, crackers						
Bagel						
Noodles, spaghetti, macaroni, Kraft dinner						
Potatoes, french fries						
Baked potato with skin						
Peas						
Cooked spinach						
Tomato sauce for spaghetti						
Broccoli						
Green peppers						
Orange, grapefruit or kiwi						
Beef or lamb						
Veal or pork						
Chicken or turkey						
Liver						
Cold cuts, sausages,						
Fish fillet, fish sticks, canned tuna or salmon, shrimp, sardines						

	Almost never	1-2 per week	3-4 per week	5-6 per week	Daily	More than once daily (How often?)
Baked beans with pork						
Other types of beans *(chickpeas, black beans, kidney beans, lentils)*						
Tofu						
Eggs						
Submarine sandwich, hamburger, cheeseburger, hot dog, breaded fish fillet, chicken pieces, pizza						
Coffee or tea						
Juice *(orange, apple,tomato, grape)*						
Instant breakfast or malted milk						
Dried fruits *(apricots, prunes, figs, raisins)*						
Nuts *(walnuts, mixed nuts, almonds,cashews or peanuts)*						

Identification No. _____

How old are you? _____ years

Were you born in Canada? 1.Yes ____ 2. No. Specify (country) _____

What is your mother tongue? 1. French _____
2. English _____
3. Other, specify _____

How many children have you given birth to? _____

Your last child before *(name)* _____ was born when? ____ _____ ____
Day / Month / Year

Race of the child		**Race of the mother**	
Caucasian		Caucasian	
Black		Black	
Asian		Asian	
Other		Other	

How many years of school did you complete? _____years

What is your employment status? Full-time work _____
Part-time work _____
Not working _____

If not working ask:
What is your source of income? Unemployment insurance _____
Social assistance _____
Other _____

If not on social assistance ask:
How many children and how many adults live with you? _____children
_____adults

Is the family income (before taxes) above:

Number of residents	Yearly income	
2	$ 23,500	_____
3	$ 29,500	_____
4	$ 35,500	_____
5	$ 39,500	_____
6	$ 44,000	_____
7 or more	$ 48,000	_____

Identification No. _____

Could I see your child's "Carnet de Santé"?

Weight at birth _____ (g) / _____ (lbs)

Most recent weight _____ (g) / _____ (lbs)

Date ____ Day / _____ Month / _____ Year

Was your child born prematurely? (less than 37 weeks gestation)
1. Yes ____ 2. No ____

The laboratory tests will be done at Saint-Luc Hospital

Has your child ever been to this hospital? 1. Yes ____ 2. No ____

If so, could I have the Hospital Card Number so that the results can go in your child's file?
1. Yes ____ 2. No ____ Card number _____

Have **you** been sick during the last 2 weeks? 1. Yes ____ 2. No ____
If yes, specify _____

Was your **child** sick during the last 2 weeks? 1. Yes ____ 2. No ____
If yes, specify _____

Have **you** ever been treated for anemia? 1. Yes ____ 2. No ____
If yes, explain (when, treatment, resolved...)

Has your **child** ever been treated for anemia? 1. Yes ____ 2. No ____
If yes, explain (when, treatment, resolved...)

Have **you** ever been diagnosed with any blood disorder such as Sickle cell anemia or Thalassemia? 1. Yes ____ 2. No ____

Blood Sample

Thank you very much for your help! You will receive the results from us very soon.

Identification No._____

Laboratory results:

Mother		**Baby**	
Hemoglobin level		Hemoglobin level	
Mean corpuscular volume (MCV)		Mean corpuscular volume (MCV)	
Serum ferritin level		Serum ferritin level	
Hematocrit		Hematocrit	
C-reactive protein (CRP)			

Notification of results:

Mother	**Baby**		
____ normal	____ normal	letter sent	_____ (date)
____ mild anemia	____ mild anemia	letter sent	_____ (date)
____ anemia	____ anemia	telephone call	_____ (date)

McGill

Ms «FirstName» «LastName»
«Address1»
Montreal, PQ «PostalCode»

Dear Ms «LastName»:

On behalf of the research team, I would like to thank you for your participation in our study of iron deficiency anemia in mothers and one-year old infants living in inner-city Montreal. We greatly appreciated your time and co-operation. The information collected will not only help us determine the rate of iron deficiency anemia, but will also give us good indicators of solutions to this problem.

The laboratory results from the blood taken from you and your baby were the following:

	Mom's value (Normal Range)	**Baby's value** (Normal Range)
Hemoglobin	«value» g/L (120-150)	«value» g/L (115-155)
MCV	«value» fL (78-98)	«value» fL (70-86)
Serum Ferritin	«value» µg/L (12-200)	«value» µg/L (12-140)

Comment:

_____

_____

_____

These tests were part of a research study and should therefore be interpreted with caution. If you think that you or your child might be sick, we strongly suggest seeing your family physician.

Sincerely,

Stephen DiTommaso, MD CMFC(F)
Assistant Professor
Head-Family Medicine Unit
CLSC des Faubourgs
Messages: 514-527-2361

January 11, 2005

To Whom It May Concern:

The purpose of the present letter is to confirm that the co-authors (Stephen DiTommaso, François Lehmann and Katherine Gray-Donald) agree that the candidate (Patricia Murphy) includes the manuscript entitled *Low rates of iron deficiency anemia in women 1-year postpartum* in her thesis.

The candidate's roles in this study included obtaining data through the *Commission d'accès à l'information du Québec* and the *Régie régionale de la santé et des services sociaux Montréal Centre* for recruitment purposes, organizing the data files, preparing mail-outs of letters of invitation and thanks, supervising the research nurse and training her in dietary measurement, developing the study questionnaire, compiling the data and conducting the analyses. The candidate wrote the manuscript under the guidance of the co-authors and made modifications to it in response to their comments.

Patricia Murphy

I, the co-author, agree that the candidate, Patricia Murphy, include the manuscript entitled *Low rates of iron deficiency anemia in women 1-year postpartum* in her thesis.

See Attached
Stephen DiTommaso

See Attached
François Lehmann

See Attached
Katherine Gray-Donald

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