A STUDY OF DIETARY AND NON-DIETARY FACTORS OF IRON DEFICIENCY ANEMIA IN HIV-POSITIVE WOMEN

by

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ABSTRACT

Anemia is one of the most common co-morbidities in human immunodeficiency virus (HIV), where approximately 50% of cases are attributed to iron deficiency anemia (IDA). IDA results from chronic inadequate iron supply to the body, whether through increased losses or decreased iron availability. In HIV, iron metabolism is greatly disrupted due to direct impacts of the virus, and therefore, prevalence of IDA is greater as compared to other populations. Women are at particularly high risk due to decreased dietary iron intakes, and increased losses due to menstruation and pregnancy. This study set out to determine the prevalence of anemia, iron deficiency and IDA, associated risk factors, and dietary iron intake patterns in a sample of HIV-positive women. Moreover, a newly-designed dietary survey was assessed for its ability to predict iron status in this population. It was found that the majority of participants had sub-optimal dietary iron intakes, with most dietary iron obtained from grain products. Prevalence of anemia, ID, and IDA were 30%, 40%, and 16%, respectively. Predictors for IDA in this sample were immune status (CD4<200 cells/μL); regular menstruation pattern; and African ethnicity. Food insecurity was quite prevalent among participants of Aboriginal and African ethnicity, with food bank use predominating as the main means of food assistance. Routine screening of iron status parameters, implementation of multivitamin use among all women with HIV, and dietary education regarding iron food sources is necessary to eliminate IDA in this population. Women with HIV should be engaged in self-management of their nutritional health, and community programs should be established that foster educational opportunities, rather than solely providing emergency food assistance.

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INTRODUCTION

1.1 Review of HIV-Associated Anemia

Anemia, defined as a hemoglobin concentration <120g/l in women and <140g/l in men, is the most common co-morbidity associated with HIV infection, with a prevalence up to 30% in the asymptomatic stage, and greater than 80% in those with advanced AIDS (1,2). There appears to be an inverse relationship between anemia prevalence and HIV disease stage, with anemia rates increasing four- to nine-fold as CD4 cell counts decrease to <200 cells/μL (3). Iron deficiency (ID) without anemia represents a moderate depletion of iron stores, without the reduction in hemoglobin concentration. Iron deficiency anemia (IDA) is a sub-type of anemia, resulting from chronic, inadequate iron supply or increased iron losses, leading to a reduction in concentrations of reticulocytes, hemoglobin, hematocrit, as well as storage iron markers such as ferritin and transferrin saturation. It is characterized microscopically by the presence of small (microcytic) and pale (hypochromic) reticulocytes (4). Thus, IDA represents the extreme end of the spectrum of ID, where body iron stores are significantly depleted in the presence of anemia (5-7).

According to the statement of Anemia in HIV Working Group, the major cause of anemia in persons with HIV is blood loss, often associated with complications of the virus, as in neoplastic disease (Kaposi sarcoma), gastrointestinal lesions, or cytomegalovirus (8). Women show higher prevalence of anemia (55%) compared to men (45%) during the course of HIV infection, where this difference relates to greater losses in women due to menstruation and pregnancy (5, 8, 9). Other causes of anemia are related directly to the adverse impacts of HIV on cellular systems. These include bone marrow suppression, thus impairing erythropoietin production and red blood cell proliferation; disruption of epithelial cell replication, thereby reducing gastrointestinal absorption of nutrients such as iron, folic acid, and B12; and the compounding effect of chronic inflammation which leads to anemia of chronic disease (2, 8, 9). Untreated anemia has been shown to have deleterious impacts on quality of life, HIV disease progression, and overall survival (1, 3, 8, 10).

Several studies have shown that, in individuals with untreated anemia, mortality rates are six times greater than in persons with normal hemoglobin levels (2, 10, 11). However, one

could hypothesize that, in light of the great developments in highly active antiretroviral therapy (HAART) over the past decade, improvements in immune status would be reflected in a drop in anemia prevalence. This, however, is not the case, and the prevalence of anemia has remained relatively unaffected despite such advancements, as well as improved access to HAART (8, 12). This phenomenon suggests that anemia is not only a medical co-morbidity of HIV, but also a reflection of social inequities and continued poor access to health care services by certain groups within the population. Women, in particular, may fall into this category, since they are the group with the greatest prevalence of anemia, and are also likely to face greater social and economic barriers when it comes to seeking treatment and care (12, 13). It is also likely that among those women who are engaged in care for their HIV management, screening for anemia may not always be a routine procedure, thereby exacerbating the impact of this condition (11).

1.2 Independent Risk Factors Associated with Anemia in HIV

As identified by the Anemia in HIV Working Group, risk factors for anemia include increasing age, African American ethnicity, female sex, low body mass index (BMI), history of pneumonia or fever, oral candidiasis, CD4 count <200 cells/μL, plasma viral load, and zidovudine (AZT) use (8). Several longitudinal studies have been conducted in the United States to determine the prevalence, incidence, and risk factors for anemia in HIV-positive women (1, 9, 14, 15).

One of the first studies to address prevalence of anemia in HIV-positive women was the Women's Interagency HIV Study (WIHS), a multi-centre prospective study of HIV disease progression in women in the United States, which was conducted from 1994 to 1995. It enrolled 2056 HIV-positive and 569 HIV-negative women from six cities (clinics, street outreach, referral from other studies, or word of mouth) across the United States (1). Risk factors for anemia in HIV-positive women were identified as black ethnicity, higher viral load, AZT use, mean corpuscular volume (MCV) <80 fl, and lowered CD4 counts, where an inverse relationship between hemoglobin level and CD4 count was elucidated (1). Although a high proportion of women, irrespective of HIV status, showed sub-optimal MCV levels, this study did not address "non-HIV-related factors", such as nutrition, but did indicate that dietary factors may play a role in the persistence of anemia among women (1).

A similar study to WIHS, the Human Immunodeficiency Virus Epidemiology Research (HER) study in the Eastern United States, a multi-centre cohort study from 1993 to 2000 and including 871 HIV-positive and 439 negative women, found that the prevalence of anemia at enrollment was 28% in HIV-positive women (74% at the 5-year follow-up) compared to 15% and 48% in HIV-negative controls, thus supporting the notion that HIV-positive women are at greater risk for anemia than their counterparts (9). In terms of risk factors, significant predictors of anemia in HIV included increasing age, African American race, CD4 lymphocyte count <200 cells/μL, zidovudine (AZT) use, weight loss, fever $>38^0$ for >2 weeks, diarrhea, oral candidiasis, *Mycobacterium avium* complex infection, bacterial pneumonia, and *Pneumocystis carinii* pneumonia (9). A possible explanation for a greater proportion of African American women with anemia may be related to the presence of inherited hematologic conditions, such as sickle cell anemia or thalassemia, but dietary factors and socio-economic may also be involved (8). Important to note is that study participants for both the WIHS and HERS were recruited from inner-city clinics, so there may have been a sampling bias in recruiting women who were at high risk for complications associated with HIV.

The use of alcohol, injection drugs, crack cocaine, or cocaine was not associated with increased anemia risk, which may be explained by disruptions in menstrual cycle that have been reported with the use of injection drugs (9, 16, 17). Protective factors for anemia included higher body mass index, use of marijuana, and HAART (9). Specifically, use of HAART for one year showed a 32% improvement in hemoglobin levels, while anemia prevalence among those on non-HAART antiretroviral therapy or no treatment did not change (18). However, these findings may be related to reporting bias in self-reported medication doses, such that over-reporting of dosages led to a shift toward observing a significant difference between anemia prevalence and HAART therapy.

Although the WIHS and HERS were able to identify major risk factors in the development of anemia, they did not explore the prevalence of ID and IDA, or the role of nutritional and social factors. Thus, further research is warranted in determining the degree to which other factors, such as nutritional and micronutrient status may be implicated in the

persistence of anemia, despite the beneficial impacts of HAART therapy on gastrointestinal absorption, immune status, and anemia of chronic disease (18).

Another major study on anemia in HIV was the AIDS Linked to Intravenous Experiences (ALIVE) study in Baltimore, Maryland, spanning from 1988 to 1994, and consisting of a large cohort of 2960 intravenous drug users (IDU), both HIV-positive and negative, who were followed every six months for health-related outcomes (5, 14, 19). In a sub-study of anemia and ID among 136 HIV-positive and 61 HIV-negative women, it was found that ID affected 40% of female IDU, irrespective of HIV status, and that IDA contributed to half of the prevalent anemia cases among women (14). Although the use and role of iron supplementation was not addressed in this study, it was suggested that iron supplementation be approached with caution in view of the risks for iron overload and hepatic complications associated with excess iron in hepatitis C virus (HCV) ((14, 20). Moreover ID and IDA in this population are likely caused by a multitude of predictors, such as poverty and poorer health outcomes due to substance use, which cannot be addressed by supplementation alone, and that improved access to food, health and nutrition education, and individualized support are needed in order to eradicate these persistent deficiencies.

A sub-sample of the ALIVE cohort was studied for risk factors implicated in the etiology of ID and IDA, and it is one of the first studies to attempt to characterize the predictors for IDA and IDA among female IDU and women with HIV (5). Of 200 women enrolled in this sub-study (134 HIV-positive, 66 HIV-negative), 37% had ID and 16% had IDA, and were characterized as being younger in age, having a positive IDU history, a CD4 count <200 cells/μL, and lower likelihood of HCV positive status (5). The notion that HCV status could be a protective agent in IDA development may be related to ferritin levels, which are often higher in the presence of HCV and thus may affect the accurate diagnosis of IDA when using ferritin as a marker (5). Again, this study did not explore the dietary factors involved in the precipitation of ID and IDA (5). Thus, each of these studies helps support the multi-factorial nature of anemia, ID, and IDA, the pressing need for prompt diagnosis through screening, as well as a new direction for public health initiatives to address social, nutritional, and lifestyle factors that are implicated in anemia, ID, and IDA etiology. Response measures need to keep in mind that it may not be sufficient to treat anemia in HIV

with HAART and iron supplementation only, but that the involvement of women in their care through ongoing education, engagement, and monitoring is also essential.

1.3 The Impact of ID and IDA in HIV

ID is the result of long-term negative iron balance and, although IDA is the most advanced form of ID, adverse impacts on functional capacity and energy level have also been demonstrated in women with milder ID cases (12, 21). For example, ID and IDA can affect cognitive performance and behavior, immune status and morbidity, and physical capacity and functional energy to perform at work and school (12, 21). In fact, there appears to be a proportional relationship between hemoglobin concentration and fatigue, such that those who have IDA are affected to a much greater degree by the consequences of their deficiency than those with ID (12).

In particular, women of reproductive age have a higher risk of developing IDA due to menstrual blood losses or pregnancy (21). It has been shown that, among women who are HIV-negative, the prevalence of ID in reproductive age is almost 20%, whereas IDA prevalence is only 3-5% (21-23). In comparison, women with HIV who inject drugs have a 38% prevalence of ID and a 20% prevalence of IDA (14). Prevalence studies in women who do not use injection drugs have not been conducted; thus, estimates are not available.

In addition to HIV status as a significant risk factor in IDA, social and economic factors may also contribute. Thus food insecurity, a critical aspect of poverty, and defined as the absence, limitation, or uncertainty of the availability of nutritionally adequate, safe foods in a personally acceptable manner through socially acceptable methods, may drive IDA in this population (13). In fact, as shown in a study of HIV-positive individuals living in British Columbia, women with HIV were more likely to experience food insecurity, particularly if they were younger in age, of Aboriginal ethnicity, if they lived with children, had unstable housing, lower education level, or a history of injection drug use, as compared to women in the general Canadian population (13). As women are often caregivers for their children, they tend to experience poorer nutritional outcomes if the household is food insecure, as women tend to feed their children first, thereby forgoing their own nutritional needs (24).

Semba has described a model for the relationship between IDA and poverty, which is illustrated as follows (12):

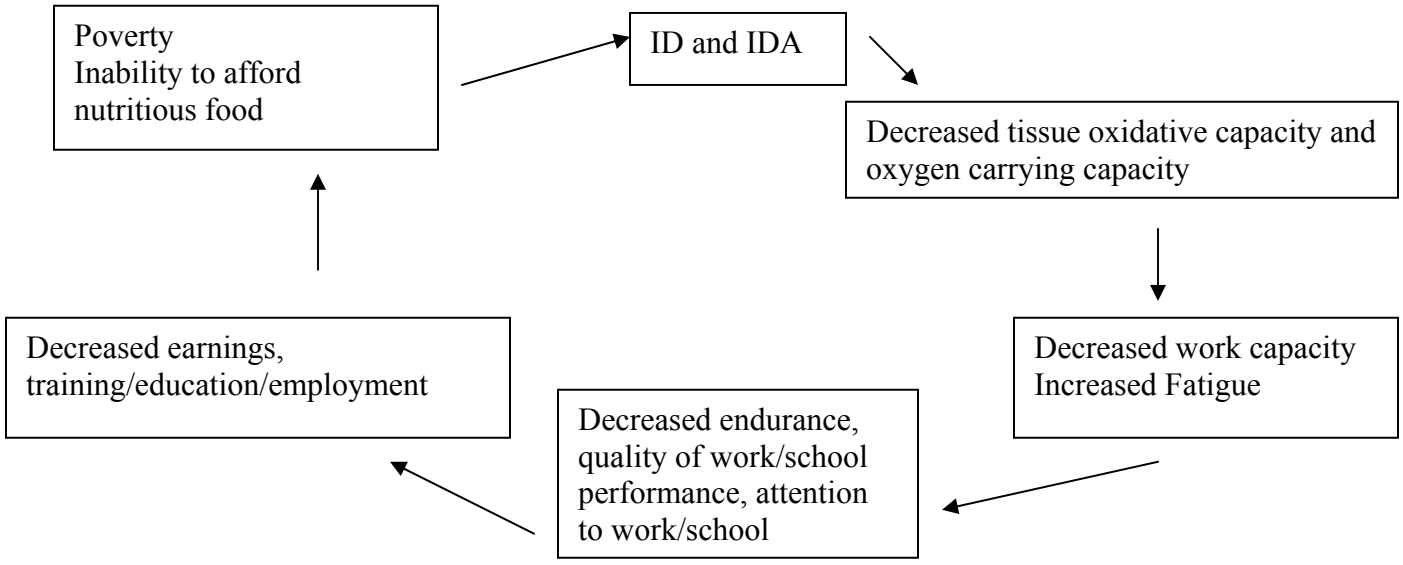

Figure 1.1 Iron Deficiency Anemia and the Cycle of Poverty (12)

As described in Figure 1.1, the presence of untreated IDA reduces functional capacity of women by affecting energy levels and cognitive performance, thereby impeding their educational and professional development, and their ability to obtain better paying jobs and acquire further educational training. In turn, the state of poverty is exacerbated, thus perpetuating the cycle of marginalization (12, 13). Thus, the impact of dietary, social and lifestyle factors associated with IDA in HIV-positive women requires further exploration, especially to quantify the impact of nutrition and food insecurity on IDA in a population that is already faced with the challenges of chronic disease management, substance use, and social stigma and marginalization.

1.4 Diagnosis of IDA in HIV

Although the definition for anemia based on laboratory markers has been established by the World Health Organization (WHO) (hemoglobin concentration <120g/L in women), there has been little consensus of which biochemical markers should be used for assessment of iron status in persons with HIV, especially in view of the fact that ferritin is also an acute phase reactant protein that rises in the presence of inflammation (25-27). Since HIV directly

activates the immune system, thereby inducing inflammation, the elevation in ferritin levels will often mask depletion in iron stores (26, 27). Serum iron is also frequently low in HIV infection, but this is a reflection of anemia of chronic disease rather than true iron deficiency, so cannot be used as a reliable indicator of IDA in HIV (11, 27). Therefore, it becomes important to distinguish between types of anemia that result from iron deficiency and those which are related directly to the effects of HIV (11). Specifically, HIV-associated anemia is similar in presentation to anemia of chronic disease, with lowered serum iron levels, normal to elevated ferritin levels, normal levels of transferrin and transferrin saturation, and normocytic, normochromic reticulocytes (2, 11).

Given masking of IDA through the impact of HIV on metabolism, the gold standard in diagnosis of IDA in HIV has been a bone marrow aspirate (2, 26). When complete blood count, ferritin, and serum transferrin receptor (TfR), a stable marker of iron status not affected by inflammation, were compared to the results from the bone marrow aspirate, the sensitivity of the blood tests was 20% and specificity 93% (26). TfR is an expensive laboratory test rarely available in hospital. Since its use does not appear to improve diagnostic accuracy, no single test of iron status has been found to be accurate enough to be clinically useful (26). It is important to note, however, that although no single test can be used to determine IDA diagnosis, it may be feasible to use a combination of iron markers, such as ferritin and transferrin saturation, to confirm a diagnosis of IDA (25). What is likely not to be feasible is the collection of bone marrow aspirates for the sole purpose of diagnosing IDA, since such a procedure is labor-intensive, requires skilled staff members to perform it, and above all, is highly invasive to the patient (26).

In general, the diagnosis of IDA in HIV in North America is defined by a plasma ferritin concentration of <30μmol/L and hemoglobin concentration <120g/L, and the effect of inflammation on ferritin levels is accounted in part by raising the ferritin cut-off for IDA from 12μmol/L to 30μmol/l (5, 6, 14). In turn, patients with serum ferritin levels >100 μg/L are not likely iron deficient, and supplements containing iron are contraindicated in view of the potential for iron overload (2, 12). In some cases, C-reactive protein (CRP) is simultaneously measured to determine whether inflammation is present, but it is not clear

how ferritin levels should be corrected based on the magnitude of inflammation, as reflected in the CRP (28, 29).

1.5 Nutrition and IDA

Dietary iron intake plays an important role in iron status, since those who consume adequate amounts of iron are less likely to develop IDA, and those with greatest iron deficits such as women of childbearing age and vegetarians are at highest risk (30, 31, 31). To date, there have been no studies reviewing iron intake in HIV-positive women, but extensive research has been conducted in the HIV-negative population. Most studies were unable to show a direct relationship between iron intake and iron status, likely in view of the complexity of iron availability from food, differences in absorption efficiency based on meal composition, individual iron status, and random errors in laboratory measurements and dietary intakes (23, 32) Thus, to evaluate accurately the effect of diet on iron status, information on both iron intake, iron status, and absorption modifiers are needed (23, 33).

In a study of European HIV-negative women, it was found that there were no significant differences among quartiles of differing iron intake with respect to iron status, indicating that the relationship between iron intake and status is likely not proportional (21). These results are also supported by a study by Zhou et al., who found that dietary intake estimated from an iron specific checklist was unable to predict iron status in pregnant and post-partum women (34). The major reason for such findings is related to the low and variable bioavailability of iron that depends on the chemical form, either heme (animal sources) or non-heme (plant sources), with iron from animal products having higher absorption rate (25-30%) than iron from plants (5-15%), as well as dietary components that either enhance or inhibit iron absorption (21, 23). Correction for such factors is often a difficult and labor-intensive task, as total meal compositions are required, and participants are asked to weigh and record all food taken over a given period of time, a task that is subject to a high degree of non-compliance (35, 36). Even if diet records were complete, absorption capacity is dependent on the individual's current iron status: in cases of ID or IDA, absorption efficiency of iron increases several-fold compared to a state where iron status is at or near normal (4, 21, 23, 37). This leads to marked individual variability in assessing iron absorption from meals, whereby differences in individual absorption rates confound the

relationship between iron intake and iron status, making it difficult to assess whether it was the meal composition or the person's iron status that lead to the observed outcomes (32).

Due to dietary factors influencing iron absorption, it is even more difficult to estimate dietary iron intakes and requirements for persons with HIV, who have higher nutritional needs in view of the direct effects of the virus on metabolism and gastrointestinal absorption, and who may, in turn, have higher iron needs compared to HIV-negative individuals (38, 39). Although general dietary reference intakes (DRI) for most populations have been established, a specific DRI for iron in persons with HIV does not yet exist. For women of childbearing age, the DRI for iron is 18mg/d, while iron requirements for vegetarians are twice this level, in view of poorer iron absorption efficiency from plant-based foods. As this is the highest DRI level compared to iron needs of other groups, it can be considered the required level at which the majority of individuals in the population would be meeting their iron needs, and thus, will be used as the DRI for women with HIV, a group that is considered at higher risk for IDA compared to other groups in the population.

As mentioned, dietary iron intake patterns of women with HIV have not previously been studied, but have been assessed for HIV-negative women. A European study has shown that women obtain the majority of dietary iron from grain products, whereby breakfast cereals, breads, crisp breads, and crackers comprised 31% of dietary iron (21). In comparison, meat and meat products comprised 12%, and vegetables 10%, respectively. These results were supported by another study showing that grains (42%) were a major dietary iron source of the UK population, with approximately 50% coming from breakfast cereals (23). Only 15% of the typical Western diet consists of meat sources, which are the only foods associated with higher ferritin levels (22, 32, 40). Therefore, it appears that although non-heme iron has a lower absorption capacity than heme sources, by virtue of its frequent consumption in the form of cereal grains, it has become the major source of iron in Western diets (23, 32).

A strategy which has been employed with significant success in communities where most of the diet consists of grain products (or non-heme iron sources) is the use of an iron skillet or cast-iron cookware, which is considered a source of "contaminant" iron due to leaching into the foods, particularly acidic foods, during the cooking process (23). In

households where cast-iron was used in place of an aluminum cookware, lower rates of anemia and higher serum ferritin levels were observed, suggesting that contaminant iron from cast-iron cookware may be a significant component in influencing iron status (23).

1.6 Estimating Iron Bioavailability from Dietary Data

There appear to be multiple factors affecting the amount of iron absorbed from the diet, and several studies have attempted to utilize statistical approaches and algorithms in hopes of obtaining dietary iron estimates that are adjusted for food inhibitors, enhancers, and individual iron status. Overall, it appears that there may be food factors that affect iron absorption which have not yet been sufficiently examined, such as soy sauce (enhancer), some flavonoids like myricetin (inhibitors), and alcohol, and of those that have been evaluated, calculation of bioavailability proves to be complex(33, 41, 42). The majority of these techniques have been developed for individual food items but, in reality, individuals consume food in combination at meals, and therefore, a better understanding of iron absorption in the context of meals is required. Thus, algorithms that focus on calculations of individual food items are somewhat obsolete, and this raises issues of whether to adjust for absorption efficiencies, if the entire meal composition is not considered.

The main enhancers of iron absorption have been identified as ascorbic acid (vitamin C), vitamin A, and beta-carotene, while inhibitors include phytic acid (fibre), polyphenols (tea, coffee), phosphorus, and calcium (dairy products) (37). In studies of iron absorption factors, it was found that the greatest impact on iron absorption were meat products (enhancers) and phytic acid (inhibitor), while calcium, ascorbic acid, and polyphenols were not consistently significant in predicting iron absorption (22, 23, 37). However, such interactions are complicated. For example, phytic acid, found in wholegrain cereals, legumes, nuts and seeds, and an inhibitor of iron absorption, may actually exhibit an attenuated effect when combined in a meal with ascorbic acid and meat, further obscuring the adjustment for the impact of dietary components on iron absorption (23). Generally, polyphenols, such as tannins found in tea and coffee, as well as calcium found in dairy products, have a tendency to reduce iron absorption, but not to levels which can lead significant iron store depletion (22, 23, 43). In the case of ascorbic acid, there may be a dose-dependent relationship of enhancing iron absorption up to 500mg per meal, after which point absorption may no

longer be affected (23). Moreover, the impact of ascorbic acid's enhancing properties on ferritin levels has not been conclusively determined by all studies (22, 43).

In a study by Pynaert and colleagues, iron intakes and food iron sources of HIV-negative European women were determined by using 2-day diet records, classifying foods eaten into animal and plant sources, and calculating iron absorption efficiency for heme sources at 25% and non-heme iron sources at 10% (21). The proportional contribution of iron intake was calculated by dividing the contribution of the particular food item over the sum of the contributions of all food items consumed by the participant from the self-reported intakes on dietary records. Other algorithms have been developed to estimate iron absorption from whole meal composition instead of individual food items, although data on the food content of particular enhancers and inhibitors are easily not available (33, 43). More importantly, algorithms may not be appropriate to use in HIV-positive individuals, as complications associated with HIV on iron absorption are not accounted for in these equations.

In view of these findings, it may be more important to adjust for iron status, as this has a significant impact on the efficiency of dietary iron absorption, whereby an inverse relationship between serum ferritin levels <60μg/L and higher degree of absorption efficiency has been identified (37, 44, 45). In fact, this process may actually play the greatest role in iron bioavailability, since it has been shown that serum ferritin concentration is a much stronger predictor of iron absorption efficiency when compared to dietary factors, where the latter contribute only a small amount to iron absorption capacity (37).

A major factor that predicts iron status in women is menstruation, and associated losses impact the degree to which iron is absorbed from the diet. In other words, high intakes of meat products are only associated with lower risk of iron deficiency in women who have lower menstrual blood losses (22, 44). Thus, marked variation in menstrual blood losses may be considered the main source of variation in the iron requirements and status of women of childbearing age (46-48), such that ID and IDA is more likely to occur in women who have regular menstruation patterns. This reasoning is supported by a cross-sectional study completed by Heath and colleagues on the role of blood loss and diet in the etiology of mild ID, whereby blood loss from menstruation, nose bleeds, intrauterine devices, and blood

donation were all significant risk factor in the development of mild ID (44). Conversely, women who use oral contraceptives are less likely to develop ID as their losses are decreased (47).

1.7 Assessment Methods of Dietary Iron Intakes

No studies to date have addressed the specific dietary factors of IDA in HIV-positive women, although recommendations have been made to address this gap (1, 5, 9, 14). Since dietary iron is a complex issue to measure, due to the variation in iron status, and its bioavailability from the diet, few dietary iron measurement tools have been validated and are available for use (Dr. Susan Barr and Ms. Diana Johansen, verbal communication)(43, 48). This issue is distinctly brought forth in an article by Gordis, who states that, even if investigators claim that a survey or measurement tool has been validated, when the time comes for other investigators to replicate a study, access to the questionnaire proves to be quite a challenge (49). Moreover, questionnaires themselves are rarely subject to critical evaluation, as they are generally not included in manuscript submissions, and therefore, do not undergo peer review (49). Although a few references on reliability and validity of iron-specific FFQ have been published to date, none included a draft of the questionnaire used, and therefore, there is no previous tool from which to draw insight (34, 43, 48).

In studies measuring dietary intake, the easiest method of assessment has been a food frequency questionnaire (FFQ), which consists of a list of food items and in some cases, their standard portion sizes, and whereby the respondent indicates the number of times per month or year a certain food item is consumed (35, 36). Most FFQs are lengthy, but, in some cases, they are abbreviated into shorter checklists which can be used to assess intake of a specific nutrient in a simpler and more efficient manner (34, 35). Food checklists can been used to estimate mean iron intake of groups and effective in establishing patterns between iron intake and status, and less accurate at assessing ‘usual’ iron intake on an individual level (21).

An iron-specific food checklist was developed by Zhou et al. to calculate daily iron intake in pregnant and post-partum women, which was then evaluated with regard to iron status, as defined by serum ferritin concentration (34). This study did not find an association

between iron intake and status, but did not adjust for factors such as ethnicity, vegetarian status, food security level, menstruation pattern, and supplement use. Moreover, this short-cut method in survey design, where participants are asked only the frequency of consumption of specific foods items, may not be equally as effective as a longer FFQ version, unless good correlations between the two can be produced (35).

In another more detailed approach to iron FFQ design, Heath et.al. from Australia and Matthys et.al. from Belgium attempted to develop computerized tools for measuring dietary iron in women, while controlling for iron status and bioavailability based on meal composition, by adjusting for enhancers and inhibitors (43, 48). This methodology was extremely detailed, involving several sections, and taking 60 to 90 minutes to complete by participants. Food patterns of meal timing throughout the day, dietary recalls, 7-day dietary records, and a list of iron-containing foods, inhibitors, and enhancers were included in the data collection process. It has been suggested that 11 to 12 days of dietary collection from diet records are needed to accurately estimate dietary iron intake (32, 43). Thus, for validation, a weighted diet record of 12 days was used as the 'gold standard', as it most accurately estimated an individual's iron intake by accounting for daily intra-individual variation (43, 48).

To date, this approach appears to be the most comprehensive methodology for estimating iron intake. Validation studies have found that due to the high degree of variation in mean iron intake between the weighted records and the iron FFQ, the iron FFQ is of limited use in estimating dietary component intakes for individuals, but that it could be used to assess median iron intakes on a group level (35, 36, 43). These results support the approach of using iron-specific surveys to measure group trends in iron intake, rather than aim to calculate specific individual intakes.

Another approach which has been used in measuring nutrient intakes is the 24-hour recall method, whereby participants recall food items and portion sizes of foods they consumed the previous day (35). Although this method is easily administered and able to show generalized trends in individual dietary choices, usual intake of iron is not well-

captured from a single day's consumption recall and multiple recalls need to be used to provide an overall representation of individual dietary iron intake (50).

In terms of assessing adequacy of iron intake from dietary collection tools, it is often thought that the dietary reference intake (DRI) for a nutrient should be used, which is 18mg/d for iron in women of childbearing age, but this value is a reflection of the upper limit of iron intake, and corresponds to the amount of dietary iron necessary to meet the needs of 97% of the population. Therefore, a better indicator of meeting minimum iron needs is the estimated average requirement (EAR), which is 8.1mg/d for iron, and represents the minimum amount of iron required by women of childbearing age to prevent deficiency (21, 23). Therefore intakes below this level are, with certainty, a reflection of sub-optimal iron intake.

1.8 Management and Supplementation in IDA

Since a significant proportion of women with HIV have ID or IDA, studies have shown that providing routine low dose iron supplementation of up to 18 mg/d in a multivitamin preparation to all HIV-positive women is successful at reducing anemia and improving iron status without increasing plasma hepatitis C virus (HCV) or HIV RNA levels (7). However, initiation of higher doses of elemental iron supplementation should be preceded by an initial assessment of the degree of deficiency through laboratory testing (51). As noted earlier, HIV is associated with disturbances in iron metabolism, and anemia in the presence of elevated ferritin levels will not only be corrected with iron supplementation, but can also result in increased viral replication due to iron overload (51). For women who have a confirmed IDA diagnosis, higher doses of supplemental iron may be required, but serum levels should be monitored regularly to prevent over-supplementation (12). Moreover, it must be noted that even if supplementation is warranted, it alone is not sufficient to engage women in the self-management of their health, and an emphasis on individualized care and nutrition education is needed, whereby dietary review and education on iron-rich diets be initiated for women with HIV who at risk for IDA.

In two randomized control trials of otherwise healthy women with ID, the effectiveness of dietary advice (recipes, cast iron frying pan, and education on iron-containing foods as

well as enhancers such as vitamin C) was compared to supplementation without dietary therapy (31, 46). Although sample size in both studies was quite small, the results showed a marginal improvement in iron status with dietary advice, but to a much lesser degree than supplementation, which still led to the biggest improvements in serum iron markers. Thus, even for those individuals who have ID without the presence of anemia, supplementation is the most effective means of correcting it and preventing further depletion of iron stores. This does not, however, underestimate the importance of individualized nutritional care and education, which is necessary to enhance engagement of women in their care, to increase compliance with supplementation therapy, and to support prevention efforts for ID and IDA (46).

1.9 Study Objectives and Hypotheses

In view of the impacts of anemia in women infected with HIV on quality of life and prognostic outcomes, as well as the gaps in research which have not explored the role of nutrition, this study was conducted with the following objectives:

1. To determine the prevalence of anemia, ID, and IDA in a sample of hospitalized and community-living HIV-positive women in the Greater Vancouver area;
2. To quantify the effect size, as measured by the odds ratio, of particular medical, social and nutritional factors on the likelihood of having IDA by using multiple logistic regression modeling;
3. To determine whether there is a proportional relationship between dietary iron intake and iron status (ferritin) using linear regression modeling;
4. To calculate sensitivity, specificity, positive and positive and negative predictive values of the daily dietary iron intake estimates derived from the diet survey to predict iron status, as defined by presence or absence of IDA;
5. To quantify dietary iron intake patterns of the sample as they relate to food groups;
6. To estimate the impact of food insecurity on iron status and nutrition.

Above all, the intent of this research is to increase the awareness of the persistence of IDA in women with HIV, as well as to highlight the importance of engaging women in nutrition and health self-management. By demonstrating the importance of prompt screening and regular monitoring for IDA, its prevalence and associated complications can

be reduced in this population, thus increasing quality of life, enhancing women's potential, and improving health outcomes.

It is anticipated that the prevalence of anemia, ID, and IDA will be in line with estimates derived from inner-city populations in the United States, since the population of interest for this study is likely similar in characteristics to other populations previously studied. It is also hypothesized that menstruation, food insecurity, vegetarian status, and ethnicity other than Caucasian, will be strong predictors of IDA, whereby the justification for the latter relates to the notion that Aboriginal and African ethnicities tend to be at greater risk for nutritional deficiencies due to the presence of greater social and health disparities (1, 9, 13, 22). From a nutrition perspective, it is hypothesized that iron intakes below 8.1mg/d will be associated with a higher risk of IDA. Conversely, it is anticipated that supplement use, such as multivitamins and iron supplements, use of an iron skillet, higher CD4 cell counts, and iron intakes of 8.1 mg/d or greater will be protective against IDA development.

2. METHODS

2.1 Study Participants and Recruitment

The participants for this study were hospitalized and community-living women with HIV who attended Vancouver-based primary care clinics, and who resided in the greater Vancouver area, which included downtown Vancouver and the Downtown Eastside, but also surrounding suburban areas, such as Burnaby, Richmond, Surrey, Abbotsford, and Mission, as well as Vancouver Island and the Sunshine Coast.

Inclusion criteria for the study was female sex, confirmed HIV-positive status, age >19 years, not pregnant, and not palliative. Although IDA appears to be in highest prevalence among women of child-bearing age, menopausal and post-menopausal women were also included in the criteria because of evidence that women with HIV in these groups are still at risk for IDA, and that duration of HIV is a possible risk factor (1, 9).

Ethical approval was obtained from the University of British Columbia and Providence Health Care Clinical Ethics Board, as well as from each of the participating community clinics through the Vancouver Coastal Health Ethics Board for Vancouver Native Health Society and the Downtown Community Health Centre Maximally Assisted Treatment Program (MAT), and through the Children's and Women's Health Centre of British Columbia Ethics Board for the BC Women's Hospital and Health Centre Oak Tree Clinic.

2.2 Study Design

This was a cross-sectional multi-centre study that spanned from October 2008 – May 2009, and consisted of a Phase 1 Pilot Study (October – December 2008) and a Phase 2 Pilot Study (January – May 2009). Sample size goals for Phase 1 were 10 participants, and 100 participants for Phase 2. A newly-designed diet survey was used to measure dietary iron intake and other nutritional factors in women with HIV. Chart and laboratory records were reviewed to assess medical factors, immune status, and anemia-related biochemical markers. Hospital and clinic staff knew of inclusion criteria, and first approached eligible participants for recruitment. If the participant agreed to take part in the study, the investigator would review the specific details of the study and obtain informed consent. Thereafter, the diet

survey was administered by the investigator, which took approximately 20 minutes to complete. Space was provided in an office (outpatient setting) or the participant's hospital room (inpatient setting) for interviews. As an added incentive during Phase 2, an honorarium of $10 was provided to all participants. Accordingly, the study protocol was amended through the clinical ethics board to include this addition.

Following the interview, laboratory measures and medical data from the paper or electronic chart were recorded. Laboratory measures, which included CD4 count, viral load, mean corpuscular volume, hemoglobin, ferritin, transferrin, transferrin saturation, and serum iron, were acceptable if taken within 3 months of the time of diet survey administration. Other tests would be ordered by the participant's physician as requested. Hospital (and clinic) protocol indicated that iron-related laboratory measures were ordered only if hemoglobin levels were below 120g/L. If hemoglobin levels were within normal range, IDA was ruled out without further investigation of iron stores. IDA was diagnosed in the presence of sub-optimal hemoglobin concentration, and in the presence of ferritin concentration <30μmol/L (5, 7). Medical data were available from the participant's medical chart. Weight and height were obtained from the participant if they knew these measures, or taken during the interview. Body mass index (BMI) was calculated using the formula of weight in kilograms divided by the square of height in meters.

Phase 1 was conducted at St. Paul's Hospital to determine whether rate of recruitment from hospital would be feasible in order to meet sample size requirements. In addition, this study would help test whether diet survey was user-friendly (i.e. easy to administer, appropriate in its length, and comprehensive and sufficient in clarity in its list of food items and supplemental questions). Finally, a 24-hour dietary recall was included at the end of the survey to gain information on usual food choices. The pilot study also helped to establish the degree to which information from medical charts and laboratory records was accessible.

Only 10 out of 24 eligible women inpatients were recruited from St. Paul's Hospital. There were several major barriers to recruitment, which included poor health status of several patients, refusal to participate, substance abuse, and mental health issues, which resulted in several of the women leaving hospital against medical advice. Thus, it was

decided that this venue alone would not be a suitable recruitment site, and that additional sites would need to be added in order to increase sample size and to result in a more representative sample. As recruitment from hospital alone would result in selection bias, with an overrepresentation of those who show more significant health complications, the addition of sites in the community for Phase 2 was clearly indicated.

In Phase 2, recruitment was broadened to include health care clinics that were major care providers for women with HIV in Vancouver. These included the Vancouver Native Health Society, the Downtown Community Health Clinic MAT program, and the Oak Tree Clinic. The Vancouver Native Health Society and the Downtown Community Health Clinic are located in Vancouver's Downtown Eastside and provide support and medical care to persons who reside in this neighborhood, many of whom are HIV-positive and are also dealing with substance abuse and mental health issues. The Oak Tree Clinic at the British Columbia Women's Hospital and Health Centre provides care to HIV-positive women and their families living in British Columbia, and is representative of a cohort of patients who are generally healthier.

2.3 Diet Survey Design

There were several reasons for designing a new instrument, rather than using a validated, generalized food frequency questionnaire (FFQ). First, there was no available electronically or print FFQ that was specific to iron. Secondly, generalized FFQs that were available were not sufficiently specific to measure dietary iron intake (52), and were quite lengthy, taking at least 60 minutes to complete, and required participants to recall foods consumed over the past year. Thus, they were not designed with the needs of this population in mind, whose HIV-associated cognitive changes affect attention span and memory recall (53). The design of a new diet survey was advantageous, in that it could be geared specifically toward women with HIV, use a shorter recall period (one week), contain a shorter list of food items, and include simplified supplemental questions to enhance accuracy of reporting. A shorter survey was also more appropriate for the clinic setting, where participants are often required to see multiple health care professionals, and therefore, the time available to be spent on completing such surveys is limited. For hospital inpatients, medical procedures call patients away during the interview, thus leading to incomplete collection of data. In all, developing a

quick screen to assess dietary iron intake in approximately 20 minutes seemed more suitable under these circumstances.

The diet survey was based on commonly consumed iron-containing foods in the Western diet and followed the traditional format of a semi-quantitative FFQ (36). The food items were drawn from the NHANES Food Questionnaire and food lists of significant iron sources (4, 52). Each food item was designated a standard serving size derived from the Nutrient Value of Common Foods tables (from the Canada Nutrient File), since these serving sizes correspond to average actual intakes of foods, rather than the recommended serving sizes (54). The interviewer would administer the survey, and participants would report the number of times the food item was consumed in the past seven days. To calculate total estimated daily iron intake from the survey, the frequency of intake of each food item during a 7-day period at a standard portion size was multiplied by the amount of iron contained in that food item as estimated from Health Canada's Nutrient Value of Some Common Foods (54). The average daily intake was calculated by using this total and dividing by seven (as food intake was reported for one-week period). Since the standard correction factors for iron bioavailability calculations are derived from research in HIV-negative populations, no correction factor for heme and non-heme bioavailability was applied in this study. The reasoning for this will be discussed in subsequent chapters.

Two drafts of the diet survey were developed. The preliminary draft, piloted in Phase 1, consisted of four sections: a list of 41 iron-containing food items; the frequency of use of iron inhibitors, such as bran, coffee, and tea; and a third section of nine supplemental questions inquiring about supplement use, cooking practices, consumption of fruit at meals (to determine whether vitamin C acted as an enhancer), menstruation patterns, vegetarianism, and food security; and a 24-hour recall to capture food patterns and choices (4). For each food item in the first two sections, participants indicated if they consumed the food "often ($\geq$2/week)", "sometimes (<2/week)", or "never" in the past 7 days. The supplemental questions in the third section consisted of "Yes/No" answers, except for one clarification question regarding regularity or irregularity of menstruation pattern. The survey took 15 to 20 minutes to complete and was administered to 10 hospitalized women.

After Phase 1, the diet survey was revised, prior to commencing Phase 2. The list of food items was expanded to include foods that were commonly consumed using information collected from the 24-hour recalls. Foods not consumed by any of the participants were removed from the list. These included blackstrap molasses, pumpkin and squash seeds, wheat germ, cocoa powder, and red wine. Other food items, such as beef or chicken liver were separated as their iron content was slightly different, and those who consumed liver were more likely to consume beef rather than chicken liver. Canned fish was separated into "canned salmon/tuna/herring", as iron content among these was different. Since breakfast cereal was consumed by the majority of participants, a line was added to indicate the type or name of breakfast cereal consumed. Although fruit, vegetables and dairy are not significant sources of iron, they were consumed frequently, so a selection of these foods was added. Section 2 of the preliminary survey regarding inhibitors of iron was removed as controlling for these inhibitors would not be appropriate unless complete meal composition could be determined. Since food records would be required for this adjustment, and poor memory recall and compliance were potential problems, correction for absorption efficiency was not applied.

Revisions also included the establishment of food categories, with each food item organized accordingly in alphabetical order. The four categories chosen were "Meats and Alternatives" (26 items); "Grains and Cereals" (14 items); "Fruit and Vegetables" (21 items); and "Dairy Products" (4 items), for a total of 65 food items. The section of supplemental questions was retained, with the following additions: one question to indicate the type of iron supplement taken; two questions to inquire about adequacy and use of kitchen facilities; and one question regarding number of days of menstruation in a cycle. These changes resulted in the inclusion of 10 supplemental questions, covering each lifestyle factor that may impact IDA risk. A copy of the revised diet survey used for Phase 2 can be found in Appendix A.

2.4 Content Validation Study of Diet Survey

Content validity refers to the extent to which items on a survey assess the relevant breadth of content material, or how well the content material was sampled (55). A content validation study is a type of investigation consisting of a comprehensive literature review to construct a survey, and then to involve a panel of subject matter experts (SMEs) to review test construction for completeness, clarity and layout (56). Content validation of a new survey is critical in its development phase, because it addresses whether items on the survey are adequately representative to be able to measure the outcome of interest (57). The SME is chosen based on a set of pre-designated criteria (55, 58). In this case, a SME was defined as a registered dietitian or a professional with a Master of Science or Doctor of Philosophy in nutrition, and professional experience in the field for a minimum of 5 years. A list of potential SMEs was identified through a review of their peer-reviewed publications in questionnaire development and design, or clinical nutrition work experience in the field of HIV (57). From related studies, it has been suggested that a minimum of 2 and a maximum of 10 SMEs would be needed for a content validation study, but that a minimum of 5 are required in order to achieve statistical significance (57, 59).

SMEs were recruited through an invitation letter, which briefly outlined the intended use of the measurement tool as well as general requirements for the evaluation (58). Eligible SMEs were asked to indicate whether they would participate or not by a given date. If agreeable to participating, a cover letter, consent form, evaluation form, and copy of the measurement tool was sent to the SMEs, who would have two weeks to complete his or her evaluation and mail responses to the investigator. A small gift (valued at $10) was provided to individuals who completed the evaluation. Approval for this study was obtained through the University of British Columbia Behavioral Ethics Board.

The evaluation form consisted of six sections, each addressing issues of content, design, and representativeness of the food items. Specifically, questions were directed toward the appropriateness of food items, the clarity of their names and what they included, as well as diet survey design and layout. Most importantly, the evaluation aimed to determine whether the food items included were sufficiently representative of food items that were considered iron sources. A copy of the evaluation form is included in Appendix B.

Since only two SMEs were able to participate in this content validation study based on eligibility criteria, written comments regarding their assessment of the survey items were used to make revisions, but the rate of agreement between their responses on the quantitative scale questions was not calculated, since the number of SMEs were too few to produce statistical differences (59). The responses will be used to make further revisions to the diet survey.

2.5 Sample Size Calculation

The following calculation was performed to determine the necessary sample size to achieve statistical power in a prevalence study (60). Since the goal of this study is to estimate prevalence of IDA in a sample of HIV-positive women, knowing that other studies have estimated 20% prevalence of IDA in this population, it is hypothesized that the following sample size is required in order to be able to estimate accurately the prevalence of IDA in this population (5, 14). The premise for this calculation is:

Ho: IDA Prevalence in HIV-positive women = 20%
Ha: IDA Prevalence in HIV-positive women ≠ 20%
$n = \frac{z^2 [(p)(1-p)]}{d^2}$, where
n = sample size
z = z statistic for a level of confidence = 1.96 (α = 0.05); 1.75 (α = 0.08); 1.65 (α = 0.10)
p = the expected prevalence of proportion of IDA = 0.20
d = precision of the estimate or level of significance (0.05; 0.08; 0.10)

Thus, for an estimated prevalence of 20% and for a level of significance of α = 0.05, a sample size of 246 participants would be required. For a level of significance of α = 0.08, a sample size of 77 participants would be required. For a level of significance of α = 0.10, a sample size of 44 participants would be required. Since it was possible to recruit over 77 participants, but not feasible to recruit 246 participants in the given time period for data collection, the sample size goal was estimated at 100 in order to approximate a 95%

confidence level. Therefore, from the expected 20%, this sample size allows only the detection of 41% of prevalent IDA cases (=100/245).

2.6 Data Presentation

All statistical analyses utilized the statistical software program SPlus® 8 Enterprise Developer for Windows (Insightful Corporation, 1988, 2007). For the generation of descriptive statistics, the sample was categorized into two groups, namely those with IDA and those without IDA. Histograms were plotted for each of the continuous variables to verify whether their distribution was normal or otherwise. For those variables with a normal distribution, mean and standard deviation was calculated and for non-normally distributed variables, the median and range were derived. Frequency distributions were determined for categorical variables and indicated as proportions and percentages. To determine whether differences between the groups were significant for continuous normally distributed variables, t-tests between two groups or analysis of variance (ANOVA) for more than two groups were conducted. For non-normally distributed continuous variables, Wilcoxon rank sum tests for two groups or Kruskal-Wallis tests for more than two groups were performed. For categorical variables, chi-square tests with Yates' continuity correction were performed if sufficient observed count (>5) were available; otherwise, the Fisher's Exact test was conducted. P-values for all tests were provided.

2.7 Calculation of Dietary Iron Intake

Since the survey divided food items into four food groups, estimated daily iron intakes from Meats, Alternatives, Grains and Cereals, Fruit and Vegetables, and Dairy Products food groups were also obtained. Proportion of iron intake from each food group was characterized for the overall sample as well as each of the five ethnicities, namely of Caucasian, Aboriginal, African, Asian, and South Asian. Calculations of percentage iron intake from a food group were derived by dividing the mean intake from a food group by the total mean iron intake for the overall sample and for each ethnicity. It was hypothesized that each of the ethnicities would exhibit dietary patterns for iron that were in line with specific cultural food choices and practices. Specifically, South Asian diets, where vegetarianism is more prevalent, intakes of meat alternatives would be higher compared to other ethnicities, and African diets would be higher in fruit and meat products, as these foods are more traditional.

2.8 Calculation of Predictive Value (61)

Sub-optimal iron intake, as estimated from the diet survey, was defined as daily iron intake <8.1mg/d, which is the estimated average requirement (EAR), or the amount of daily iron intake required for 50% of the population to meet their needs (23). The dietary reference intake (DRI), or optimum level to meet iron needs in women of childbearing age, is 18mg/d (62). This level was chosen for all participants in the Phase 2 study, irrespective of age, as it has been indicated that micronutrient needs may be higher in individuals with HIV in view of direct impacts of the virus on gastrointestinal enterocytes (63, 64). The degree to which the diet survey was able to predict iron status was quantified through measures of sensitivity, specificity, positive, and negative predictive value. Unadjusted dietary iron intakes were considered, since it was unclear what cut-off should be used for absorbable iron requirements, and the EAR does not consider absorbable iron in its calculation (46, 62).

A two-by-two contingency table was designed to calculate predictive values with IDA status from laboratory tests as the criterion for IDA diagnosis, and dietary iron intake estimates from the diet survey as the comparator (Table 2.1). Sensitivity is defined as the proportion of women with IDA who have sub-optimal iron intakes as calculated by (a/a + c). Specificity is the proportion of women without IDA who have adequate iron intakes as calculated by (d/b + d). The positive predictive value (PPV) is the proportion of women with sub-optimal iron intake who have IDA as calculated by (a/a + b). The negative predictive value (NPV) is the proportion of women who have at least minimal iron intakes who do not have IDA as calculated by (d/c + d).

Table 2.1 Two-by-Two Contingency Table for Predictive Value of Dietary Iron Survey

	IDA Present	IDA Absent	Total
Iron Intake <8.1mg/d	a	b	a + b
Iron Intake ≥ 8.1 mg/d	c	d	c + d
Total	a + c	b + d	N

2.9 Exploration of Linear Relationship between Ferritin and Meat Intake

Based on previous research on iron deficiency and dietary intake, a linear relationship has been described between meat intake and serum ferritin (21, 22, 31). To determine whether there was a linear relationship between ferritin concentration (μg/L) and meat intake (mg/d) calculated from the diet survey, each variable was first assessed for normality using histograms, and for linearity using a scatter diagram. The Pearson's correlation coefficient was also calculated. In the case that the distribution was non-normal for either ferritin or meat intake, these would be transformed into a logarithmic function to assess linearity.

2.10 Logistic Regression Modeling for Predictors of IDA

To determine whether the assumption of proportionality for IDA and iron intake was observed, iron intake was first categorized into three tertiles. Then, the log-odds of IDA were plotted against the midpoint of each iron intake tertile to determine whether there was a proportional relationship between IDA and iron intake (65). A logistic regression model was used to adjust for multiple factors associated with IDA to elucidate the relationship between iron intake, as estimated by the diet survey, and iron status, as identified by the presence or absence of IDA. It was hypothesized that there would be an inverse relationship between iron intake and IDA, such that the odds of IDA would decrease with increasing iron intake. The outcome variable, IDA, was coded as a dichotomous variable, where the presence of IDA was coded as 1 and absence of IDA as 0. Iron intake was the primary predictor variable, and treated as a continuous variable (mg/d) because of its normal distribution. By retaining iron intake as a continuous variable, a more accurate estimation of IDA across levels of iron intake could be quantified.

Based on previous findings in the literature of the factors that impact iron status, the covariates included in the multiple logistic regression model were CD4<200 cells/μL, ethnicity (Caucasian, Aboriginal, African, Asian, and South Asian), past or present IDU, and menstruation pattern (regular/irregular). CD4 was coded as 1 if <200 cells/μL and 0 otherwise. Ethnicity was coded as 0 for Caucasian (referent group), 1 for Aboriginal, 2 for African, 3 for Asian, and 4 for South Asian women, respectively. Past or present IDU was coded as 1 and 0 otherwise. Menstruation pattern was coded as 1 if regular, and 0 for

amenorrhea (referent group). Nutritional factors which were hypothesized to impact iron status were also adjusted for, and included multivitamin use, iron supplement use, vegetarianism, and use of food assistance programs. The use or presence of each of these factors was coded as 1, and otherwise as 0. Missing values in the laboratory and anthropometric measures of the data set were excluded from the analysis. Regression coefficients were obtained for each of the independent variables, which were then converted to odds ratios (OR), whose significance was indicated by the 95% confidence interval (CI). The multiple logistic regression model was also analyzed for significance using the likelihood ratio test (LRT) and corresponding p-value at $\alpha=0.05$.

3. RESULTS

3.1 Content Validation Study

Six subject matter experts (SMEs) were approached for recruitment into the content validation study, but only two agreed to participate based on fulfillment of eligibility criteria. After reviewing the evaluations of the SMEs, as summarized below, it became clear that the survey would require major revisions before it could be subjected to further content validation and application. Recommendations were used to revise the diet survey and a future recruitment of SMEs is planned for another content validation study.

The comments of the SMEs were summarized for each of the content areas of the evaluation form in Table 3.1: Layout of Instrument, Clarity of Food Categories, Clarity of Names of Food Items, Dietary Representativeness of Food Items, Response Format, and Additional Comments.

Table 3.1 Summary of Written Comments from Content Validation Study

Content Area	Comments
1. Layout of Instrument	• Overall layout acceptable • Uncertainty regarding instructions (whether interviewer or participant should complete survey)
2. Clarity of Food Categories	• Individual food items lead to underreporting: combination foods/mixed dishes should be included in each category • "Fruit and Vegetables" should include fewer items (not iron sources)
3. Clarity of Names of Food Items	• Different forms of food items should be specified: "Beans, cooked" should include pinto/kidney/lima/lentils to avoid confusion with green beans • Forms that are iron sources should be included: "Mushrooms, canned"

Content Area	Comments
4. Dietary Representativeness of Food Items	• Survey was quite representative in terms of single food items • Further information required on reasoning behind inclusion of fruit and dairy products, and omission of other iron-rich sources (i.e. combination foods with beef)
5. Response Format	• One week not representative of usual intake: one month better to assess usual intake
6. Additional Comments	• Another column to indicate portion sizes for each of the food items • Survey redesign requires clear purpose and indication of target population; information on other available validated instruments; and, the assessment of bioavailability, supplement use, and portion sizes

3.2 Characteristics of the Sample

Data were collected on 102 participants, who were recruited from January – May 2009. Sixty-one participants (60%) were recruited from Oak Tree Clinic, 26 participants (25%) from Vancouver Native Health Clinic (where one participant was excluded due to absence of medical information), 11 participants (11%) from the Downtown Community Health Clinic MAT program, and 4 participants (4%) from St. Paul's Hospital. Although a sample size of 246 participants would have resulted in the desired power, a goal of 100 participants was designated under the timeline for recruitment. Participants were predominantly Caucasian (53%), followed by Aboriginal (30%), African (11%), South Asian (4%), and Asian (2%). Of the eleven women of African ethnicity, 10 were from the African continent, while one woman who was from a Caribbean country. Age range of participants was 20 years to 60 years.

3.3 Anemia Prevalence

Hemoglobin levels were available for 102 participants, but ferritin levels only for 42, since criteria at clinics for testing iron storage parameters was only conducted for hemoglobin concentrations <120g/L. Anemia prevalence was 30% and IDA prevalence 16%. Estimates of ID prevalence were more difficult to elucidate, since ferritin levels were unavailable for most participants whose hemoglobin levels were normal. Of those on whom ferritin levels were available, 22 (52%) had ferritin levels <30μg/L. The prevalence of ID among 20 participants who had both normal hemoglobin and available ferritin levels was 40%.

3.4 Demographic Characteristics

Table 3.2 provides the overall distribution of characteristics for the sample as well as the characteristics of participants with and without IDA. Values are expressed as mean (standard deviation) for normally distributed variables and median (range) for non-normally distributed continuous variables, and proportion (percentage) for categorical variables. The asterisk (*) indicates significant results at $\alpha = 0.05$. Of the women with IDA, the majority were of Aboriginal, African, or South Asian ethnicity, although sample size in the latter two groups was small. Mean age of participants was 40 years, and the mean BMI approximately 24 kg/m^2. In terms of medical indicators, only 23 participants had body mass index (BMI) <20 kg/m^2 (22%), which is indicative of an overall healthy weight range for the participants. Other measures which supported an improved health status include a high prevalence of use of antiretroviral therapy (ARV) (75%), an absence of current opportunistic infections (OI), and a relatively lower prevalence of active intravenous drugs (37%). However, nearly half the participants were hepatitis C positive (46%), and although mean age was 40 years and of childbearing age, half the sample reported amenorrhea.

With respect to nutritional demographic characteristics, there was a low prevalence of past or present vegetarianism (25%), although these practices prevailed among South Asian women, likely due to cultural and religious preferences. Multivitamin use was high (57%), but only 25 participants were on an iron supplement (25%), and of the 16 participants with IDA, only 5 were taking iron supplements (28%). In terms of types of supplements taken, 15

participants took ferrous gluconate (60%), while 10 took ferrous sulfate (40%), indicating that a lower dose iron supplement was more often prescribed. With regard to food security indictors, 62 participants used food assistance programs in a one-month period (61%), which is reflective of significant food insecurity in this sample, despite the fact that the majority of recruitment occurred outside of the Downtown Eastside. Of those participants who used food assistance programs, six used free meal delivery programs (10%), 42 used food banks (68%), and 14 used free meal programs (22%).

There were significant differences between IDA status between Caucasian and African ethnicity using a Fisher's Exact test comparison, where the latter group was more likely to have IDA. There were no significant differences among other ethnicities in terms of IDA risk, although sample size among Asian and South Asian groups was small. Women with regular menstruation pattern were more likely to have IDA in a chi-square comparison, while higher median days of menstruation also yielded significantly higher likelihood of IDA in Wilcoxon Rank test. T-tests showed significantly lower hemoglobin and MCV levels among those with IDA, as did the results of the Wilcoxon Rank test for ferritin and transferrin saturation levels. The groups did not differ significantly on any other factors, and were similar in terms of age, body mass index, antiretroviral therapy use, past or present IDU, HCV status, CD4 cell count, food security indicators, and supplement use.

Table 3.2 Characteristics of Study Participants

	Overall Distribution (n = 102)	Participants with IDA (n = 16)	Participants without IDA (n = 86)	P Value for Difference
Mean Age (years)	40.5 (8.8)	40.3 (9.1)	41.3 (7.3)	0.62 (NS)
Body Mass Index (kg/m^2)	24.0 (5.4)	23.7 (6.6)	24.0 (5.2)	0.84 (NS)
	Overall Distribution (n = 102)	Participants with IDA (n = 16)	Participants without IDA (n = 86)	P Value for Difference
Ethnicity				
• Caucasian (n = 56)	56 (55%)	5 (28%)	51 (60%)	Reference
• Aboriginal (n = 30)	30 (29%)	5 (28%)	25 (29%)	0.31 (NS)
• African (n = 10)	10 (10%)	4 (22%)	6 (7%)	0.02*
• Asian (n = 3)	3 (3%)	1 (5%)	2 (2%)	0.28 (NS)
• South Asian (n = 3)	3 (3%)	1 (17%)	2 (2%)	0.28 (NS)
Past or present intravenous drug use (IDU)	38 (37%)	2 (12%)	36 (42%)	0.03*
Present opportunistic infection (OI)	0 (0%)	0(0%)	0(0%)	NA
Gastrointestinal disorder/bleeding (GI)	6 (6%)	1 (6%)	5 (6%)	0.61 (NS)
Hepatitis C Virus (HCV)	47 (46%)	5 (31%)	42 (49%)	0.28 (NS)

	Overall Distribution (n = 102)	Participants with IDA (n = 16)	Participants without IDA (n = 86)	P Value for Difference
Antiretroviral Therapy (ARV)	76 (74%)	12 (75%)	64 (74%)	0.80 (NS)
Use of Non-Steroidal Anti-Inflammatory Drug (NSAID)	17 (17%)	2 (12%)	15 (17%)	0.90 (NS)
Menstruation	53 (52%)	13 (81%)	40 (46%)	0.08 (NS)
Regular	45 (44%)	13 (81%)	32 (37%)	0.02*
Irregular	7 (7%)	0 (0%)	7 (8%)	0.28 (NS)
Amenorrhea	50 (49%)	5 (31%)	45 (52%)	0.03*
Number of days (range)	2.5 (0.0 – 8.0)	4.0 (0.0 – 7.0)	0.0 (0.0 – 8.0)	<0.001*
Hemoglobin (g/L)	125.5 (14.7)	108.6 (9.1)	128.6 (13.4)	<0.001*
Mean Corpuscular Volume (fL)	91.9 (8.4)	86.2 (8.0)	93.0 (9.0)	0.03*
Viral Load (copies/mL)	21628 (100309)	63091 (229705)	14717 (56183)	0.44 (NS)
CD4 (cells/μL) (range)	380 (10 – 940)	340 (40 – 810)	380 (10 – 940)	0.11 (NS)
Ferritin (μg/L) (range)	29 (3 – 344)	11 (3 – 40)	58 (10 – 344)	<0.001*
Transferrin (g/L) (range)	2.46 (1.00 – 3.47)	2.73 (1.31 – 3.47)	2.04 (1.00 – 2.52)	0.18 (NS)
Transferrin Saturation (%) (range)	13.5 (4.0 – 30.0)	9.0 (5.0 – 22.0)	17.0 (4.0 – 30.0)	0.04*
Serum Iron (mg/L) (range)	7.5 (1.0 – 19.0)	5.0 (4.0 – 19.0)	11.0 (1.0 – 16.0)	0.17 (NS)
Estimated Iron Intake (mg/d)	13.1 (6.1)	13.3 (4.6)	13.1 (6.4)	0.48 (NS)

	Overall Distribution (n = 102)	Participants with IDA (n = 16)	Participants without IDA (n = 86)	P Value for Difference
Past or present vegetarianism	25 (24%)	5 (31%)	20 (23%)	0.53 (NS)
Calcium Supplement	34 (33%)	3 (19%)	31 (36%)	0.25 (NS)
Fibre Supplement	5 (5%)	0 (0%)	5 (6%)	1.00 (NS)
Iron Supplement	25 (24%)	5 (31%)	20 (23%)	0.53 (NS)
Multivitamin Supplement	58 (57%)	11 (69%)	47 (55%)	0.44 (NS)
Use of Iron Skillet	31 (30%)	5 (31%)	26 (30%)	0.99 (NS)
Adequate Kitchen Facilities	76 (75%)	13 (81%)	63 (73%)	0.99 (NS)
Kitchen Facilities used for Meals	76 (75%)	15 (83%)	61 (73%)	0.72 (NS)
Afford Groceries in One Month	75 (74%)	12 (75%)	63 (73%)	0.87 (NS)
Use of Food Assistance Program	62 (61%)	10 (63%)	52 (60%)	0.90 (NS)

3.5 Dietary Iron Intake Estimates

Adjustment for heme and non-heme iron sources was attempted in order to correct for differences in bioavailability, using a correction factor of 25% and 10%, respectively (21, 23). However, when this adjustment was performed, iron intakes were extremely low, with a mean dietary intake of 1.2mg/d of iron. Therefore, due to this low value after adjustment, this correction factor is questionable in its application to a population where iron intakes might be well below that of the average population. For the purpose of the analysis, unadjusted iron intakes were used and associated limitations and implications discussed in Chapter 4.

Using unadjusted intakes from the diet survey, 18 participants (18%) did not meet the estimated average requirement (EAR) or minimum requirement of 8.1mg/d, while 65 participants (63%) were able to meet minimum iron requirements, but not the dietary reference intake (DRI) for iron, and 19 participants (19%) met or exceeded the DRI. Among participants with IDA, 2 (12%) did not meet the EAR, while 11 (69%) exceeded the EAR but did not meet the DRI, and 3 participants exceeded the DRI (19%). The results are similar among those without IDA: 16 (19%) did not meet EAR, 54 (63%) exceeded the EAR but not the DRI, and 16 (19%) exceeded the DRI (Figure 3.1). Hence, a statistically significant difference in iron intake was not observed between IDA and non- IDA groups, as shown in Table 3.2, although it is noteworthy that the majority of the participants were unable to meet their daily requirements for iron intake.

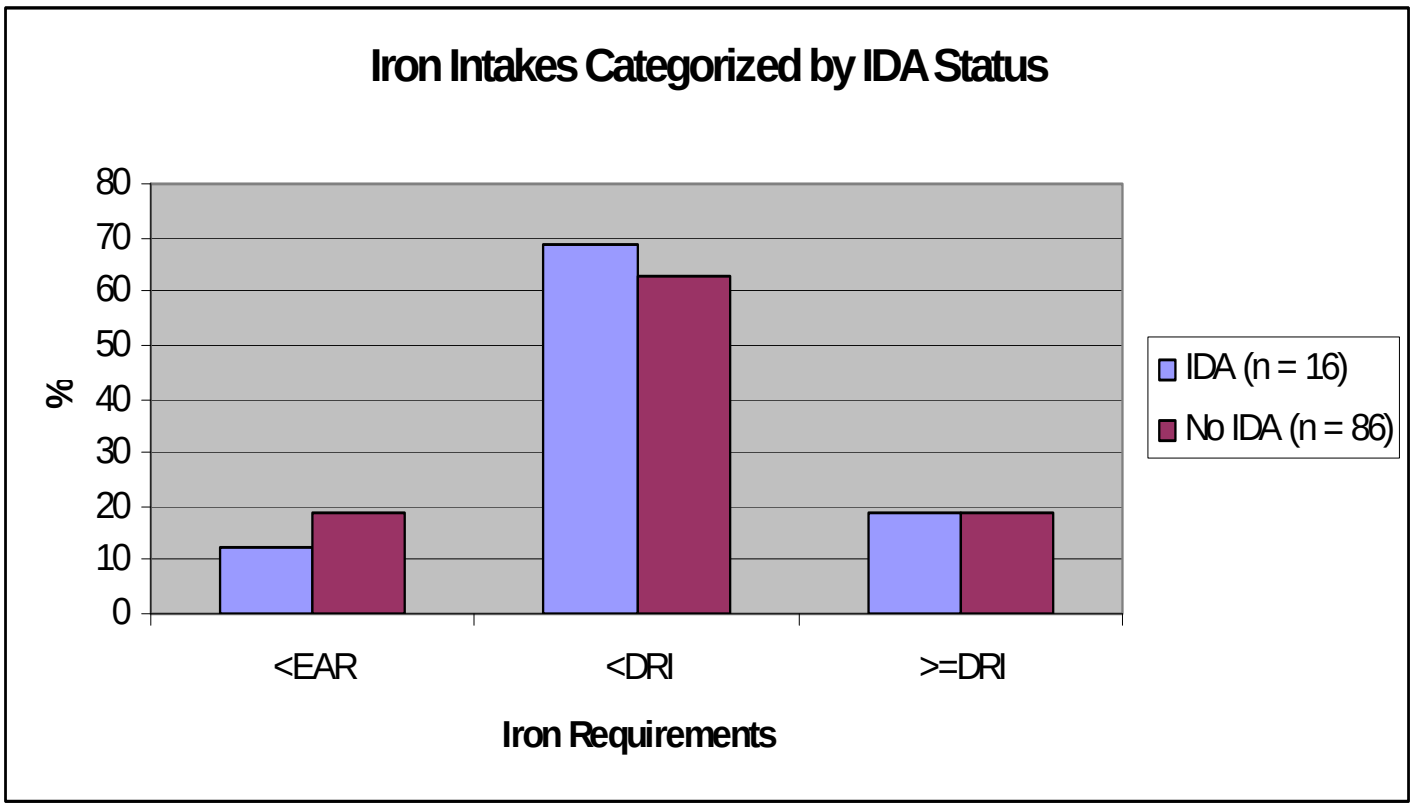

Figure 3.1 Distribution of Iron Intakes by IDA Status (Chi-square p-value 0.89)

The percentage contribution of iron for each food group was defined in terms of the proportion of iron intake for Meats, Alternatives, Fruit and Vegetables, and Dairy divided total iron intake (Figure 3.2). Overall, iron intakes were greatest from Grains at 55%, followed by 17% from Fruits and Vegetables, 12% from Meats, 12% from Alternatives, and 4% from Dairy. Iron intake from meats was highest among African and South Asian women at 13% and 15%, respectively, but these percentages are likely higher due to the smaller sample size in these groups. In other words, mean intakes could have been greatly affected by one participant's intake, as may have been the case for the South Asian group, where only one participant was a current non-vegetarian. Among Caucasian, Aboriginal, and Asian participants, meat intakes were 12%, 12%, and 11%, respectively. No significant differences were observed between mean meat intakes among ethnicities (ANOVA >0.50).

Alternatives, including nuts, seeds, and legumes comprised 42% of South Asian, 12% of African, 11% of Asian, 11% of Caucasian, 10% of Aboriginal dietary iron intakes, with significant differences observed among ethnicities (ANOVA p-value < 0.02). Grain products

comprised 62% of Aboriginal and 58% of Caucasian dietary iron intakes, followed by 44% of South Asian, and 33% of Asian, and African diets, respectively, with no significant differences observed (Kruskal-Wallis p-value = 0.09). Significant differences were observed in Fruit and Vegetables, where intakes were highest in African women's diets at 27%, followed by Aboriginal and South Asian diets at 17%, Caucasian diets at 16%, and Asian diets at 14% (Kruskal-Wallis p-value = 0.04). Iron intake from dairy products comprised the smallest contribution to diet composition, or 6% of Aboriginal diets, 4% of Asian diets, and nearly 3% of Caucasian, African and South Asian diets with no significant differences between groups (ANOVA p-value = 0.97).

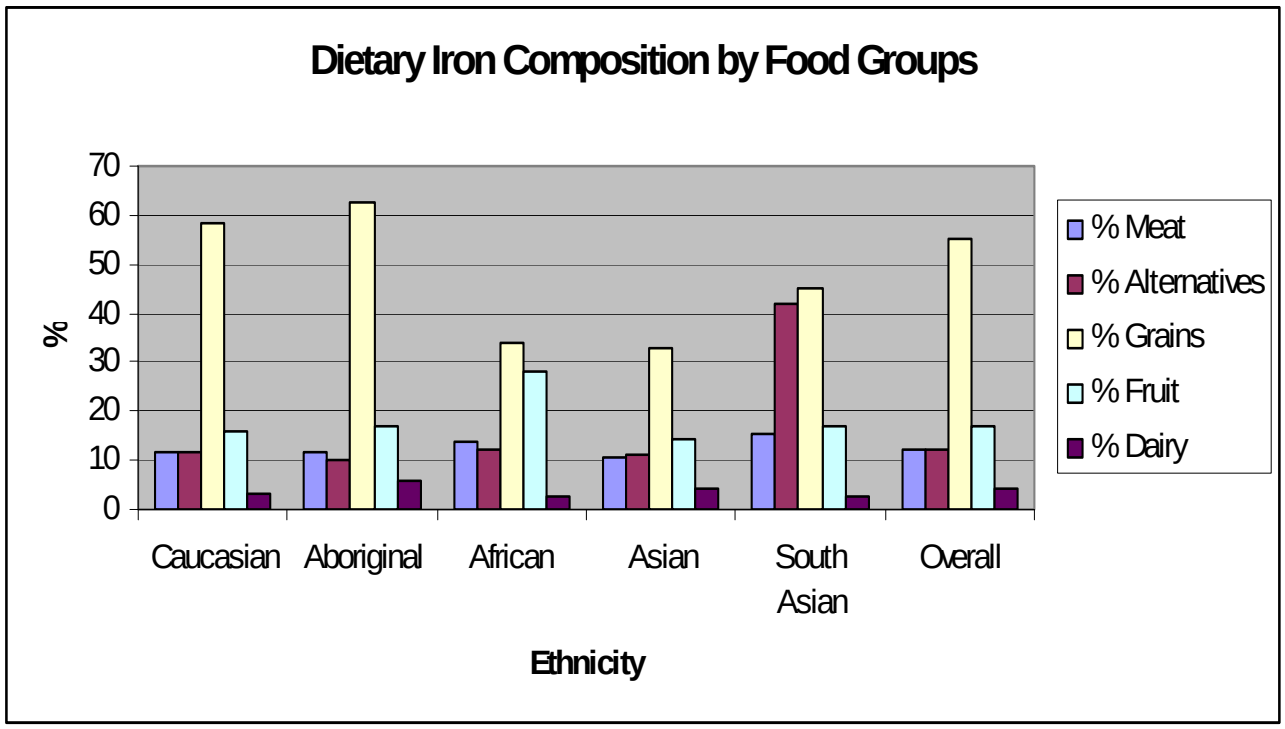

Figure 3.2 Dietary Iron Composition of Food Groups by Ethnicity

3.6 Vegetarianism and Iron Intake Patterns

Since vegetarians tend to obtain the majority of their dietary iron from non-heme sources, such as Alternatives and Grains, the prevalence of vegetarianism in the sample was compared to the prevalence of IDA by ethnicity to support the hypothesis that as vegetarianism increases, so would the prevalence of IDA. Prevalence of past or present vegetarianism was highest in South Asian women at 100%, likely due to cultural and

religious reasons. Prevalence of vegetarianism among other ethnicities was 33% among Asian, 23% among Caucasian, and 20% among Aboriginal and African women, respectively (Figure 3.3). Between ethnicities, where the reference group was Caucasian women, there were no significant differences in vegetarian status compared to Aboriginal women (Chi-square p-value = 0.94), African women (Fisher's Exact p-value = 1.00), and Asian women (Fisher's Exact p-value = 0.56). However, significant differences in vegetarianism were observed between Caucasian and South Asian groups (Fisher's Exact p-value = 0.02). Testing the hypothesis of a proportional relationship between vegetarianism and IDA, results do not indicate a significant differences in terms of vegetarian status and IDA when stratified by ethnicity (Mantel-Haenszel p-value = 0.89). Clearly, these results could be related to the small sample size of African, Asian, and South Asian participants, as well as the absence of a clear definition for vegetarian status. Specifically, the question relating to vegetarian status only asked about past or present vegetarianism, and did not explore specific dietary habits and their duration.

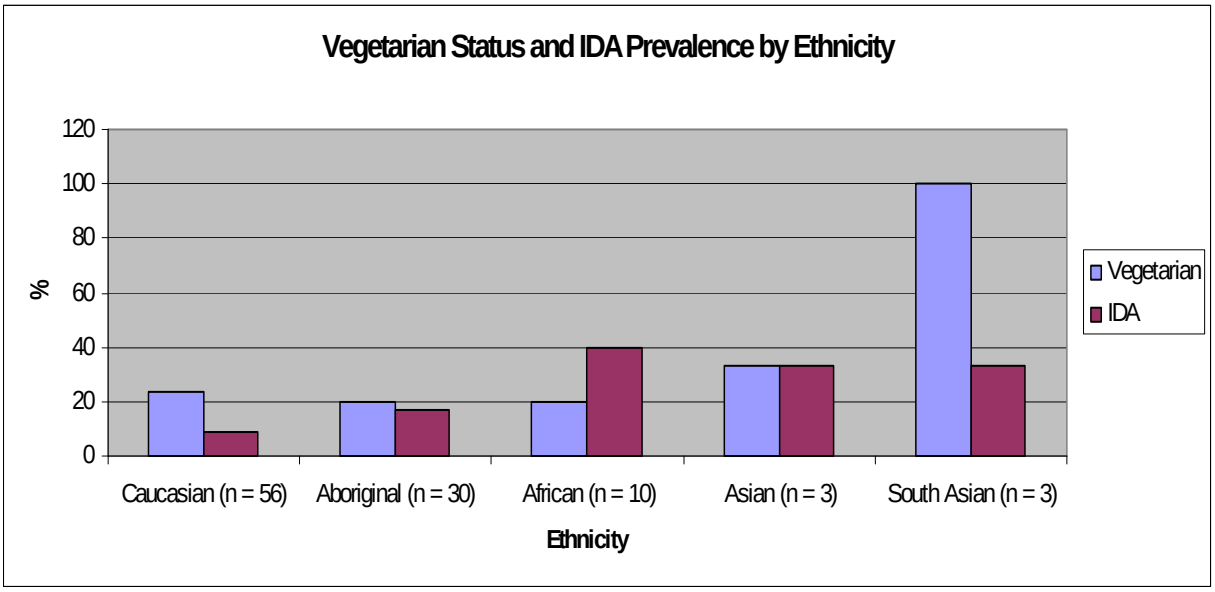

Figure 3.3 Vegetarian status and IDA by Ethnicity

3.7 The Relationship between Iron Status and Food Insecurity

Figure 3.4 shows the prevalence of two food insecurity status indicators, the inability to afford food in a 1-month period and use of food assistance programs, stratified by ethnicity. Asian and South Asian women did not report food insecurity, and were therefore not included in the analysis. Eighteen percent of Caucasian women, 43% of

Aboriginal women, and 40% of African women reported inability to afford food in a one-month period. Significant differences among Caucasian women and Aboriginal women (Chi-square p-value = 0.02) were observed, but not between Caucasian and African women (Fisher's Exact p-value = 0.18), nor among Aboriginal and African women (Fisher's Exact p-value = 0.86) due to low number of African participants in this sample. The use of food assistance programs was highest among Aboriginal women at 90%, followed by Caucasian women at 57%, and African women at 30%. Significant differences were observed between the Caucasian and Aboriginal women (Chi-square p-value = 0.04), and Aboriginal and African women (Chi-square p-value = 0.007), indicating that use of assistance programs was significantly greater among Aboriginal women than among any other groups. No significant differences were found between Caucasian and African women, however (Chi-square p-value = 0.21) in terms of food assistance program use.

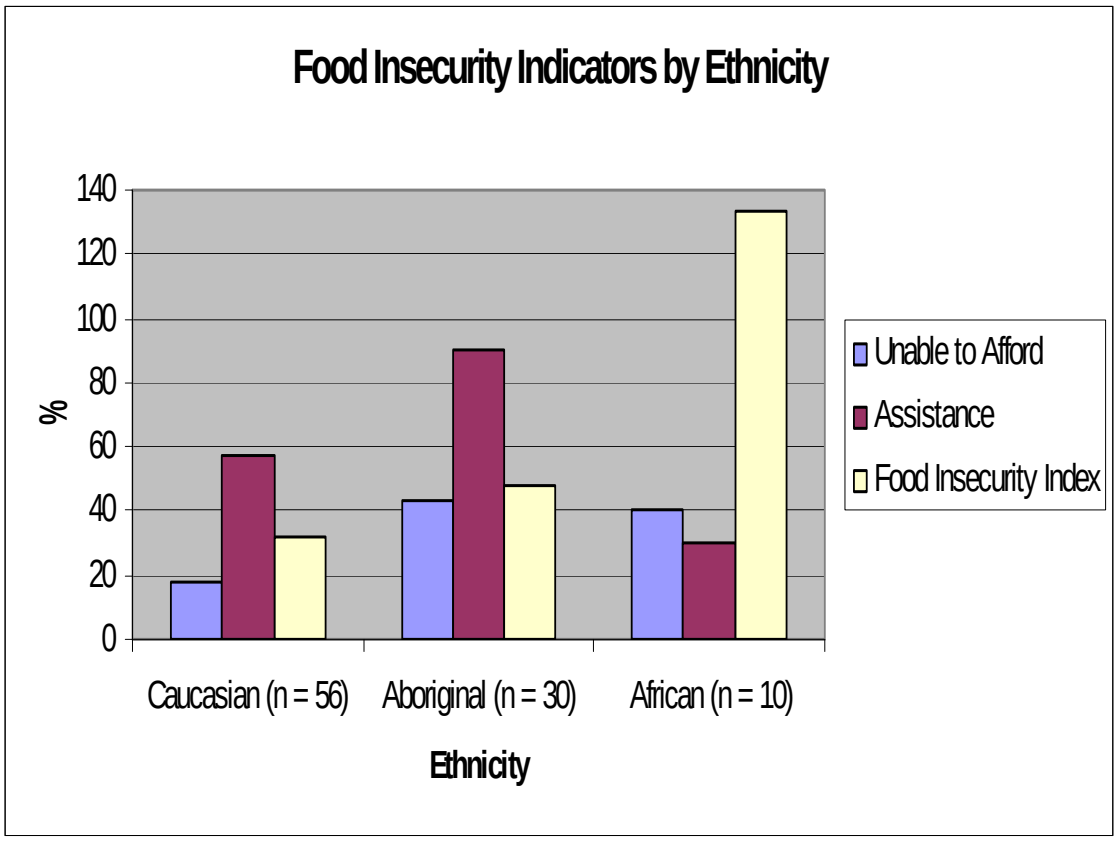

Figure 3.4 Food Security Indicators by Ethnicity

A food insecurity index was calculated, which was defined as the proportion who could not afford food over the proportion that used food assistance programs. This indicator, if high, would then be able to quantify the degree of food insecurity that was present, despite food assistance use. As shown in Figure 3.4, for Caucasian and Aboriginal women, the food insecurity index is 31% and 48% respectively, while it is 133% for African women. These results indicate that despite inability to afford food, Caucasian and Aboriginal women are able to supplement their food intake through the use of food assistance programs. On the other hand, African women are still food insecure because there is a greater proportion who cannot afford food compared to the number accessing food assistance programs.

3.8 Differences in Menstruation Pattern by Ethnicity

Irregular menstruation patterns were reported only by 5 Caucasian (9%) and 2 Aboriginal women (6%), while 22 (39%) Caucasian, and 12 (40%) Aboriginal women had regular menses, and the remaining 27 (48%) and 17 (57%), respectively, reported amenorrhea. Among African women, 8 (80%) reported regular periods, while one Asian (33%) and two South Asian women (67%) reported regular menstruation (Figure 3.5). Differences between regular menstruation patterns among ethnicities were not significant between Caucasian and other ethnicities. Differences in irregular menstruation pattern between Caucasian and Aboriginal women were not significant, nor were patterns of amenorrhea. In term of median number of days of menstruation, there were no significant differences between groups (Kruskal-Wallis p-value = 0.63).

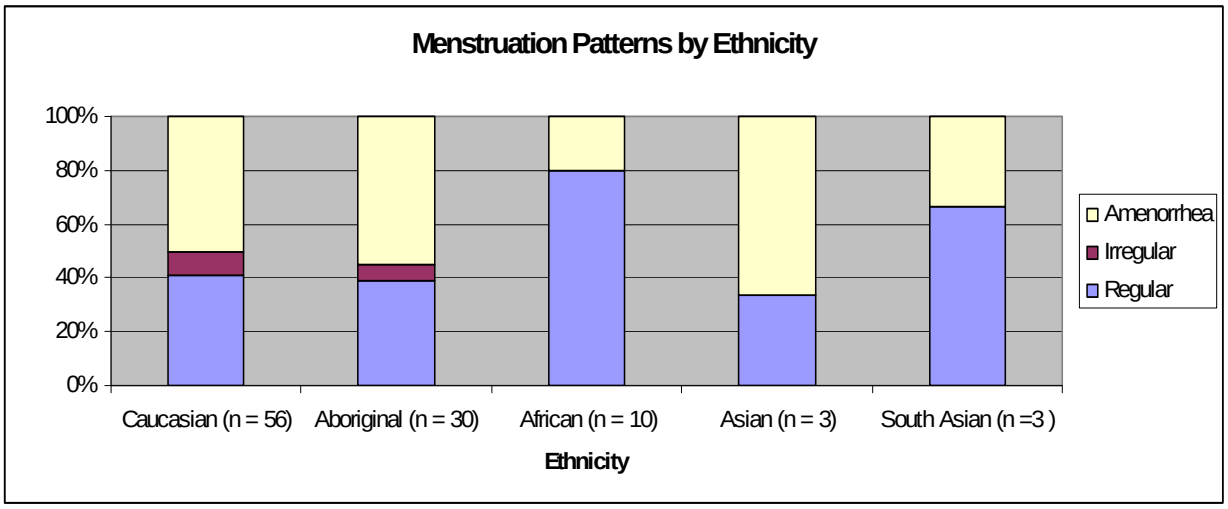

Figure 3.5 Menstruation Pattern by Ethnicity

3.9 Predictive Value of Diet Survey

Table 3.3 depicts the populated two-by-two contingency table for the calculation of sensitivity, specificity, PPV, NPV of the diet survey, where the counts for categories of iron intakes are compared to IDA status in a contingency table. The sensitivity of the survey to correctly classify those with IDA as exhibiting suboptimal iron intakes was 12.5% (sensitivity =2/16*100%).The specificity of the survey to correctly classify those without IDA as exhibiting adequate dietary iron intakes was 81% (specificity = 70/86*100%). The PPV of the survey was 11% (PPV = 2/18*100%) and NPV 83% (NPV = 70/84*100). These results indicate that the diet survey does not have sufficient sensitivity to predict iron status in this sample and that iron status in women with HIV is more strongly predicted by other variables other than dietary iron intake.

Table 3.3 Contingency Table for Predictive Value Calculations

	IDA Present	IDA Absent	Total
Iron Intake <8.1mg/d	2	16	18
Iron Intake $\geq$ 8.1 mg/d	14	70	84
Total	16	86	102

3.10 Linear Relationship between Ferritin and Meat Intake

Based on graphical representation on histograms with density plots, meat intake was normally distributed, while ferritin had a non-normal distribution. Using a scatter diagram, the majority of the data were clustered at the lower end of the graph, eventually creating a "fanning" effect, indicating that the linear relations did not hold. The Pearson correlation for ferritin concentration and meat intake was r = -0.09, an indication that there was no association between the variables in this study. When ferritin concentrations were logarithmically transformed, it was still not possible to observe a linear relationship. Since only 43 observations in this sample were available on ferritin, there is likely too much variation in this measure to produce statistically sound relationships. A high degree of random error in dietary meat intakes, inflated by too few observations, also contributed to the inability to elucidate this relationship.

3.11 Logistic Regression Modeling for Predictors of IDA

To determine whether there is a proportional relationship between iron intakes and IDA to support the use of a logistic regression model, iron intake were separated into three levels (tertiles), and each tertile midpoint graphed against the log-odds of IDA (Figure 3.6). The midpoint for each tertile was calculated as 7.9 mg/d, 12.1 mg/d, and 18.1 mg/d, respectively. Although no significant linear relationship was found between iron intake and the log-odds of IDA (F statistic = 4.9, 1 degree of freedom, p-value = 0.27), there appears to be some degree of proportionality between the log-odds of IDA and iron intake (Multiple $R^2 = 0.83$). Although this relationship is non-significant, its direction is counterintuitive to the hypothesis that iron status should improve with iron intake, as it appears to be the opposite case shown in Figure 3.6. There are several confounders and sources of bias, including random error due to the high variability in daily iron intake among individuals as well as differences in iron bioavailability that are shifting the relationship between IDA and iron intake in a positive direction, and hence, the opposite direction as initially hypothesized.

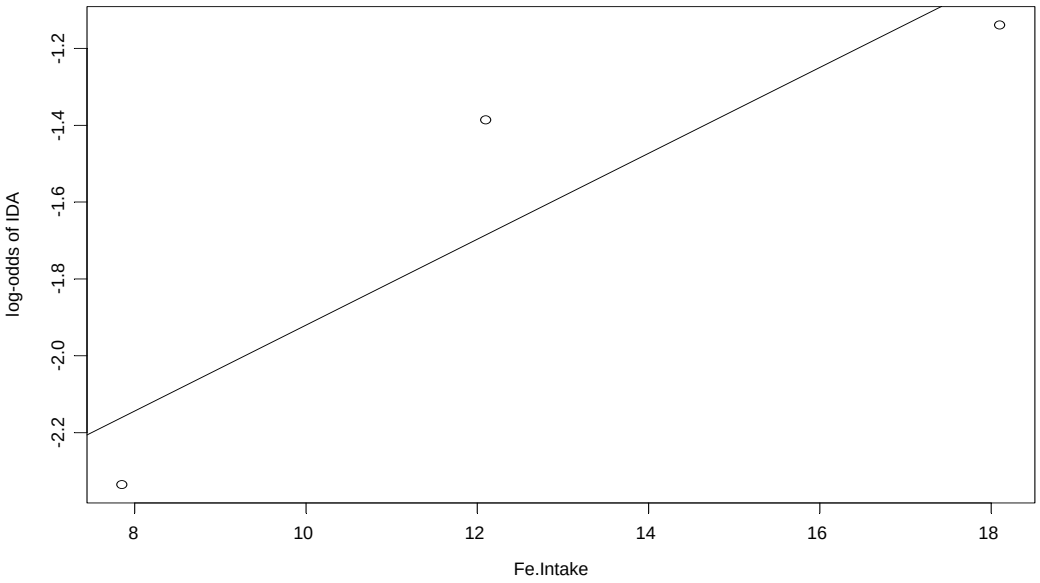

Figure 3.6 Relationship between Iron Intake the Log-Odds of IDA

The results of the logistic regression analysis are provided in Table 3.4, whereby each variable is adjusted for the other in a multiple logistic regression model. Variables were chosen based on the hypothesis that they were significant predictors of IDA, based on previous findings in the literature. Adjusted odds ratios (OR) and 95% confidence intervals (95% CI) for each variable are provided. The following variables were significant predictors in IDA risk: African ethnicity, regular menstruation pattern, and CD4 cell count<200 cells/μL. Despite adjustment for these and other independent predictors identified from the literature, there was no significant association between iron intake from the diet survey and likelihood of having IDA. In fact, the OR of 1.03 indicates that there is likely no relationship between iron intake and iron status as measured by the diet survey. It is likely then, that a biologically plausible relationship between iron intake and iron status could not be elucidated due to the following factors: iron intake was not captured adequately by the diet survey; intakes shows a great degree of variation; and that there are likely more confounders that impact this complex relationship of iron intake and status in this population than were adjusted for. The log-likelihood ratio test statistic for the multiple logistic regression model was 26.4, degrees of freedom = 12, and p value <0.009, indicating that this model is significant to describe the relationship between iron intake and IDA.

Table 3.4 Adjusted Predictor Variables of Logistic Regression Model

Variable	Odds Ratio (OR)	95% CI	P-Value for OR
Estimated Iron Intake (mg/d)	1.03	(0.92, 1.17)	0.57 (NS)
Ethnicity			
Caucasian	1.00	(reference)	
Aboriginal	1.12	(0.21, 6.1)	0.89
African	8.6	(1.1, 68.0)*	0.04*
Asian	17.1	(0.63, 463)	0.09
South Asian	11.7	(0.32, 428)	0.18
IDU	0.47	(0.06, 3.8)	0.48
Menstruation			
Regular	8.1	(1.5, 44.0)*	0.02*
Amenorrhea	1.00	(reference)	
CD4 (cells/μL) <200	8.8	(1.7, 46)*	0.01*
Multivitamin Use	2.15	(0.47, 9.8)	0.32
Iron Supplement Use	3.1	(0.57, 17.0)	0.21
Vegetarianism	2.0	(0.44, 9.0)	0.37
Food Assistance Program Use	3.0	(0.52, 17.0)	0.22

4. DISCUSSION

4.1 Major Findings

The objectives of this study were to determine the prevalence of anemia, ID, and IDA in a sample of HIV-positive women; to quantify the effect size of nutritional, medical, and social predictors on the likelihood of having IDA; to determine whether there is a proportional relationship between meat intake and ferritin level; to calculate the predictive value of an iron-specific diet survey; to estimate dietary trends; and to quantify the impact of food insecurity on iron status and nutrition. All objectives were achieved, with one overall finding that the impact of iron intake on iron status in women with HIV is extremely complicated, and that several unmeasured factors, such as the impact of HIV on iron absorption and metabolism, may play a greater role in iron status than nutrition alone. Moreover, the significantly lower iron intakes in this population compared to results from studies on HIV-negative women indicate that there are likely greater social barriers that affect HIV-positive women in comparison. Thus, more routine interventions for screening and treatment of ID and IDA, as well as greater attention to this research area are required to help alleviate this condition in HIV-positive women.

4.1.1 Characteristics of Participants

General demographic characteristics of the sample showed satisfactory health status, as indicated by a normal mean body mass index (BMI), a median CD4 count >350cells/μL, and a high proportion of participants who used antiretroviral therapy (ARV) (74%). As the majority of participants were recruited from a clinic outside of the Downtown Eastside, a selection bias may be the reason behind such relatively good health status measures. Specifically, compared to hospital patients and participants from the Downtown Eastside, participants at Oak Tree Clinic were more likely to be healthier, attend routine follow-up visits, be compliant with ARV regimens, and to have stable housing and support systems. Participants recruited from Downtown clinics (nearly 40% of the sample) were more likely to be of Aboriginal descent and to experience social issues such as substance abuse and poverty. Also, although recruitment at all sites was random, it is likely that women who were healthier in general, more interested in nutrition, and more literate in terms of health knowledge would be more likely to

participate. Overall, however, this study was successful at representing the health status spectrum of women with HIV.

4.1.2 Prevalence Estimates

One objective of this study was to determine the prevalence of anemia, ID, and IDA in a sample of hospitalized and community-living HIV-positive women in the Greater Vancouver area. Prevalence of anemia was 30%, prevalence of ID was 40%, and prevalence of IDA was 16%, whereby IDA accounts for 53% of anemia cases. These estimates are similar to those reported in other North American studies of HIV-positive women who used injection drugs, where two studies reported anemia prevalence at 44%, ID prevalence at 43%, and IDA prevalence at 21% (5, 14); and others showed that ID accounted for 50% of anemia cases (14, 28). It is surprising, then, that despite the healthier status of this sample, these prevalence estimates would not be significantly different from those obtained in more marginalized populations.

4.1.3 Predictors of IDA

A second objective was to quantify the effect size, as measured by the odds ratio, of medical, social and nutritional factors on the likelihood of having IDA by using multiple logistic regression modeling. The sample size of this study was too small to achieve statistical power, especially for the multivariate logistic regression model, where observations in the IDA group were less than 10 events per variable for most covariates. However, several statistically significant predictors for IDA were identified, which included African ethnicity, regular menstruation pattern, and CD4 count <200cells/μL. Specifically, women of African ethnicity were nearly 9 times as likely as Caucasian women to have IDA, likely due to socio-economic disparities and the potential for higher prevalence of thalassemia in this population, although records of this condition were not found in chart reviews (1, 8, 9). Because food insecurity was a major issue for African women in this sample, this may also contribute to higher IDA risk in this group.

Women with CD4 counts<200cells/μL were nearly 9 times as likely as those with higher CD4 levels to have IDA, where low CD4 cell counts have been shown in other studies to predict anemia risk in persons with HIV (1, 9, 15). Menstruation pattern has

not been as widely researched in the HIV-positive population, but is a significant factor in determining iron status: those with regular menstruation patterns were 8 times as likely as those with amenorrhea to have IDA. In HIV-negative female populations of childbearing age, menstruation has been identified as a major predictor of iron status, and this likely applies to this sample as well (21, 22, 44, 66).

Dietary iron intake, when adjusted for other potential confounders, showed no relationship to iron status (adjusted OR = 1.03). It was not appropriate to adjust for absorption factors (heme and non-heme iron sources) since iron intakes for the majority of the sample were so low, that adjustment led to extremely low iron intake values. Also, using the minimum daily requirement of 8.1mg/d, a level which does not account for individual absorption differences of dietary iron, it was assumed to be best to leave iron intakes as unadjusted. Had a level of "minimum daily absorbable iron requirement" existed, which accounted for adjustment of absorption modifiers then, this would have likely supported the application of an adjustment factor. Another reason for not observing a biologically plausible relationship between iron intake and status was that a large proportion of participants did not consume adequate amounts of iron (<DRI), and so, there was little variation in intakes to be able to observe a relationship, as compared to the case where intakes would have had more distinct variation.

Based on studies of iron intake in HIV-negative women, it has been suggested that iron intake be adjusted for both iron status and presence of absorption modifiers found in other foods (21-23, 32, 33, 43). However, in this sample, unadjusted iron intakes from heme and non-heme sources were so low that when correction factors were applied, results showed very low values compared to what has been reported elsewhere. Specifically, this sample showed a mean heme iron intake of 0.14 mg/d compared to levels obtained from other studies: in a study by Galan and colleagues among French healthy women, heme iron intake was 1.1mg/d, whereas, in a study by Pynaert and colleagues, mean heme iron intake was 0.6mg/d (21, 22); in Australia, a study by Heath, Skeaff, and Gibson showed a mean heme iron intake of 0.8 mg/d, whereas a study by Patterson et al found levels of 0.9mg/d (31, 46). This marked difference in intakes between HIV-positive and negative groups is likely related to social and economic

differences among them such as a higher degree of food insecurity, substance abuse, as well as poorer dietary habits and limited access to nutritional services in HIV-positive women as compared to healthy women.

In view of these differences, it was concluded that the correction factors for heme and non-heme iron foods may not apply to populations where iron intakes are significantly below required levels. Moreover, differences in absorption physiology between HIV-negative and HIV-positive persons may be different and adjustment algorithms designed thus far may not be adequate enough to account for this.

Although attempts were made to adjust iron status of the individual by including the variables for menstruation and vegetarian status in the logistic regression model, there may be other factors, such as the impact of HIV on nutrient absorption and variations in absorption efficiency based on iron status (IDA vs. no IDA), that were not adequately adjusted for and greatly affected iron absorption estimates. Specifically, inability to correct for the latter would underestimate iron absorption in those who have IDA, and overestimation of iron absorption in those with normal iron status (4, 23, 32, 33, 66).

Other nutritional predictors such as supplement use and food insecurity were not significantly associated with IDA due to a small sample size. This resulted in low statistical power to detect differences in variables. Multivitamin (MVM) supplement use was frequent in this sample, although not significantly protective against IDA in the logistic regression model. However, when iron intakes were combined with multivitamin use, 53% of the sample was able to meet their DRI for iron. Although bioavailability of iron from MVM was not accounted for, thus overestimating its iron contribution, and the fact that iron supplements were excluded from total dietary iron intake are limitations, significant differences between total mean iron intake including a MVM, and excluding a MVM were observed (t-test p value<0.001). These results highlight the benefit of a daily MVM for the improvement in dietary iron intakes, which may otherwise not reach recommended amounts in this population.

4.1.4 Association between Dietary Iron Intake and Iron Status

It was hypothesized that a proportional relationship between meat intake and ferritin would be observed in this study, whereby ferritin levels would increase with increasing dietary iron intake from meat (22, 30, 44-47). However, such a linear relationship was not observed in this study. Possible explanations for these findings are related to the complex nature of iron absorption and metabolism in persons with HIV, which may play a much stronger role than dietary intake on iron status. Therefore, a dose-response or linear relationship cannot be expected without adjustment for these confounders. Moreover, the limited number of ferritin measurements available (42 observations), increased the variability in this measure, thus subjecting it to random error. The presence of inflammation may also have prevented the observation of an association, as this can mask true IDA with increased ferritin levels. Also, as mentioned, meat intake in this sample was significantly lower as compared to other studies, which may have led to low values, rendering it more difficult to observe differences.

4.1.5 Predictive Value of Diet Survey

The sensitivity of 39% and positive predictive value of 16% of the diet survey to predict IDA status indicate that in its current form, the former is unable to predict iron status. It is possible that there are too many other factors involved in the relationship between iron intake and status that would prevent the design of a survey that could be used as a screening tool for IDA in women with HIV. In particular, dietary intakes should be adjusted for aforementioned factors such as individual gastrointestinal absorption ability, iron status, and general dietary composition.

Other possible sources of error included reporting bias due to memory recall issues, social desirability, and social approval, as well as factors such as dieting behavior. The degree of underlying food insecurity may have actually resulted in lower iron intakes across the entire sample, irrespective of iron status. Iron intakes may also have been underestimated due to the limitation of the diet survey to include food combinations, which are more likely to elicit accurate reporting than single food items alone (36). This underreporting may have led to an underestimation of iron intake among all participants, and likely a misclassification bias towards no difference between groups (the null), such

that a greater proportion of participants' intakes (although above minimum requirements) were categorized into the category of sub-optimal iron intakes.

Another potential source for misclassification is the cut-off level for sub-optimal iron intake, which in this study was set at the estimated average requirement (EAR) of 8.1mg/d (21, 23). This level is defined as the minimum amount of total iron required in the diet in order for 50% of the healthy population to meet their needs. Because iron intakes among the majority of participants were sub-optimal irrespective of iron status, the EAR may not have been a sensitive enough limit at which to classify low and high intakes. On a related note, the ferritin cut-off level chosen for diagnosis of IDA (<30μg/L) may not have adequately accounted for inflammation in this sample (67). Thus, those who had depleted iron stores and inflammation may have been classified as not having IDA, even though their stores were depleted. This would have also resulted in an attenuation of the effect between low iron intake and IDA status, thus reducing sensitivity of the diet survey.

4.1.6 Dietary Iron Intake Patterns and Food Habits

Results for dietary intake patterns in this study are in line with those from previous investigations. For example, as reported in European studies (21, 23), the majority of participants in this sample obtained their dietary iron from grain products (55%), followed by meats and alternatives (combined 24%) and fruit and vegetables (17%). In this study, the likely reason for fruit and vegetables ranking as a potential iron source despite the fact that these foods are very low in iron content, may be related to the higher frequency of their consumption, as well as the fact that 21 out of the 65 food items included on the survey were from this category.

Grain products likely comprised the main dietary iron source because they are affordable, create satiety when overall access to food is inadequate, and are often provided in greater amounts at food assistance programs. Since the majority of participants relied on such services to supplement what they could not afford to purchase, it is likely the reason why foods such as grains and fruit and vegetables dominate the iron sources in the diet over meat products. Other possible explanations include that the

greatest proportion of caloric intake is derived from carbohydrates; that carbohydrate portions are often larger than that of other food groups; and that meat consumption has declined globally over the last decade in view of the associated health risks of consuming red meat (23, 30, 66, 68).

Vegetarian food habits were noted in this sample, but only among one-quarter of the participants. Other studies have shown that vegetarians tend to have lower iron stores and are at higher risk for ID and IDA (30, 69). Primarily, vegetarian food practices were most pronounced among South Asian women, who for cultural and religious reasons tend to consume a diet free of meat products, poultry, and shellfish. In this study, vegetarianism was not associated with IDA due to a series of factors. First, the group among whom vegetarianism was most common (i.e. South Asians) was very small in sample size, thus lowering the power of the comparison. Vegetarian status was also not precisely defined on the diet survey, so some participants may have included themselves in this category if they did not consume red meat, but consumed poultry, fish and seafood. Furthermore, the length of time the vegetarian diet was followed was not requested and this can significantly impact iron status as long-term vegetarians adjust to lower iron intakes by increasing absorption efficiency compared to new vegetarians (45, 69).

Other studies have also reported a lack of association between dietary iron intake and vegetarian status, whereby confounding by poor dietary habits rather than the vegetarianism was suspected (44, 66). In fact, vegetarian diets can be adequate in iron if foods that enhance non-heme iron absorption, such as ascorbic acid, are paired with grains, nuts, and legumes (31, 44). Because overall dietary information was not recorded in this study, such food pairings and other dietary habits could not be investigated.

4.1.7 Menstruation Patterns

Regular menstruation pattern was a significant predictor of IDA, but the role of irregular menstruation patterns could not be studied in detail due to low numbers of participants in this category. Mainly, irregular menstruation was reported in Caucasian and Aboriginal participants, where such irregularities may be linked to disruptions in the menstrual cycle through intravenous drug use (IDU) (5, 16, 17). In fact, IDU was

reported in 36% of Caucasian, and 60% of Aboriginal women, but not reported by participants of other ethnicities. Although a significant relationship between IDU (and the likelihood of IDA) was not found in the logistic regression model, a significant difference in IDU was found between participants who had IDA and those who did not, where IDU was higher in those without IDA. It is plausible that IDU serves as a protective mechanism against IDA by disrupting the menstrual cycle. In a simple logistic regression model, women who used intravenous drugs were less likely to have regular menstruation patterns (OR = 0.21, 95% CI (0.09, 0.51)).

This study also showed differences in menstruation pattern by ethnicity, supporting the evidence that African American women are more likely than European American women to have episodes of heavier menstrual bleeding (70). In this sample, African women showed the highest percentage of regular menstruation pattern of all ethnicities, although sample size in this group was too small to detect significant differences.

4.1.8 Food Insecurity

There have been several studies indicating that food insecurity is a significant driver of poorer nutrition status and IDA in HIV-positive women (7, 12, 13, 15). There was no significant association between use of food assistance programs (the indicator of food insecurity in this study) and IDA, but these results could have been attributed to low statistical power, such that the majority of participants, irrespective of IDA status, used food assistance programs. In fact, the prevalence of food insecurity in this sample (61%) was nearly triple that of lone parent food-insecure households (22%) (71).

Thus, the impact of food insecurity on nutrition status of HIV-positive women cannot be understated, particularly among Aboriginal and African women who used food assistance programs most frequently (13). The difference between these two groups, however, lies in the fact that Aboriginal participants are more well-connected with community services and thus able to supplement the food they purchase with food assistance programs (hence the majority in this group reported ability to afford groceries in a one-month period). In other words, food assistance use may narrow the gap in food insecurity among Aboriginal as compared to African women, as supported by the lower

food insecurity index in the former group. On the other hand, African women, the majority of whom are new to Canada, may not yet be familiar with the social services programs, are unaccustomed to the food assistance environment and culture or fearful of the stigma associated with using such programs, and thus may find it more of a challenge to connect with supportive services.

The type of food assistance program most often used was the food bank (68% of those who used food assistance programs). This is an important finding, since the intent of such services was to serve as an emergency measure for those unable to afford food but instead seems to be a mainstay for supplementing food costs among participants. This is problematic, because food supply to food banks is inconsistent; their environment is not always supportive of families or cultural needs; and by relying on donations, they often do not provide balanced or nutritious foods. For the most part, these foods are low in iron, highly processed, and consist of refined carbohydrates that may not necessarily be fortified with iron. From an emotional perspective, food bank use can also be the most embarrassing and socially undesirable experience for women, who consider themselves the providers of food for their children.

Therefore, food bank use can actually serve as a vehicle for disconnecting women from food from a philosophical perspective, and dissuading them further from engaging in the enhancement of nutritional wellbeing. Even foods that are high in iron, such as canned foods, may be viewed as “charity foods” and therefore excluded from the diet. Often, the quality of the food provided at food banks is poor, with food spoilage cited as a major problem (72). In turn, the impacts of poor food quality place persons with HIV at risk for iron deficiency in two ways: the resultant gastrointestinal effects, such as diarrhea and vomiting, due to food-borne illness, if the food is consumed, or the dietary deficiency from insufficient food supply if the food is discarded. Such deficiencies are compounded in larger food insecure households, where women feed their children first before attending to their own nutritional needs (24).

4.1.9 Utility of Diet Survey

At this time, the diet survey requires major revision before it can be used as a diagnostic tool for IDA. As iron absorption and metabolism in HIV is a complicated matter, it may be that standard measures of dietary iron intake will not be appropriate for this population. However, for this study, the survey has served as a useful screening tool for identifying the impact of different factors on IDA development, as well as been a successful means by which to engage women in a discussion about their health, nutrition status, and lifestyle factors. In future, the survey could be used as a vehicle for delivery of health and nutrition education: as participants are responding to questions, areas that may particularly affect iron status can be highlighted and discussed. Also, the survey is quickly administered, but able to measure the impact of multiple health indicators which may not otherwise be asked about during routine clinic visits, such as menstruation pattern, food security status, and compliance with use of supplements. Thus, this could result in an open dialogue, whereby women have the opportunity to discuss health issues which are of greatest relevance to them. Finally, if routine blood tests are ordered, the responses on the survey could be used as a guide to understand why the resultant iron storage levels are observed.

4.2 Recommendations

4.2.1. Routine Screening Measures and Medical Management

As supported by the results of this study, women with HIV are at high risk for low iron stores due to changes in immune status, inadequate dietary intake, financial barriers, and losses due to menstruation. In this study, the protocol for most clinics was to test ferritin and other storage iron indicators only if hemoglobin levels were sub-optimal. Therefore, this approach was only able to screen those women who were at risk for IDA, but overlooked many others whose iron stores may have been low, but whose hemoglobin levels had not yet progressed to anemia. Even if hemoglobin levels were normal, this should not preclude the fact that the woman may have ID, and would still benefit from medical and nutritional intervention to prevent a further drop in iron stores. In addition to overlooking ID, complete blood count measures cannot serve as the only guide for screening, as they may show a normal MCV, despite the fact that IDA is

present, since oftentimes, persons with HIV also have concurrent B_{12} deficiency, which results in masking of low cell volume.

The consequences of untreated IDA are generally manifested in reduced work capacity as a consequence of declining physical and cognitive functioning, which first manifest symptomatically in mild ID (12, 73). Prompt diagnosis of low iron stores can help identify and treat those at risk, thus improving work capacity in women, enhancing their potential to engage in employment and school opportunities, and to be better mothers to their children. Such improvements in health may be sufficient to push some women out of the cycle of poverty. In terms of other health impacts, any unprecedented events such as surgery, infection, or pregnancy in the presence of normalized iron stores, could be better tolerated compared to complications that could result when women have low iron stores. With normal iron status, mortality risk in the case of surgery or infection is reduced, and in pregnancy outcomes, cognitive and developmental damage to newborns may be prevented (10, 11, 15, 30, 73-76). Thus, routine screening of iron storage tests, which cost approximately $10 per person, are an insignificant sum to pay when compared to the burden of disease that would be incurred in untreated ID or IDA.

Currently, there is clearly a gap between screening for low iron stores and diagnosis, since only those who have anemia are screened. New protocols in primary care should be put in place so that during each routine clinic visit (every 3 months), complete blood count, ferritin, transferrin and transferrin saturation are ordered for all women with HIV, irrespective of hemoglobin level (25, 77). All HIV-positive women should be considered high risk for IDA due to the likelihood that dietary iron needs are not being met, and the fact that their losses are significant, particularly through malabsorption, disrupted metabolism, and menstruation. Laboratory results for iron status should be more thoroughly reviewed, and supplementation (with the goal of restoring iron levels as soon as possible) initiated. For those who are already receiving iron supplements, iron storage tests should be taken once a month, and discontinued once levels have normalized.

As menstruation status has been shown to greatly affect iron status in women with HIV, those with heavy menstruation patterns should be advised as appropriate to taking

oral contraceptive pills rather than IUDs, since the former has been shown to reduce the risk for IDA, while the latter increases blood loss, and thereby IDA risk (44, 45, 47, 78).

4.2.2 Nutritional Management of IDA

There is evidence that excess iron supplementation in persons with HIV may lead to iron overload and advance viral progression (20, 74, 79), but studies are weakened by small sample size, design (observational studies rather than randomized control trials), and type of iron supplements used, which have mainly been in parenteral form (hence more potent than oral supplements) (74). In fact, low dose oral supplementation with elemental iron up to 18mg/d in a small randomized control trial has not been shown to have deleterious effects on viral replication in HIV, hepatitis C virus (HCV) replication, and iron overload (7, 74). Thus, it is safe to recommend a daily MVM to women with HIV, especially since the majority of such preparations available in Canada contain only 10mg of elemental iron, of which only a fraction is absorbed. Concerns of iron overload in this population should not outweigh the fact that most women have inadequate iron stores. Specifically, prevalence of iron overload in HIV is estimated at 7%, while prevalence of ID can be up to 50%, so the benefits of mass supplementation clearly outweigh the consequences of iron overload (14, 79).

In women of childbearing age who have regular menstruation, even moderate daily doses of iron supplementation (14 - 20 mg/d) were able to influence iron status in those with low iron stores (47). Several publications have also demonstrated the beneficial effect of MVM supplementation on overall survival of persons with HIV by improving CD4 counts and reducing viral load (38, 80, 81). It is well-established that persons with HIV have higher needs for micronutrients due to poorer dietary intakes, higher dietary requirements due to malabsorption, and increased requirements to maintain healthy immune function (38, 39, 63, 64, 80, 82). In this study, it was also shown that MVM supplementation had a significant impact on daily iron intake, and resulted in over half the sample's ability to meet their DRI for iron. Therefore, MVM supplementation is a relatively inexpensive intervention that will not only be beneficial in reducing the risk of IDA, but can also maintain the normal status of other micronutrients, whose concentrations may already be lower in persons with HIV (39).

Although to a lesser degree than supplementation, dietary intervention can also improve iron status in women with mild ID, especially among women with low compliance to an iron supplement. The impact of dietary intervention should not be underestimated, as it can engage women to comply with supplement regimens by providing a rationale for nutrition recommendations, address behaviors associated with dieting and poor dietary habits, and provide education on food pairings and choices which improve dietary iron intake. Dietary education is especially important for women with HIV in view of sub-optimal iron intakes and a tendency to consume a higher proportion of non-heme iron sources, whose absorption efficiency is significantly impacted by meal inhibitors. Thus, dietary education to women should emphasize consumption of juice or fruit with meals to improve iron absorption, and reserve consumption of inhibitors such as milk, coffee, tea, and alcohol to between meals (31). The inclusion of meat, poultry, and fish, when possible, will also enhance the absorption efficiency of dietary iron (66). Although meat consumption may be linked to cardiovascular disease and cancer risk, the benefits of liberalizing meat intake in diets of women with HIV outweighs adverse impacts of such health risks, mainly because diets of women are inadequate in meat products, and also because meat consumption does not have to become excessive to enhance iron stores. In fact, up to 90g/d (3 ounces) of meat per day would be sufficient enough to improve iron levels in this population (66). The cost of meats may be a barrier to intake in this group, in which case low-sodium processed or canned products could be substituted. Finally, although no significant protective effect of iron skillet use on IDA was observed in this study, it has been shown to improve iron status in households, and therefore, is a reasonable recommendation (23).

4.3 Limitations

Due to the constraints of the recruitment timeline, it was not possible to recruit the required sample size of 246 participants as needed for a prevalence study. Instead, only 102 participants were recruited. In turn, the prevalence estimates calculated can only account for about 41% of the prevalent anemia, ID and IDA cases. Moreover, the sample size potentially limited the number of significant predictors of IDA as several predictors, such as supplement use, food insecurity, and antiretroviral use have been shown elsewhere to impact

iron status, but did not reach significance in this study. Sample size of ethnicities such as African, Asian, and South Asian were also too small to observe differences in IDA risk that could be present in each of these groups.

Aside from the low sample size, estimates of ID prevalence were not representative of actual prevalence because, based on clinic protocols, ferritin levels were obtained only when hemoglobin concentrations were below normal. Thus, prevalence of ID could only be calculated for a fraction of participants. Anemia prevalence estimates were limited in ability to distinguish between anemia due to iron deficiency and anemia due to other etiologies, such as B_{12} deficiency, opportunistic infection, immune compromise, medication-induced anemia, or HIV-associated hemopathies (11, 83). Therefore, it was assumed that when both low hemoglobin levels and storage iron markers were low, IDA was present, without exploration of other potential anemia causes.

As one goal of this study was to evaluate potential predictors for IDA, an estimate of incident IDA cases would have provided a more accurate measure to prevent incidence-prevalence bias, a bias which blurs the differences between newly diagnosed IDA cases (who may have had the condition for some time) and actual new onset cases (84). In this study, incidence would have been calculated retrospectively by reviewing laboratory records of those with IDA to determine when the first diagnosis could have been made. However, in the absence of comprehensive records of previous laboratory measures of storage iron markers for the majority of participants, incidence of ID and IDA could not be determined.

It was also a challenge to adjust for factors that impacted dietary iron intake, such as absorption modifiers found in foods and iron status. Meal composition was not assessed through the diet survey, nor was the heme and non-heme adjustment factor for food items appropriate in this sample where the majority had very low unadjusted iron intakes. Because iron metabolism in HIV cannot be compared to that in healthy individuals, it may be necessary to develop adjustment algorithms that are specific to the HIV-related factors that affect iron absorption, rather than applying correction factors that lead to questionable outcomes (such as very low iron intakes after applying adjustment in this sample).

Total daily caloric intake was also not assessed by the diet survey, as participants would not have been able to complete food records. However, it may have been possible to estimate average caloric intake by collecting one 24-hour recall and using total calories to adjust iron intake accordingly, instead of relying on absolute iron intakes for comparison.

Although an advantage of this study was the design of a diet survey that was able to focus specifically on measuring iron intake, compared to generalized national surveys (such as the NHANES and Nurses' Health Study (NHS) questionnaires), which lack accuracy in their estimation of specific micronutrients, it requires significant revisions before it can be considered a reliable tool for producing valid estimations of dietary iron intake (70). The inclusion of combination foods or mixed dishes would improve estimates, as they may be significant dietary iron sources that are frequently consumed by participants, and improve accuracy of reporting through prompting. In other words, a list of single food items may lead participants to underestimate intakes by forgetting to report combination dishes that include the food item. For example, although 82% of participants consumed beef, an iron-rich food source, it is likely that exact number of times beef was consumed in a week was underreported because examples of common beef-containing foods, such as spaghetti or hamburgers, were not provided. Moreover, items may not have fully represented food choices made by the participants, despite the attempt to determine frequently consumed foods through the phase 1 pilot study.

Aside from the incompleteness of the food list, errors in underreporting could also in part be due to memory recall issues among some participants who may have had difficulty recalling food items consumed up to one week prior to the interview, as well as other sources of reporting bias, social desirability bias (SDB) and social approval bias, which may have attenuated the impact of diet on IDA. SDB is defined as tendency of an individual to convey an image in keeping with social norms in order to avoid criticism in a testing situation, while social approval is the tendency for an individual to seek a positive response in the testing situation and, therefore, a less defensive response (85). Women are more likely to exhibit SDB and social approval bias compared to men in dietary assessments, especially because the former are more vulnerable to erratic eating patterns such as binge eating, emotional eating, or excessive dietary restraint (85). Thus, underreporting due to

embarrassment or fear of criticism can significantly distort dietary iron intake measurements. Specifically, if SDB was present in this study, there would be a tendency toward non-association of dietary iron intake to IDA through underreport of both general and food-specific intakes (for example canned or processed foods) which are viewed as less nutritious choices.

Since vegetarianism was not a predictor of iron status, this raises the question of whether provision of a more precise definition would have led to more biologically plausible outcomes (i.e., that iron stores are lower among vegetarians). In this study, participants were asked about past or present vegetarianism, but a specific definition of this dietary lifestyle, or the duration of such a diet were not provided. Therefore, the term 'vegetarianism' was left to interpretation by participants, and thus, may have resulted in false reporting.

A confounder that directly affects iron status via changes in menstruation pattern is the use of oral contraceptives, intra-uterine devices (IUD), and injections of Depo-Provera®, all of which will influence the frequency and the duration of menses. Specifically, oral contraceptive use decreases menstrual losses by 50% and IUD may increase losses by as much as 100%, while injections may have variable effects (44, 45, 47). However, data on oral contraceptive use and type were not collected in this study, although their use may have played a significant role in the frequency and duration of menstruation patterns.

4.4 Implications for future research

Factors that impact iron absorption are complicated to study in populations such as HIV-positive individuals since information on meal composition was required (from food records) to assess the impact of absorption modifiers. As mentioned, cognitive changes, non-compliance, and memory recall issues were barriers to obtaining accurate food records from this group. Even if food records were available, however, the existing validated algorithms for adjusting for iron status and absorption modifiers have not been designed for HIV-positive individuals. Therefore, these equations do not account for the major effects of HIV on iron metabolism and gastrointestinal absorption, and, therefore, may not be applicable for use in such cases.

Further studies are required in HIV-positive groups to determine what additional adjustments to the algorithm are necessary so that it can be applied to this population with a more precise estimate of absorbable iron. Moreover, studies should also address modifications in correction factors for heme and non-heme iron that are more appropriate for iron intakes which are suboptimal, such that corrected values are more useful for analysis, instead of being extremely low and therefore devoid of variation for comparison in a statistical analysis.

In its current state, iron intakes calculated from the diet survey are too simplistic to assess the complexity of iron utilization and absorption in a population with HIV, whose gastrointestinal health is much different than a healthy population, and whose immune status plays a significant role in iron metabolism. Therefore, future investigations should focus on identifying specific predictors that influence dietary iron intake in persons with HIV and to develop algorithms specific to HIV-positive individuals. Specific methodologies developed by Hallberg using a variety of test meals and radio-labeled iron could be used to assess absorption efficiency of meals in this population (23, 33). Moreover, a consensus on a standardized minimum level of absorbable iron that accounts for adjustment of absorption modifiers in its calculation should be determined.

In terms of the benefits of MVM and iron supplementation, further randomized control trials (RCT) are required, that separate participants with low iron stores into three arms: control, MVM, and iron supplementation to determine which of the three treatments have better outcomes on improving iron status without increasing iron overload. Other RCTs could compare the use of MVM to control (no MVM) to determine whether the former has a significant impact on iron status. Such RCTs should attempt to include arms with larger sample size for improved statistical power.

It would also be valuable to have further qualitative investigations on HIV-positive women regarding body image dysmorphia and dieting behavior, since these would have significant impacts on nutrition status and quality of life. Although dieting behavior was not discussed in the diet survey, a substantial proportion of women cited dieting as a reason for their low dietary intake reports. Since dieting behavior is already an area of concern among

most women, it may be even more of an issue in a population that is struggling with the side effects of highly active antiretroviral therapy (82, 86, 87).

4.5 Conclusions

Women with HIV are vulnerable to IDA for the following reasons: 1) their iron intakes are inadequate to meet iron needs; 2) a significant proportion are dealing with food insecurity specifically and issues of poverty more generally; 3) their dietary needs are greater due to chronic iron losses, either directly related to HIV infection, or to factors such as menstruation and pregnancy (64). Therefore, even though baseline serum measures may be normal at a given time, these measures may be subject to dramatic changes within a short period of time. As shown in this study, even though estimates of iron intake were subject to error, the majority of women with HIV were not only failing to meet their iron requirements, but a notable proportion had dietary iron intakes well below the minimum requirement of 8.1mg/d.

Therefore, screening for IDA in all women with HIV should become a routine procedure in all primary care clinics, and risk factors thoroughly reviewed from a medical and nutritional standpoint. Preventative measures such as supplementation with MVM and dietary counseling should also be routine for women with HIV. If untreated, the major adverse impacts of IDA on women are poor work performance and reduced functional capacity, translating IDA from a health problem to a barrier in economic and educational advancement (12, 74). Conversely, improvement of iron status can reverse IDA symptoms quickly and at low cost to the health care system (77).

In addition to access to medical and nutritional interventions, such as screening, supplementation, and dietary counseling for women with HIV, initiatives also need to focus on their social milieu in order to improve their quality of life and ability to provide for themselves and their children. Prevention becomes just as important as treatment in order to reduce IDA in this population.

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APPENDIX A: Copy of Diet Survey used for Phase 2 Pilot Study

Respondent, please indicate how often you ate the following foods <u>in the last week</u>.
Interviewer, please indicate the appropriate frequency.

Meats and Alternatives	
Food Item	**Number of Times Consumed Last Week**
Beans (canned)	
Beans (dry and cooked)	
Beef (e.g. hamburger, steak)	
Beef Liver	
Chicken	
Chicken Liver	
Clams	
Cold Cuts **Type:_____**	
Eggs	
Fish (canned: salmon/tuna/herring)	
Fish (fresh)	
Ham	
Hot Dogs	
Lamb	
Nuts	
Oysters	
Peanut Butter	
Pork	
Sardines	
Sausage	
Sesame Seeds	
Shrimp/Prawns	
Tofu	
Turkey	
Veal	
Venison	

Grains and Cereals	
Food Item	**Number of Times Consumed Last Week**
Bagel	
Bran	
Bread (multigrain) **How many slices per meal?**	
Bread (white) **How many slices per meal?**	
Bread (whole wheat) **How many slices per meal?**	
Breakfast Cereal **Type:_____**	
Cookies	
Crackers	
Muffins	
Oatmeal	
Pasta (white)	
Pasta (whole wheat)	
Rice (brown)	
Rice (white, jasmine)	

Fruit and Vegetables	
Food Item	**Number of Times Consumed Last Week**
Apple	
Banana	
Beets (boiled)	
Broccoli	
Blackberries	
Blueberries	
Carrots	
Celery	
Corn	
Fruit, Dried	
Grapes	
Lettuce	
Mushrooms (fresh)	
Onions	
Tomatoes (canned)	
Tomatoes (fresh)	
Peas (frozen)	
Potato (baked, not boiled or fried)	
Raspberries	
Strawberries	
Spinach (fresh)	

Dairy Products	
Food Item	**Number of Times Consumed Last Week**
Cheese **Type:_____**	
Milk	
Soy milk	
Yogurt	

Supplemental Questions		
1. Do you take calcium supplements regularly?	**Yes**	**No**
2. Do you take fiber supplements regularly (Metamucil or bran)?	**Yes**	**No**
3. Do you take iron supplements regularly? **If yes, which type?** **Ferrous Gluconate/Ferrous Sulfate/Ferrous Fumarate**	**Yes**	**No**
4. Do you use an iron pot or cast iron skillet?	**Yes**	**No**
5. Do you have periods/menstruation? **If yes, are they irregular or regular?** **If yes, how many days per month?**	**Yes** **Regular**	**No** **Irregular**
6. Are you or have you ever been a vegetarian?	**Yes**	**No**
7. Do you have adequate kitchen facilities (stove/hot plate/microwave)?	**Yes**	**No**
8. Do you use your kitchen facilities to prepare meals?	**Yes**	**No**
9. Are you able to afford groceries in a one month period?	**Yes**	**No**
10. Do you use food assistance programs in a one month period? **If yes, which one do you use? (Loving Spoonful/VNH/DEWC)**	**Yes**	**No**

APPENDIX B: Evaluation Form for Content Validation Study

Subject Matter Expert Initials: _____

Evaluation Form
Content Validation Study of the Iron Food Frequency Questionnaire

Please rank each of the items below as follows:
0 = not at all; 1 = somewhat; 2 = mostly; 3 = very

Section A.
Layout:
1. How practical or user-friendly is the layout of the instrument?

Please circle the most appropriate response:

0 1 2 3

Comments on the layout:
_____
_____
_____
_____

Title:
2. How indicative is the instrument's title, "Iron Food Frequency Questionnaire", of its intended purpose of measuring iron intake from foods?

Please circle the most appropriate response:

0 1 2 3

Comments on the title:
_____
_____
_____
_____

Section B.
Clarity of Food Categories:
3. How helpful is it to have food items grouped in these specific categories (Meats and Alternatives; Grains and Cereals; Fruits and Vegetables; Dairy Products)?

Please circle the most appropriate response:

0 1 2 3

Comments on clarity of food categories:
_____
_____
_____
_____

Please rank each of the items below as follows:

0 = not at all; 1 = somewhat; 2 = mostly; 3 = very

4. Overall, how well do the food items fit into the categories presented?

Please circle the most appropriate response:

0 1 2 3

Comments:

_____
_____
_____
_____

Clarity of Names of Food Items:

5. If you were administering this instrument, please indicate how you would rate this item on clarity (e.g. How clear is it to you what "Beans (canned)" includes?). Also, for each food item, please indicate what exactly you would include as part of this item (e.g. what types of beans would you include in "Beans (canned)"?

<u>Meat and Alternatives</u>

Food Item (as presented in instrument)	**Please circle the most appropriate response:**				**Please indicate what food item you think this includes:**
Beans (canned)	**0**	**1**	**2**	**3**	
Beans (dry and cooked)	**0**	**1**	**2**	**3**	
Beef (e.g. hamburger, steak)	**0**	**1**	**2**	**3**	
Beef Liver	**0**	**1**	**2**	**3**	
Chicken	**0**	**1**	**2**	**3**	
Chicken Liver	**0**	**1**	**2**	**3**	
Clams	**0**	**1**	**2**	**3**	
Cold Cuts/Deli Meats Type:_____	**0**	**1**	**2**	**3**	
Eggs	**0**	**1**	**2**	**3**	
Fish (canned: salmon/tuna/herring)	**0**	**1**	**2**	**3**	
Fish (fresh)	**0**	**1**	**2**	**3**	
Ham	**0**	**1**	**2**	**3**	
Hot Dogs	**0**	**1**	**2**	**3**	

Please rank each of the items below as follows:

0 = not at all; 1 = somewhat; 2 = mostly; 3 = very

Meats and Alternatives (continued)

Food Item (as presented in instrument)	Please circle the most appropriate response:				Please indicate what food item you think this includes:
Lamb	0	1	2	3	
Nuts	0	1	2	3	
Oysters	0	1	2	3	
Peanut Butter	0	1	2	3	
Pork	0	1	2	3	
Sardines	0	1	2	3	
Sausage	0	1	2	3	
Sesame Seeds	0	1	2	3	
Shrimp/Prawns	0	1	2	3	
Tofu	0	1	2	3	
Turkey	0	1	2	3	
Veal	0	1	2	3	
Venison	0	1	2	3	

Grains and Cereals

Food Item (as presented in instrument)	Please circle the most appropriate response:				Please indicate what food item you think this includes:
Bagel	0	1	2	3	
Bran	0	1	2	3	
Bread (multigrain) How many slices per meal?	0	1	2	3	
Bread (white) How many slices per meal?	0	1	2	3	
Bread (whole wheat) How many slices per meal?	0	1	2	3	

Please rank each of the items below as follows:

0 = not at all; 1 = somewhat; 2 = mostly; 3 = very

Grains and Cereals (continued)

Food Item (as presented in instrument)	Please circle the most appropriate response:				Please indicate what food item you think this includes:
Breakfast Cereal Type:_____	0	1	2	3	
Cookies	0	1	2	3	
Crackers	0	1	2	3	
Muffins	0	1	2	3	
Oatmeal	0	1	2	3	
Pasta (white)	0	1	2	3	
Pasta (whole wheat)	0	1	2	3	
Rice (brown)	0	1	2	3	
Rice (white, jasmine)	0	1	2	3	

Fruits and Vegetables

Food Item (as presented in instrument)	Please circle the most appropriate response:				Please indicate what food item you think this includes:
Apple	0	1	2	3	
Banana	0	1	2	3	
Beets (boiled)	0	1	2	3	
Broccoli	0	1	2	3	
Blackberries	0	1	2	3	
Blueberries	0	1	2	3	
Carrots	0	1	2	3	
Celery	0	1	2	3	
Corn	0	1	2	3	

Please rank each of the items below as follows:

0 = not at all; 1 = somewhat; 2 = mostly; 3 = very

Fruits and Vegetables (continued)

Food Item (as presented in instrument)	Please circle the most appropriate response:				Please indicate what food item you think this includes:
Fruit, Dried	0	1	2	3	
Grapes	0	1	2	3	
Lettuce	0	1	2	3	
Mushrooms (fresh)	0	1	2	3	
Onions	0	1	2	3	
Tomatoes (canned)	0	1	2	3	
Tomatoes (fresh)	0	1	2	3	
Peas (frozen)	0	1	2	3	
Potato (baked, not boiled or fried)	0	1	2	3	
Raspberries	0	1	2	3	
Strawberries	0	1	2	3	
Spinach (fresh)	0	1	2	3	

Dairy Products

Food Item (as presented in instrument)	Please circle the most appropriate response:				Please indicate what food item you think this includes:
Cheese Type:_____	0	1	2	3	
Milk	0	1	2	3	
Soy milk	0	1	2	3	
Yogurt	0	1	2	3	

Additional Comments or Suggested Changes regarding Clarity of Food Items:

_____
_____
_____
_____
_____

Section C.
Dietary Representativeness of Food Items:
6. Overall, how representative are the food items in this instrument as <u>potential</u> sources of iron?

Comments on dietary representativeness of food items:

_____
_____
_____
_____

Please rank the item below as follows:

0 = not at all; 1 = somewhat; 2 = mostly; 3 = very

Section D.
Cultural Representativeness of Food Items:
7. Overall, how well do the items represent culturally diverse food choices?

Please circle the most appropriate response:

0 1 2 3

Comments on cultural representativeness of food items:

_____
_____
_____
_____

8. Item Deletion:
Are there any food items that you would recommend deleting from the list? Please identify these items <u>and</u> explain your recommendations.

_____
_____
_____
_____
_____

9. Item Addition:
Are there any food items that you would recommend adding to the list? Please identify these items <u>and</u> explain your recommendations.

_____
_____
_____
_____

10. Item Revision:
Are there any food items that you would recommend revising? Please identify these items <u>and</u> explain your recommendations.

_____
_____
_____

Please rank the item below as follows:

0 = not at all; 1 = somewhat; 2 = mostly; 3 = very

Section E.
Response Format:

11. As you can see, the response format of this instrument involves recording the number of times a food item was consumed during the previous week. How easy do you think it would be for respondents to recall the number of times they consumed a food item in the last week?

Please circle the most appropriate response:

0 1 2 3

Comments on ease of response for respondents:

_____
_____
_____
_____

12. Do you recommend a different response format for this instrument? If so, please provide comments.

_____
_____
_____
_____

Section F.

Additional Comments or Feedback:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Thank you for your time!

APPENDIX C: Ethics Approval Certificate for Phase 1 Pilot Study

UBC-Providence Health Care Research Institute
Office of Research Services
11th Floor Hornby Site - SPH
c/o 1081 Burrard St.
Vancouver, BC V6Z 1Y6
Tel: (604) 806-8567
Fax: (604) 806-8568

ETHICS CERTIFICATE OF EXPEDITED APPROVAL: AMENDMENT

PRINCIPAL INVESTIGATOR:	**DEPARTMENT:**	**UBC-PHC REB NUMBER:**
Mark W. Tyndall	UBC/Medicine, Faculty of Medicine	H08-01311

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
Providence Health Care	St. Paul's Hospital

Other locations where the research will be conducted:
N/A

CO-INVESTIGATOR(S):
Samuel B. Sheps
Dani Shahvarani

SPONSORING AGENCIES:
University of British Columbia

PROJECT TITLE:
A Study of Iron Deficiency Anemia in Marginalized Women of Vancouver's Downtown Eastside

REMINDER: The current UBC-PHC REB approval for this study expires: August 1, 2009

AMENDMENT(S):	**AMENDMENT APPROVAL DATE:**
	October 9, 2008

Document Name	Version	Date
Protocol:		
Amended Clinical Research Protocol	N/A	September 23, 2008
Consent Forms:		
Subject Consent Form Controls	N/A	September 23, 2008
Amended Subject Consent Form	N/A	September 8, 2008

CERTIFICATION:

1. The membership of the UBC-PHC REB complies with the membership requirements for research ethics boards defined in Part C Division 5 of the Food and Drug Regulations of Canada.
2. The UBC-PHC REB carries out its functions in a manner fully consistent with Good Clinical Practices.
3. The UBC-PHC REB has reviewed and approved the research project named on this Certificate of Approval including any associated consent form and taken the action noted above. This research project is to be conducted by the principal investigator named above at the specified research site(s). This review of the UBC-PHC REB have been documented in writing.

The amendment(s) for the above-named project has been reviewed by the **UBC-PHC Research Ethics Board Chair or Associate Chair** , as presented in the documentation and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

Approval of the UBC-PHC Research Ethics Board or Associate Chair, verified by the signature of one of the following:

[redacted]

Dr. I. Fedoroff,	Dr. J. Kernahan,	Dr. Kuo-Hsing Kuo,
Chair	Associate Chair	Associate Chair

APPENDIX D: Ethics Approval Certificate for Phase 2 Pilot Study

PROVIDENCE HEALTH CARE Research Institute

UBC-Providence Health Care Research Institute
Office of Research Services
11th Floor Hornby Site - SPH
c/o 1081 Burrard St.
Vancouver, BC V6Z 1Y6
Tel: (604) 806-8567
Fax: (604) 806-8568

ETHICS CERTIFICATE OF EXPEDITED APPROVAL: AMENDMENT

PRINCIPAL INVESTIGATOR:	DEPARTMENT:	UBC-PHC REB NUMBER:
Mark W. Tyndall	Medicine	H08-01311

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
Children's and Women's Health Centre of BC (incl. Sunny Hill)	Children's and Women's Health Centre of BC (incl. Sunny Hill)
Providence Health Care	St. Paul's Hospital
Vancouver Coastal Health (VCHRI/VCHA)	Vancouver Community

Other locations where the research will be conducted:
This research may also be conducted at Vancouver Native Health under the supervision of Dr. Tyndall, who is the infectious diseases physician there.

CO-INVESTIGATOR(S):
Samuel B. Sheps
Dani Shahvarani
Diana Johansen
Neora Pick

SPONSORING AGENCIES:
University of British Columbia

PROJECT TITLE:
A Study of Iron Deficiency Anemia in Marginalized Women of Vancouver's Downtown Eastside

Request for Amendment of Title from above to "A Study of Dietary and Non-Dietary Factors of Iron Deficiency Anemia in HIV-Positive Women"

REMINDER: The current UBC-PHC REB approval for this study expires: 01 August 2009

AMENDMENT(S):	AMENDMENT APPROVAL DATE: December 16, 2008	
Document Name	**Version**	**Date**
Protocol:		
Amended Clinical Research Protocol	N/A	December 5, 2008
Consent Forms:		
Amended Consent form for SPH, Oak Tree, and VNH	N/A	December 5, 2008
Questionnaire, Questionnaire Cover Letter, Tests:		
Iron Food Checklist	N/A	December 3, 2008
Other Documents:		
Dr. Kozac School of Population and Public Health	N/A	October 2, 2008
Chair Report School of Population and Public Health	N/A	October 2, 2008
Dr. Teschke Report School of Population and Public Health	N/A	October 2, 2008

CERTIFICATION:

1. The membership of the UBC-PHC REB complies with the membership requirements for research ethics boards defined in Part C Division 5 of the Food and Drug Regulations of Canada.
2. The UBC-PHC REB carries out its functions in a manner fully consistent with Good Clinical Practices.
3. The UBC-PHC REB has reviewed and approved the research project named on this Certificate of Approval including any associated consent form and taken the action noted above. This research project is to be conducted by the principal investigator named above at the specified research site(s). This review of the UBC-PHC REB have been documented in writing.

The amendment(s) for the above-named project has been reviewed by the **UBC-PHC Research Ethics Board Chair or Associate Chair** , as presented in the documentation and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

Approval of the UBC-PHC Research Ethics Board or Associate Chair, verified by the signature of one of the following:

[redacted]

Dr. I. Fedoroff,	Dr. J. Kernahan,	Dr. Kuo-Hsing Kuo,
Chair	Associate Chair	Associate Chair

APPENDIX E: Ethics Approval Certificate for Content Validation Study

The University of British Columbia
Office of Research Services
Behavioural Research Ethics Board
Suite 102, 6190 Agronomy Road,
Vancouver, B.C. V6T 1Z3

CERTIFICATE OF APPROVAL - MINIMAL RISK AMENDMENT

PRINCIPAL INVESTIGATOR:	DEPARTMENT:	UBC BREB NUMBER:
Anita M. Hubley	UBC/Education/Educational & Counselling Psychology, and Special Education	H08-03070

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
UBC	Vancouver (excludes UBC Hospital)

Other locations where the research will be conducted:
Not Applicable.

CO-INVESTIGATOR(S):
Dani Shahvarani
Mark W. Tyndall

SPONSORING AGENCIES:
N/A

PROJECT TITLE:
Content Validation Study of the Iron Food Frequency Questionnaire

Expiry Date - Approval of an amendment does not change the expiry date on the current UBC BREB approval of this study. An application for renewal is required on or before: February 26, 2010

AMENDMENT(S):	AMENDMENT APPROVAL DATE: March 17, 2009	
Document Name	**Version**	**Date**
Consent Forms:		
Consent Form	Version 2	March 12, 2009

The amendment(s) and the document(s) listed above have been reviewed and the procedures were found to be acceptable on ethical grounds for research involving human subjects.

Approval is issued on behalf of the Behavioural Research Ethics Board

Dr. M. Judith Lynam, Chair
Dr. Ken Craig, Chair
Dr. Jim Rupert, Associate Chair
Dr. Laurie Ford, Associate Chair
Dr. Anita Ho, Associate Chair