Correlates of iron status, hemoglobin and anemia in Inuit adults

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ABSTRACT

Iron deficiency and anemia have been paradoxically observed among circumpolar Inuit populations consuming diets rich in animal-source foods for decades, yet representative data are lacking to clarify the extent and association of both conditions. Little is known about the degree to which iron deficiency anemia can explain anemia for Inuit adults, who are at nutritional risk given the ongoing nutrition transition throughout the Arctic. The objectives of this thesis were: (i) to determine the iron status of Inuit adults (from depleted iron stores to iron overload), the prevalence of anemia, and the extent to which both iron deficiency and anemia occur together for men and women throughout adulthood; (ii) to assess dietary intakes of nutrients required for erythropoiesis and associations between traditional Inuit foods, iron status and anemia; and (iii) controlling for the effect of inflammation, to identify dietary and non-dietary correlates of iron status, hemoglobin, and risk of anemia. Data for this work were from the International Polar Year Inuit Health Survey, 2007-2008. This was a cross-sectional survey, with stratified random sampling of 2550 Inuit adults (60.9 % female) 18-89 years of age with an overall household response rate of 68 %. For objective (i) hemoglobin, serum ferritin, serum high-sensitivity C-reactive protein, and (on a subset n=1039) serum soluble transferrin receptor were measured. For objective (ii), a single 24 hour recall and a 42-item semi-quantitative food frequency questionnaire were utilized. Dietary iron inadequacy was calculated by adjusting the dietary iron intake distribution from the 24 hour recall by within-subject coefficients of variation for iron intake from previous dietary surveys with this population. For objective (iii) multivariate modeling was performed for serum ferritin, iron deficiency, elevated iron stores, hemoglobin, and anemia unexplained by iron deficiency (UA), controlling for potential confounders. Results showed that iron deficiency was pervasive among pre-menopausal women and explained a significant portion of the anemia in this lifestage despite adequate iron intake. For men, body iron stores were lower

than expected based on dietary intake but not depleted. Rates of UA increased with age, being highest amongst men >50 years of age (30 %). Traditional food intake was an important correlate of iron status for both men and women, and was associated with reduced risk of iron deficiency among food insecure women. UA prevalence was highest in the most traditional Inuit region and was characterized by factors associated with a more traditional lifestyle including higher red blood cell eicosapentonoic acid (RBC EPA) proportions, elevated blood lead concentrations, low education levels, infections, and inflammation. The relationship between RBC EPA % and UA is a potentially important finding and should be further investigated for impact on RBC stability and hemolysis. Regional differences and correlates of UA across age-groups do not support the hypothesis that physiologically lower hemoglobin concentrations can explain the high rates of anemia observed among Inuit. Without clear evidence for revising the WHO cut-off for Inuit, anemia without evidence of iron deficiency should not be dismissed. Iron deficiency and anemia remain important public health concerns for Canadian Inuit adults.

RÉSUMÉ

La carence en fer et l'anémie ont été observées parmi les populations Inuits circumpolaires qui, paradoxalement, consomment des diètes riches en aliments d'origine animales depuis des décennies. Cependant, il manque de données représentatives clarifiant l'ampleur et l'association de ces deux conditions. Très peu est connu quant au degré avec lequel la carence en fer anémique peut expliquer l'anémie chez les Inuits adultes, qui courent un risque nutritionnel étant donnée la transition alimentaire qui est en cours à travers l'Arctique. Les objectifs de cette thèse étaient : (i) de déterminer le statut en fer chez les Inuit adultes (depuis l'insuffisance des réserves jusqu'à la surcharge en fer), la prévalence de l'anémie, et la mesure dans laquelle la carence en fer et l'anémie coexistent chez les hommes et les femmes durant toute la période adulte; (ii) d'évaluer l'apport diététique requis pour l'érythropoïèse et les liens entre les aliments Inuit traditionnels, statut en fer et l'anémie; et (iii) tout en contrôlant pour les effets de l'inflammation, d'identifier les corrélats diététiques et non-diététiques du statut en fer, l'hémoglobine, et le risque d'anémie. Les données utilisées dans cette étude proviennent du Sondage sur la Santé des Inuit (2007-2008) de l'Année Polaire Internationale. Il s'agissait d'un sondage cross-sectionnel avec échantillonnage aléatoire stratifié de 2550 adultes Inuit (60.9% féminin) âgés entre 18 et 69 ans, avec un taux de réponse ménagère global de 68%. Pour l'objectif (i) l'hémoglobine, la ferritine dans le sérum, la protéine C-réactive hautement sensible dans le sérum, et (pour une fraction n=1039) le récepteur de transferrine soluble dans le sérum, ont été mesurés. Pour l'objectif (ii), un unique rappel après 24 heures et un questionnaire sur la fréquence alimentaire semi-quantitatif comprenant 42 items ont été utilisés. L'apport inadéquat en fer a été calculé en ajustant la distribution de l'apport en fer du rappel après 24 heures par rapport aux coefficients de variabilité intra-sujets en apport en fer tirés de précédents sondages alimentaires effectués sur cette population. Pour l'objectif (iii), le modelage multivarié a été effectué pour la

ferritine, la carence en fer, les réserves en fer élevées, l'hémoglobine et l'anémie qui n'est pas expliquée par une carence en fer (AI), tout en contrôlant pour les potentiels facteurs de confusion. Les résultats ont montré que la carence en fer était répandue chez les femmes pré-ménopausées et expliqué une proportion significative de l'anémie dans cette tranche d'âge, en dépit d'un apport en fer adéquat. Pour les hommes, les réserves en fer étaient plus basses que ce qu'on s'attendrait par rapport à leur diète, mais n'étaient pas insuffisantes. Les taux d'AI augmentaient avec l'âge, étant au plus haut chez les hommes de plus de 50 ans (30%). L'apport en nourriture traditionnelle était un important corrélat du statut en fer pour les hommes et les femmes, et était associé à un risque de carence en fer réduit chez les femmes ayant une insécurité alimentaire. La prévalence de l'AI était la plus haute dans les régions Inuits les plus traditionnelles et était caractérisée par des facteurs associés avec un mode de vie plus traditionnel, incluant de plus hautes proportions d'acide eicosapentonoic dans les globules rouges (AEP GR), des concentrations sanguine de plomb élevées, de faibles niveaux d'éducation, des infections et de l'inflammation. La relation entre le % AEP GR et l'AI est une trouvaille potentiellement importante qui devrait étudiée davantage en rapport à l'impact sur la stabilité des GR et l'hémolyse. Les différences régionales et les corrélats de l'AI à travers les groupes d'âges ne supportent pas l'hypothèse par laquelle une différence génétique résultant en une concentration physiologique d'hémoglobine réduite pourrait expliquer les hauts taux d'anémie observé chez les Inuit. Sans évidences claires pour réviser la limite de l'OMS pour les Inuit, l'anémie sans carence en fer évidente ne devrait pas être exclue. La carence en fer et l'anémie demeurent des préoccupations de santé publique importantes pour les Inuits Canadiens adultes.

ADVANCE OF SCHOLARLY KNOWLEDGE

1. Original contribution to knowledge

This doctoral dissertation is the first representative study to examine iron status and anemia among Inuit adults with comprehensive dietary, biochemical, clinical, and socio-demographic measures. Prevalence estimates of iron deficiency (ID), iron deficient erythropoisesis, iron deficiency anemia (IDA) and anemia unexplained by iron deficiency (UA) were established across adult sex and age-groups after accounting for the influence of inflammation on nutritional biomarkers. The Inuit population is known to be at increased risk for various infectious diseases; yet, previous surveys have reported iron status assessment without accounting for inflammation. Independent associations between iron status and anemia with dietary and non-dietary variables were also reported for the first time. It was shown that traditional food intake is an important correlate of iron stores but that red blood cell EPA status (a marker of traditional food intake) is negatively associated with hemoglobin in men. Elevated, but not overtly toxic, blood lead concentrations were also associated with anemia in men but not women. With the exception of pre-menopausal women, anemia prevalence was not explained by iron intake or iron stores, despite lower than expected iron stores based on heme and total dietary iron intake estimates. Moreover, this thesis was able to demonstrate that physiologically lower hemoglobin levels do not explain the clinically mild but pervasive anemia observed, in opposition to the argument for a revised anemia cut-off for Inuit. The Inuit population is in a period of dietary and health transition, with implications for micronutrient deficiency in addition to overnutrition.

2. Research publications in refereed scientific journals

Manuscript 1: THE PARADOX OF ANEMIA WITH HIGH MEAT INTAKE: A REVIEW OF THE MULTIFACTORIAL ETIOLOGY OF ANEMIA IN THE INUIT OF NORTH AMERICA.

Jennifer A. Jamieson and Harriet V. Kuhnlein

Nutrition Reviews. 2008; 66(5):256-71.

Manuscript 2: TRADITIONAL FOOD IS CORRELATED WITH IRON STORES IN CANADIAN INUIT MEN

Jennifer A. Jamieson, Hope A. Weiler, Harriet V. Kuhnlein & Grace M. Egeland

The Journal of Nutrition. 2012; 142(4): 764-770.

3. Research publications submitted to refereed scientific journals

Manuscript 3: n3-FATTY ACID STATUS AS A MARKER OF TRADITIONAL FOOD INTAKE IS ASSOCIATED WITH LOWER RISK OF IRON DEPLETION AMONG FOOD INSECURE CANADIAN INUIT WOMEN.

Jennifer A. Jamieson, Harriet V. Kuhnlein, Hope A. Weiler & Grace M. Egeland

4. Research publication to be submitted to refereed scientific journals

Manuscript 4: MILD ANEMIA PREVALENT AMONG INUIT MEN AND POST-MENOPAUSAL WOMEN IS ASSOCIATED WITH CHARACTERISTICS OF A MORE TRADITIONAL LIFESTYLE

Jennifer A. Jamieson, Hope A. Weiler, Harriet V. Kuhnlein & Grace M. Egeland

5. Abstracts and presentations

1) Prevalence and determinants of iron depletion and anemia among Canadian Inuit. Poster: Experimental Biology Annual Meeting. Washington, D.C.; April 9-13, 2011.

2) Risk Factors for Iron Deficiency and Anemia in Inuit Adults of Inuvialuit Settlement Region, Nunavut, and Nunatsiavut. Poster: International Polar Year Oslo Science Conference. Oslo, Norway; June 8-12, 2010.

3) High iron intake from traditional foods and anemia in Inuit: The paradox. Presentation: 19th International Congress of Nutrition (ICN 2009), Bangkok, Thailand, 4-9 October, 2009. Symposium: Indigenous Peoples' Food Systems and Nutrition.

4) The multifactorial etiology of anemia among Inuit women: Preliminary results from the Inuit Health Survey. Presentation: International Congress on Circumpolar Health (ICCH14), Yellowknife, NT, July 11-16, 2009.

5) The paradox of anemia with high meat intake: is there a multifactorial etiology for anemia among the Inuit? Presentation: Nassivik Graduate Student Conference, Quebec City; June 23, 2008.

CONTRIBUTIONS OF AUTHORS

For manuscript one, the candidate was primary author, conceived of the review topic (in collaboration with the thesis committee) and wrote the first draft. Dr. Kuhnlein contributed to the writing of the manuscript and editorial revisions.

For manuscripts two-four, the candidate collected data (dietary and questionnaire interviews, blood sample processing), analyzed iron status biomarkers, developed the research questions, performed statistical analyses, was the primary author, wrote the first draft of the manuscripts, and contributed to interpretation of the data.

Dr. Egeland, the principal investigator and candidate's committee member, obtained funding, designed the survey, oversaw data collection, and contributed to study design, data analysis and interpretation, as well as manuscript revisions.

Dr. Kuhnlein, a co-investigator and candidate's co-supervisor, provided consultation for dietary data collection and contributed to data analysis, interpretation and manuscript revisions.

Dr. Weiler, a co-investigator and candidate's co-supervisor, provided consultation for dietary data collection and biological sample analyses, and contributed to data analysis, data interpretation, as well as manuscript revisions. Dr. Weiler was involved in training the candidate for laboratory analysis of serum ferritin and other biomarkers by auto-analyzer.

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LIST OF ABBREVIATIONS

25(OH)D=25-hydroxy vitamin D

AOI=anemia of inflammation

ASA=acetylsalicylic acid

EAR=estimated average requirement

EBL=elevated blood lead concentration

CANDAT=Research orientated nutrient calculation system

CCHS=Canadian Community Health Survey

CINE=Centre for Indigneous Peoples' Nutrition and Environment,

CNF=Canadian Nutrient File

CRP=C-reactive protein

DALY=disability-adjusted life-years

DFE=dietary folate equivalents

DHA =docosahexaenoic acid

DRI=dietary reference intake

EAR=estimated average requirement

ELISA=enzyme-linked immunosorbent assay

EPA=eicosapentaenoic acid

FFQ=food frequency questionnaire

Hb=hemoglobin

hs-CRP=high-sensitivity C-reactive protein

ID=iron deficiency

IDA=iron deficiency anemia

IDE=iron deficient erythropoiesis

IDWA=iron deficiency without anemia

ISR=Inuvialuit Settlement Region

LC-PUFA=long-chain polyunsaturated fatty acids (EPA + DHA)

MCV=mean cell volume

MF=market food

n-3 PUFA= n-3 (omega-3) polyunsaturated fatty acids

NSAID=non-steroidal anti-inflammatory drug

NTD=neural tube defects

RBC=red blood cell

RDA=recommended daily allowance

SF=serum ferritin

SIDE=software for intake distribution estimation

sTfR=soluble transferrin receptor

TF=traditional food

UA=anemia unexplained by iron deficiency

USDA=United States Department of Agriculture

WHO=World Health Organization

CHAPTER 1 – INTRODUCTION

1.1 Background and rationale

Inuit are an Indigenous Peoples residing in the Circumpolar North: Alaska (USA), Arctic regions of Canada, Greenland and Russia. Although there are regional differences in lifestyle and customs, Inuit share strong cultural and environmental characteristics across national boundaries. Inuit have been experiencing a rapid health and nutrition transition as a result of changing lifestyles, diet, and environment. This transition has been associated with high rates of food insecurity (Krümmel, 2009; Egeland et al., 2011b) and unsatisfactory access to traditional food species (Krümmel, 2009). The emergence of contaminants in traditional foods and the availability of market foods high in refined carbohydrate and saturated fat but limited in nutrient density have further contributed to this dietary transition. Together these factors place Inuit at risk for the so-called double burden of malnutrition; the co-existence of over- and undernutrition in developing regions undergoing a rapid nutrition transition (Delisle, 2008; Sharma, 2010).

Indigenous Peoples living within a mainstream society are generally worse off in terms of population health indicators, with ethnicity and poverty being strong determinants of health (Kuhnlein et al., 2004). Within Canada, Inuit are a small population (approximately 50 000) (Statistics Canada, 2006) living in geographically remote communities throughout the Territories, Northern Quebec, and Labrador; but comprising one-third of the global Inuit population (Wilkins et al., 2008). In all Inuit regions, life expectancy rates are lower and infant mortality rates higher than national average population statistics (Wilkins et al., 2008). In Canada, population health indicators (Jenkins et al., 2003) and living conditions (Statistics Canada, 2006; Egeland et al., 2010a) for Inuit continue to lag behind national estimates. Compared to other regions, the gap in

life expectancy between Inuit and national statistics is greatest in Canada (approximately 10 years) (Krümmel, 2009) and infant mortality in Nunavut is second only to Chukotka (Russia) (Krümmel, 2009). Tuberculosis infection rates are particularly disparate, with a 140-time greater rate observed among self-identified Inuit than non-Indigenous Canadians (Krümmel, 2009).

While the emerging issues of obesity and chronic disease associated with the nutrition transition for Inuit are currently being investigated, there has been limited attention on the risk of undernutrition. Micronutrient deficiency is often considered a "hidden hunger" as the consequences are more subtle, yet insidious. The co-existence of obesity and iron deficiency anemia (IDA) is one of the major phenotypes of the double burden of malnutrition now under investigation in lower and middle income developing regions (Delisle, 2008) but has not been assessed in Arctic populations. Although the inflammation associated with obesity may be involved in IDA, it is distinct from the phenotype of the anemia of inflammation (AOI) (Tussing-Humphreys and Braunschweig, 2011). Obesity in adults and children has been associated with lesser iron status, higher hepcidin levels, and reduced iron absorption (Lecube et al., 2006; Yanoff et al., 2007; Menzie et al., 2008; Zimmermann et al., 2008; Tussing-Humphreys et al., 2009). Obesity, however, does not seem to involve iron sequestration in the reticuloendothelial system, which is a defining characteristic of AOI (Tussing-Humphreys and Braunschweig, 2011). Although the mechanism explaining this relationship between obesity and iron status is not yet known, the main iron regulator hepcidin is thought to be involved (McClung and Karl, 2009). Hepcidin is a small, hepatic peptide hormone believed to regulate gastrointestinal iron absorption, hepatic iron storage, as well as iron recycling by macrophages (Zhang et al., 2011). Hepcidin, whose expression is induced by iron overload, inflammation, and erythropoiesis, accomplishes this through post-translational regulation of ferroportin, the cellular iron export protein, found on enterocytes,

hepatocytes, and macrophages (Zhang et al., 2011). Together these actions result in hypoferremia and reduced iron stores. Pro-inflammatory cytokines (tumor-necrosis factor-α and interleukin-6) released during the chronic inflammation of obesity may be responsible for increased hepatic hepcidin release and diminished iron status, although further research is required.

National population health data in Canada, as with most developed countries, is not available segregated by cultural subgroup. In fact, on-reserve First Nation and populations in remote regions are excluded from sampling in the Canadian Community Health Survey (CCHS) and the 2004 Nutrition module of the CCHS further excluded all three Canadian territories (Statistics Canada, 2011). The 2007-2009 Canadian Health Measures Survey, which will report biochemical iron status measures in 2012, included sampling from Yellowknife and Whitehorse, but not Inuit communities or First Nations Reserves (Statistics Canada, 2010). A large proportion of the Canadian Aboriginal population is therefore excluded from the national population health monitoring system, despite being perhaps the most vulnerable population to poor health outcomes.

Anemia as a public health issue for Inuit in Canada (in males and females of most age-groups) has been recognized since the 1970-72 Nutrition Canada Survey, although more recent literature is limited and confined to high risk sub-populations (infants, young children and pregnant women). Similarly, the US Centre for Disease Control has observed anemia and ID among Alaska Natives over the past several decades, yet the etiology remains unknown. Representative data are needed to clarify the extent of the problem across sex and age-groups for Inuit and to identify potential etiological factors. While IDA may be the primary cause of anemia among infants and pregnant women (Willows and Gray-Donald, 2000; Hodgins et al., 1998), it will not likely explain anemia among older adults, and especially among males.

Estimates of global ID (1.5 – 2 billion) are extraordinary given that the causes of nutritional ID have been known for more than five decades (Lynch, 2011b). However, as methods of assessment improve and estimates are no longer based on hemoglobin (Hb), an indicator with low sensitivity for IDA, these rates are expected to decline. Current indicators for iron status assessment are hampered by non-specificity. Infections, inflammation, lead exposure, liver disease, and rate of erythropoiesis, for example, influence the main biomarkers of iron status (Lynch, 2011a). Consequently, classification of iron status by multiple-parameter models is common place, though difficult to accomplish in remote and developing regions. Alternatively, the sTfR:SF ratio can be used to categorize individuals with ID, normal iron stores, and high iron stores, especially in areas with minimal infectious disease burden (Lynch, 2011b). This approach may be preferable to the multiple parameter index, although sTfR methodology does require standardization as well as further development to make reliable assays available at reasonable costs (Lynch, 2011b).

ID occurs when there is inadequate bioavailable dietary iron taken in to meet body iron requirements. This is generally attributed to a lack of dietary diversity (due to poverty) and the shift in dietary iron sources from primarily animal source foods to a reliance on cereals and legumes that accompanied the agricultural revolution (Lynch, 2011b). The fortification of wheat flour, breakfast foods, infant formulas and complementary foods in North America has paralleled a decline in IDA among young children and infants (Lynch, 2011b), although ethnic differences do still exist (Zacharski et al., 2000). For Inuit, however, traditional foods provide rich sources of bioavailable iron and market foods are subject to Canadian fortification regulations. Although diet and lifestyle are clearly in transition for Inuit, the extent to which this transition has impacted iron status is not known.

Women are particularly vulnerable to malnutrition as a result of the higher nutrient requirements for pregnancy and lactation, as well as inequities in socioeconomic status (Delisle, 2008). The prevention and treatment of IDA in pregnant women and infants is a known public health priority (WHO/UNICEF/United Nations University, 2001), necessary for healthy child development. Most critically, IDA during infancy and early childhood has well known negative effects on cognition, motor and social development (Gleason and Scrimshaw, 2007). Anemia during pregnancy is associated with increased maternal and child mortality, preterm birth and low birth weight. Furthermore, evidence is accumulating that malnutrition *in utero* (low birth weight) may result in permanent metabolic changes with future health implications (Fall, 2009). Consequently, the iron status of women of child-bearing age has significant public health importance.

In contrast to women, ID among men is rare with iron overload being more of a public health concern. Literature on the iron status of Inuit men has been conflicting with low iron stores observed among Alaska Native men (Petersen et al., 1996) and high iron stores reported in Greenlandic Inuit men (Milman et al., 1992). If IDA is observed among Inuit men, sources of chronic blood loss should be investigated. Iron overload, whether related to hereditary hemochromatosis or not, is also a concern as it may lead to oxidative tissue damage and significant morbidity (Mainous et al., 2011), including metabolic disturbances such as insulin resistance and cardiovascular disease progression (Dongiovanni et al., 2011).

Although ID is the primary cause of anemia in the world, the need to identify population-specific causes of anemia prior to intervention is increasingly recognized by public health organizations (WHO/UNICEF/United Nations University, 2001). Iron supplementation will not correct anemia when the cause is inflammatory or non-iron related and may have negative consequences.

Adverse effects of iron supplementation have been noted among iron-replete children with high infectious disease burden (Iannotti et al., 2006).

Anemia, whether due to ID or not, is more significant than the common symptoms of weakness and fatigue. A decline in physical work capacity impacts an individual's well-being and productivity (Lynch, 2011b). There is a clear relationship between anemia and mortality in older adults, independent of underlying disease processes (Roy, 2011). Cognitive decline, fatigue, muscle weakness, gastrointestinal dysfunction, and cardiac stress have also been associated with anemia among the elderly (Roy, 2011; Turkoski, 2003a). Therefore, it is important to determine the magnitude of the problem and explore potential contributing factors to anemia for older adults as well as young women. If prevalence of anemia and ID is observed to be high for Inuit, such data will be useful to identify risk factors and guide future research and intervention strategies.

1.2 Statement of purpose

The overall objective of this study was to establish the prevalence of anemia, ID, and IDA and subsequently identify correlates of iron status and anemia among Inuit adults in Nunavut, Inuvialuit Settlement Region (ISR), and Nunatsiavut. It was hypothesized that ID will be the main cause of anemia among pre-menopausal women but would not explain the anemia of older adults and men. On the basis of these hypotheses specific objectives included:

- to conduct a comprehensive literature review of potential causes of anemia for Inuit adults *(Manuscript 1);*
- to assess iron status and hemoglobin in a representative, cross-sectional sample of Inuit adults across sex and age-groups *(Manuscripts 2-3);*
- to assess dietary intakes of several nutrients essential for erythropoiesis and the association of traditional food intake with iron status *(Manuscripts 2-3)* and hemoglobin concentrations *(Manuscript 4);*
- to determine important correlates of ID *(Manuscripts 2-3)* and anemia *(Manuscript 4)* specific to sex and age-groups for Inuit adults that may explain the prevalence of anemia unrelated to ID.

CHAPTER 2: REVIEW OF LITERATURE. MANUSCRIPT 1

Published in *Nutrition Reviews*, 2008

The Paradox of Anemia with High Meat Intake: A Review of the Multifactorial Etiology of Anemia in Inuit of North America[1]

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2.1 INTRODUCTION

The purpose of this chapter is to review the literature on Inuit-specific risk factors for anemia. To meet this objective it was first necessary to describe more broadly, the determinants of health for Inuit and the social and economic challenges currently faced. This was followed by a description of hematopoiesis and anemia, with special focus on the essential nutrients required. After which, a survey of Inuit-specific iron deficiency and anemia prevalence in North America was presented, with an emphasis on investigation into the type of anemia observed when possible. Studies investigating the dietary and/or biochemical assessment of relevant nutrients for Inuit were summarized and potential non-nutritional causes of anemia considered.

2.2 ABSTRACT:

Anemia is a serious concern among indigenous populations in North America, and it appears to be widespread among Inuit despite abundant intakes of heme iron. It is therefore hypothesized that anemia for Inuit involves other dietary factors not usually associated with animal foods, such as low intakes of vitamin A and/or, folate, riboflavin, and vitamin C. Also, *Helicobacter pylori* infection and/or parasitosis may result in gastrointestinal blood loss and/or functional iron deficiency. It is our purpose to describe factors which may cause anemia in Inuit despite high meat intakes, abundant bioavailable iron, and other important hematological nutrients.

2.3 DETERMINANTS OF HEALTH AMONG NORTH AMERICAN INUIT

Inuit is defined as "the Inupiat (Alaska), Yup'ik (Alaska), Inuit, Inuvialuit (Canada), Kalaallit (Greenland) and Yup'ik (Russia)" in the charter of the Inuit Circumpolar Conference. For the purpose of this review we have used the term "Inuit" to refer to the Inupiat and Yup'ik (Alaska) and in Canada, the Inuvialuit (Northwest Territories[2]), and the people of Nunatsiavut (Labrador), Nunavik (northern Quebec), and Nunavut. Although Inuit of Alaska and northern Canada reside in separate nations and the Yup'ik are linguistically distinct, they are considered together in this nutrition literature review because of strong similarities in culture, geography, diet and life circumstances. In addition, the term Native American will refer to the Indigenous Peoples of the continental United States and First Nations for the Aboriginal Peoples of Canada who are neither Inuit nor Métis. When the specific culture of Alaskan Peoples is unclear or several cultures have been included in the same study, the term "Alaskan Native" is used. Inuit of Canada have a population of approximately 55 000 (Statistics Canada, 2006) and the Yup'ik and Inupiat of Alaska number approximately 38 000 (Strome, 2005).

Although migration to urban areas is occurring, Inuit are widely dispersed across many rural communities, covering vast areas of land often geographically isolated and only accessible by sea or air (Natural Resources Canada, 2007). This has importance for food security and access to health care. Basic health services in more remote northern communities are often provided by a nurse or community health worker, with intermittent visits from physicians (Jenkins et al., 2003). In more serious cases, patients must be transported to larger centres for treatment. Many Arctic Indigenous Peoples are not likely to be reached by

[2] The lands of Nunavut were included in the Northwest Territories until the official creation of the new Nunavut Territory in April, 1999.

mainstream public health initiatives and health care services (Jenkins et al., 2003).

Arctic Indigenous Peoples are experiencing a health and nutrition transition, (Kuhnlein et al., 2004) that has been greatly accelerated over the last 50 years (Bjerregaard et al., 2004). This transition involves a shift away from nutrient-dense traditional foods to less nutrient-dense but energy-rich market foods (Kuhnlein et al., 2004) and reflects an interaction between genetic and environmental variables (Bjerregaard et al., 2004). Not surprisingly, this shift has been associated with increased rates of obesity and chronic disease, as well as an increased risk of vitamin and mineral inadequacy (Kuhnlein et al., 2004). In general, nutrients identified of concern for inadequacy include vitamins A, C, and E, folate, magnesium, fibre, n-6 fatty acids, and calcium (Kuhnlein et al., 2007) in northern Canada, and folate, calcium, vitamin D, and fibre in Alaska (Nobmann and Lanier, 2001).

There is a general discrepancy between Inuit populations and the overall North American population in terms of various population health indicators. Life expectancy remains much lower among Inuit than the national average in Canada. In 1996, life expectancy was approximately 8 years lower in the Northwest Territories and 14 years lower in Nunavik than the Canadian average of 78.4 years, (Jenkins et al., 2003) thought to be partially attributable to the 3-fold higher rate of infant mortality in these regions compared to the Canadian average (Jenkins et al., 2003). Furthermore, the traditional protection from cardiovascular disease and type 2 diabetes provided by the Inuit lifestyle and genetic heritage appears to be eroding (Bjerregaard et al., 2004). This protection was thought to be related to the high consumption of fish and marine mammals and strenuous physical activity (Bjerregaard et al., 2004). Thus, the movement away from traditional dietary patterns and lifestyles likely contribute to the declining health profile in the North.

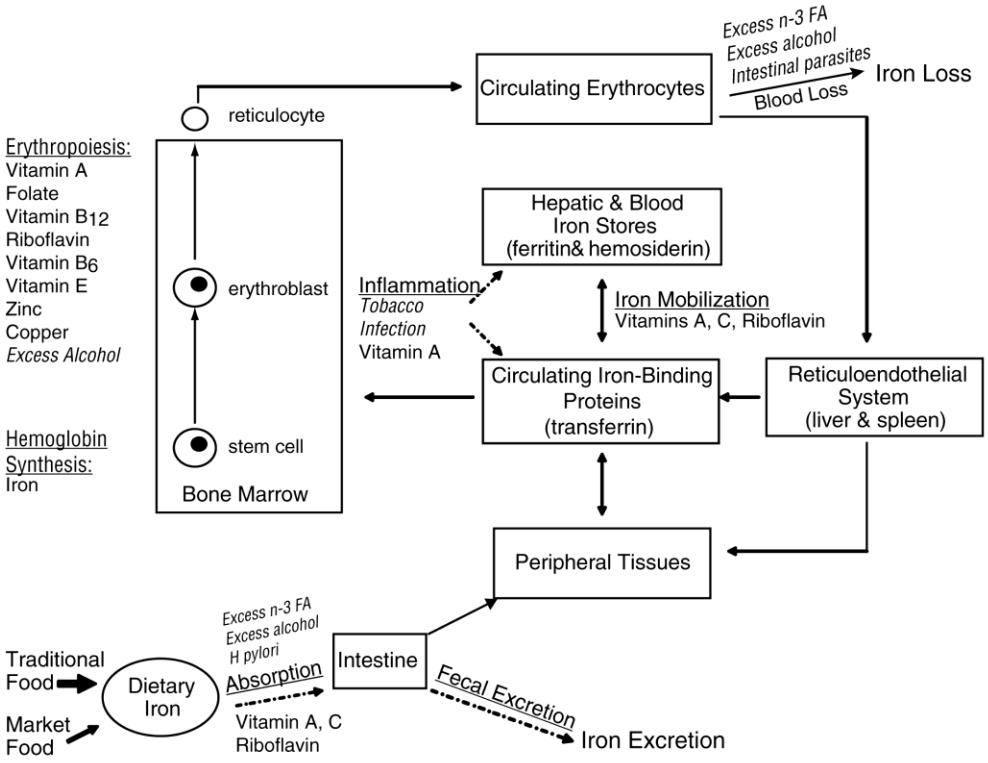
Poverty and ethnicity have been shown to be highly predictive of poor health status (Kuhnlein et al., 2004). Nunavut society is predominantly Inuit, estimated at about 85% of the population and the traditional Inuktitut language is spoken by the majority (80%) (Natural Resources Canada, 2007). The unemployment rate is relatively high (22.9%) and more than half of adults (56.2%) reported not finishing high school (Statistics Canada, 2001). A recent analysis has demonstrated a strong link between economic factors and food availability among Arctic Indigenous women (Lambden et al., 2006). Access to hunting and fishing, as well as the expense of market foods were found to be significant barriers to food security among the women. Transportation issues associated with accessing remote northern communities result in foods that cost more but provide less nutritional value, quality and variety (Lambden et al., 2006). These expenses are then compounded by higher living costs and lower incomes. Thus, food security is a significant concern in the Arctic, with food insecurity related to poor health outcomes documented by many researchers (Hamelin et al., 2002; Hamelin et al., 1999; McIntyre and Tarasuk, 2003; Bhattacharya et al., 2004).

Poverty also relates to housing conditions, over-crowding, and hygiene, which further contribute to poor health outcomes. Over-crowding and inadequate housing, water supply, and sewage disposal have been reported as serious concerns in the North (Jenkins et al., 2003). These conditions are thought to impact upon infection rates and disease transmittance. Inuit children are disproportionately affected by respiratory infections, otitis media, tuberculosis, meningitis, and gastrointestinal infections (Jenkins et al., 2003). The bacterium *H. pylori* has been identified in northern water supplies (McKeown et al., 1999) and there is a high prevalence of infection with this bacteria among Aboriginal populations (McKeown et al., 1999; Bernstein et al., 1999; Parkinson et al., 2000).

2.4 ANEMIA IN INUIT

Anemia is considered to be a condition, rather than a disease, which develops when either the number of circulating red blood cells (RBC) is decreased or the amount of available hemoglobin for RBC production is reduced (Turkoski, 2003a). The process of erythropoiesis (RBC production) is included in Figure 2.1. Briefly, RBC originate from pluripotent stem cells in the myeloid lineage. Through differentiation into the premature erythroblast and finally to the reticuloycte the nucleus is lost. Differentiation and maturation is dependent on folate, vitamins B_{12}, B_6, E, A, riboflavin, zinc and copper (Fishman et al., 2000). Hemoglobin synthesis also takes place in the developing erythroblast and is dependent on available cellular iron to complete the functional protein (Figure 2.1).

Figure 2.1. Main risk factors for anemia among Inuit including the roles of nutrients, immunological and environmental factors in erythropoiesis and iron metabolism. (Adapted from Fishman *et al* 2000)**.** Italics are used for inhibitors of good iron status. Abbreviations: n-3 FA, omega 3 fatty acid.



The symptoms of anemia are related to tissue hypoxia and will vary with the extent of oxygen deprivation as well as the body's ability to compensate (Turkoski, 2003a; Turkoski, 2003b). In mild cases, an individual is often asymptomatic during normal activities but may experience clinical symptoms when challenged by physical exertion or infection (Turkoski, 2003a). The most common symptom is fatigue but others include dyspnea, palpitations, tachycardia, vertigo, muscle weakness, depression, anorexia, social withdrawal, and a general diminished capacity to cope with stress (Turkoski, 2003a). The ability to carry out the normal activities of daily life, including work capacity, becomes impaired. Thus, it is important to account for both morbidity and mortality when measuring the impact of anemia on a population by using indicators such as disability-adjusted life-years (DALY) (Lewis, 2005).

In addition to the physiological presentation of the condition, chronic anemia during key developmental periods can negatively affect motor and mental development (From the Centers for Disease Control and Prevention, 2002), leading to poor cognitive performance and behavioural difficulties (Cooper et al., 2006). Maternal iron deficiency anemia (IDA) has been associated with outcomes of low birth weight and premature delivery (Cooper et al., 2006; From the Centers for Disease Control and Prevention, 2002). The immune response and susceptibility to infections are also compromised in IDA (Cooper et al., 2006). Due to the severity and breadth of these effects, which have been thoroughly reviewed elsewhere (Gleason and Scrimshaw, 2007), it is critical to address the unacceptably high rates of anemia within vulnerable populations of North America and across the globe.

Anemia remains a major public health issue worldwide, with wide-reaching clinical health effects, as well as socioeconomic consequences. Many factors that are nutritional, environmental, and genetic are associated with anemia and are summarized in Table 2.1. The etiology of anemia is much more

complex than insufficient dietary iron, however. The World Health Organization (WHO) estimates the global prevalence of anemia at an overwhelming two billion people, of which approximately 50% of cases are thought to be attributed to ID.

Table 2.1. Summary of Nutritional, Environmental and Genetic Factors Relevant to Anemia

Dietary Intake	**Environmental**	**Genetic**
Iron	Infections	Hemoglobinopathies
Copper	Hookworm	Thalassemias
Zinc	Malaria	Sickle Cell Anemia
Selenium	Shistosomiasis	Hemoglobins C, D & E
Vitamin A	*Helicobacter pylori*	
Vitamin B_6	Inflammation	
Vitamin B_{12}	Tobacco	
Folate		
Niacin		
Pantothenic Acid		
Riboflavin		
Thiamin		
Vitamin C		
Nutrient Bioavailability		
Protein Energy Malnutrition		
Alcohol		

The United Nations General Assembly special session on children (May 2002) adopted a goal to reduce by one-third the prevalence of anemia (2 billion) by 2010 (WHO, 2004). However, despite this goal and many initiatives little

progress has been made on lowering anemia rates worldwide (WHO, 2004). Global and regional estimates of anemia tend to vary considerably, owing in large part to the paucity of accurate data available (WHO, 2004). However, in low-income and resource-poor populations, the presumption that a significant percentage of young women and children will be anemic is generally considered to be true (WHO, 2004). Studies documenting anemia prevalence in pregnant Indigenous North American, Inuit women at 52-79% and infants at 19-47%, support this proposition (Willows and Gray-Donald, 2000; Willows and Gray-Donald, 2004; Whalen et al., 1997; Christofides et al., 2005). Furthermore, studies with the Alaskan Yup'ik found anemia prevalence at 9-17% suggesting that anemia and/or ID may also be a public health concern in the general adult population, including both men and women (Yip et al., 1997; Parkinson et al., 2000).

In North America the prevalence of anemia in Indigenous populations from representative samples is not known. The most recent survey data which included biochemical analyses is over 30 years old and comes from the 1975 Nutrition Canada Survey, where data were drawn from Canadian Inuit samples collected between 1970 and 1972 (Canada Bureau of Nutritional Sciences, 1975). Moreover, the sample size for the Inuit portion of the survey was small, with 100 individuals randomly selected from four major urban centres. Pregnant women were included, but not selected randomly. Nonetheless, the results of the study were of concern, as they were consistent across many age groups and in both sexes. Anemia was more prevalent in the Inuit sample than the national Canadian sample, with 8% of Inuit children, 15% of teenage girls, 7% of women aged 20-39 years, 14% of women aged 40-54 years, 22% of women over 55 years, and 38% of pregnant women classified as anemic according to WHO classification criteria (Canada Bureau of Nutritional Sciences, 1975). There was also a high prevalence (32%) of anemia among Inuit men over 55 years(Canada

Bureau of Nutritional Sciences, 1975). These findings were counterintuitive to the dietary intake survey results, which suggested that Inuit populations, including pregnant women, consumed equivalent or higher dietary iron levels than the national sample. Thus, other causes of anemia were likely but co-existing nutritional deficiencies such as folate and vitamin A were not further investigated (Canada Bureau of Nutritional Sciences, 1975).

ID is recognized as the main single cause of anemia worldwide. As such, a high prevalence of anemia in populations has generally been attributed to IDA. The terms IDA and anemia, in fact, are often used interchangeably (WHO, 2004). However, this may not be appropriate in regions with an unknown or complex etiology of anemia or where folate, vitamin B_{12}, or vitamin A deficiencies may coexist (WHO, 2004). Infectious diseases including malaria, parasitosis, tuberculosis, and HIV/AIDS have made significant contributions to anemia prevalence (WHO, 2004). Hemoglobinopathies must be considered, most notably in Mediterranean and Asian regions (Bagchi, 2004; Thurlow et al., 2005). Among Alaskan Natives, anemia has been recognized for years as a major public health issue (Parkinson et al., 2000). Despite this, the etiology of anemia in this population remains unclear as dietary iron intake has repeatedly been assessed as more than adequate, while iron stores appeared low (Parkinson et al., 2000; Yip et al., 1997). Thus, iron supplementation will not likely be effective in these populations and may be detrimental if infection or inflammation are simultaneously present (Sazawal et al., 2006). Effective intervention planning will therefore require a multidisciplinary approach to the diagnosis and treatment of anemia. Strategies should be evidence-based, targeted toward local conditions and the complex etiology of anemia within the population assessed (WHO, 2004).

2.5 POSSIBLE CAUSES OF ANEMIA IN INUIT

As a result of climate and high meat consumption in the Arctic, many of the factors listed in Table 2.1 are not relevant or applicable to anemia among Inuit. Specific genetic predispositions to defects in hemoglobin have not been reported. Consequently, this review will focus on only those factors of greatest public health importance in the North, including iron metabolism, vitamin A, folate, riboflavin, vitamin C, and high infection rates, with special attention to *H. pylori*, and parasitosis associated with raw meat or fish consumption. In addition to the potential inadequacy of specific nutrients, overall diet quality must also be considered as a contributing factor to nutrient malabsorption and malnutrition. Further issues such as the high intake of n-3 fatty acids that may interact with iron metabolism and hematopoiesis are discussed. Alcohol and tobacco use is reportedly high in the North (Segal, 1999; Hesselbrock et al., 2003) and may play a role in malnutrition. In this review we address both essential nutrients and inhibitors as they relate to Inuit of North America.

2.5.1 DIETARY IRON DEFICIENCY & ANEMIA

Iron is an essential dietary mineral required for oxygen transport, immune function, and energy production in the body. It is essential throughout life, but is especially critical during periods of growth and development (Cooper et al., 2006). When erythropoiesis is impaired by nutritional ID or blood loss hemoglobin synthesis is limited and iron is the optimal treatment (Weiss and Gordeuk, 2005b; Labbe and Dewanji, 2004). However, when the underlying cause of anemia is a functional or metabolic iron deficiency, iron treatment can be more detrimental than when no therapy is given (Weiss and Gordeuk, 2005b; Labbe and Dewanji, 2004). Functional ID is associated with inadequate iron delivery to the marrow or problems with iron utilization within the marrow

(Labbe and Dewanji, 2004). Thus, it is critical to identify the precise cause of anemia in a given population before implementing iron therapy. Generally, IDA is characterized by a hypochromic, microcytic anemia accompanied by a depletion of body iron stores (Brugnara, 2003). However, biochemical indicators of iron depletion such as serum ferritin and serum iron, are affected by infection and various nutrient deficiencies (Brugnara, 2003). The gold standard for assessing iron stores is considered to be iron staining of bone marrow biopsies (Brugnara, 2002), but this method is highly invasive and not suitable for population studies. The ratio of serum transferrin receptor protein to serum ferritin is a reasonable indicator of iron status and is increasingly used (Thomas and Thomas, 2002). For a comprehensive review of the diagnosis of IDA the reader is referred to the chapter by Biesalski & Erhardt (Biesalski and Erhardt, 2007).

The problem of IDA, and anemia in general has not been systematically reported in Inuit; some data are available from studies with Alaskan Native children and adults (Table 2.2). Petersen *et al* (1996) compiled data from multiple cross-sectional surveys conducted across Alaska in 1988-89 and showed anemia and depleted iron stores to be much higher in Alaskan Natives than the general U.S. population. This trend was found in all regions and across all demographics, including school-age children and adult men (Table 2.2) (Petersen et al., 1996). Surprisingly, dietary iron intake (obtained from at least one 24h recall per participant in each of 4 seasons in 1987-88) was found to be adequate and included many foods high in bioavailable iron (Petersen et al., 1996). The authors also reported elevated stool heme in a small, non-random sample, leading to the hypothesis that gastrointestinal blood loss may be responsible for the ID and anemia in this population. Since Alaskan Natives consume a relatively high quantity of marine mammals and fish (Petersen et al., 1996). Petersen *et al* (1996) speculated that a high intake of (n-3) fatty acids may promote blood loss through altered platelet function and/or hemostasis. In a follow-up study, Yip *et*

al (1997) conducted a descriptive survey (n=140) of Yup'ik adults from three rural villages, recruited on a voluntary basis (Table 2.2) (Yip et al., 1997). A high prevalence of ID (33% females; 16% males), but not anemia, was found, despite adequate dietary iron. The dietary survey here, however, was based on a single 24 h recall conducted in autumn (1992), whereas blood was collected from Sept 1992-Aug 1993. ID appeared to be highly pervasive, irrespective of age and gender, although anemia rates were not higher than the reference U.S. population. This was unusual and led the authors to suspect occult blood loss as the cause. Fecal hemoglobin was elevated in 90% of subjects, of which 70 of these subjects underwent endoscopy and gastric biopsy. Biopsy analysis revealed chronic active gastritis and infection with *H. pylori* in 99% of subjects. *H. pylori* infection causes a non-erosive and non-hemorrhagic gastritis which is not visible through endoscopy (Wood and Feldman, 1997). Erosive and hemorrhagic gastritis may be caused by overuse of alcohol or nonsteroidal anti-inflammatory drugs (NSAIDs), which were not fully accounted for in the report by Yip *et al* (1997). Parisitosis and genetic defects in hemoglobin were also not ruled out, although hemoglobinopathies are not considered to be present in this population (Yip et al., 1997). Parisitosis is a potential risk factor due to the common practice of raw meat and/or fish consumption and should be further investigated, especially with respect to low iron or hemoglobin status in men (Figure 2.1).

In one of the few investigations of anemia in Nunavut, there was an elevated prevalence of anemia (11.5%) reported in children aged 9 months to 17 years in 8 communities of the Keewatin region in 1994 (Table 2.2) (Thika et al., 1994). Although the etiology was not fully characterized, preliminary analysis suggested that macrocytosis was more common than hypochromic microcytosis, suggesting a vitamin B_{12} or folate deficiency. Furthermore, the anemia was thought to be nutritional in origin as it was independently associated with low

height-for-age scores and strongly associated with poverty (Thika et al., 1994). Another study of pregnant, Arctic Canadian women found ID to be present in 34% of women by the second trimester and 25% by term (Table 2.2) (Godel et al., 1992). Dietary iron intake, assessed by two 24 h recalls and food frequency questionnaire, was lower when a "southern" diet was followed compared to a traditional food diet. Notably, there was poor correlation between dietary iron intake and iron stores, the extent to which this could be explained by errors in food intake assessment or other genetic, absorption or bioavailability-related issues was not known.

Further studies on ID and anemia have been completed in northern communities of Manitoba, Ontario, and Quebec, including Nunavik. Whalen *et al* (1997) reported a 52-79% prevalence of anemia (defined as Hb < 110 g/dL) in 3 to 5 year old First Nations children from Northwestern Ontario (Table 2.2)(Whalen et al., 1997). However, this rate may be an overestimation due to the use of non-representative, stored blood samples and the lack of control for infection, which can independently depress hemoglobin concentration. Hodgins *et al* (1998) studied IDA in pregnant women and infants in Nunavik (Hodgins et al., 1998). Data and samples were drawn retrospectively from hospital charts and stored blood. The prevalence of anemia and iron depletion was found to be much higher in the Nunavik sample than a comparison group from Southern Quebec (Table 2.2) (Hodgins et al., 1998). Anemia was present in 40% of pregnant women at term and 58% of infants. In addition, more than 50% of non-anemic infants were iron deficient.

More recently, a cross-sectional study (n=115 children) was carried out in two First Nations Cree communities and one Inuit community to assess both the prevalence of ID and anemia, as well as their associated risk factors (Christofides et al., 2005). Although dietary intake was not quantified, 70 % of Inuit children consumed meat, liver, or blood in the past seven days, suggesting good sources

of bioavailable iron in the diet although seasonal variability and quantity of consumption was not assessed. Nonetheless, 48% of Inuit, and 26% of First Nations children were classified as anemic. Iron deficiency (sTfR>8.5 mg/L) was also highly prevalent (27%) and 53% of the children had depleted iron stores (SF<12 μg/L). Iron status may have been underestimated due to the high prevalence of *H. pylori* infection (39%) and elevated C-reactive protein (CRP) levels (29%). Anemia was significantly associated with *H. pylori* infection, consumption of cow's milk, and prolonged breastfeeding, although causative relationships were not established (Christofides et al., 2005). Of note, ID was reported as the main cause of anemia; however, 10% of anemic children were not ID, implicating one or more additional etiologies in these communities. Given a relatively small sample size, the ability to generalize these results was limited.

Finally, the etiology of anemia in Inuit and Cree infants was reported for Northern Quebec (Table 2.2) (Willows and Gray-Donald, 2000; Willows and Gray-Donald, 2003). IDA was found to be a significant health issue for Inuit infants of 6 months of age and older (Willows and Gray-Donald, 2000) and 9 month old Cree infants, with prevalence's of 21-48%, depending on age (Table 2.2) (Willows and Gray-Donald, 2003). Prevalence's of ID or IDA approached the level of anemia in infants 6 months of age and over, suggesting that IDA was a main explanatory factor in these cases. However, characterizing the type of anemia present in these communities was complicated by the pervasiveness of infection (Willows and Gray-Donald, 2004).

Table 2.2 Iron deficiency and anemia in North America northern populations

Subjects	N	Age (y)	Prevalence of anemia	Prevalence of ID	Dietary iron intake	Other comments	*H. pylori* status	Stool heme	Reference
Alaskan Native									
Children	355	< 12[1]	9-17%	50-70%[2]	NR	NR	NR	NR	Petersen
Females, youth	222	12-17[1]	15%	70%[2]	NR	NR	NR		et al 1996
Females, adults	415	18-44[1]	17%	45%[2]	Adequate	NR	NR	Elevated in 23 adults	
Females, adults	218	≥ 45[1]	11%	12%[2]	Adequate	NR	NR		
Males, youth	227	12-17[1]	9%	55%[2]	NR	NR	NR		
Males, adults	410	18-44[1]	7%	10%[2]	Adequate	NR	NR		
Males, adults	174	≥ 45[1]	15%	8%[2]	Adequate	NR	NR		
Alaskan Native Adults	140	18-86							
Females	83		NR	33%[3]	Adequate	NR	68/69 positive	Elevated in 90%	Yip et al
Males	57		NR	16%[3]	Adequate	NR			1997
Inuit children, Nunavut	399	9 mo – 17	11.5%; macrocytosis (n=23); hypochromia (n=11)	NR	NR	Low height-for-age scores with anemia	NR	NR	Thika et al 1994

Arctic pregnant women, NWT									
Pregnant	121		NR	34% [4]	Inadequate unless high TF diet	NR	NR	NR	Godel et al 1992
At Delivery	79		NR	25% [4]		NR	NR	NR	
Post-natal	29		NR	52% [4]		NR	NR	NR	
Infants, post-natal	29	≥ 4 mo	NR	31%	NR	NR	NR	NR	
First Nations Children, Northern Canada	284	6 mo – 24 mo	52-79%[5]	NR	NR	NR	NR	NR	Whalen et al 1997
Inuit, Nunavik									
Non-pregnant Women	NR[6]	< 25	NR	NR	Inadequate, 8 mg/d (mdn)	NR	NR	NR	Hodgins et al 1998
Pregnant, first Trimester	187	NR	31%[7]	Clinically suspected IDA	NR	NR	NR	NR	
Pregnant women	411	NR	40%[5] 24%[8]	NR	NR	85% prescribed Fe supplement	NR	NR	
Infants	116	< 4 mo	35%[9]	NR	NR	NR	NR	NR	
	123	4 mo - 9 mo	49%[10] (20% mild) [11]	~50%	NR	NR	NR	NR	

	128	9 mo - 14 mo	36% (23% mild)[11]	64% (non-anemic)	NR	NR	NR	NR	
Cord blood	100						8 positive; 19 equivocal; 73 negative		
Inuit and Cree Children	115	4 mo – 18 mo	36%[8]	27%[12]	NR	High prevalence of infection (29% ↑ CRP)	39% positive	NR	Christofides et al 2005
Cree	65	11 mo ±4	26%[8]	28%[12]	NR	NR	NR	NR	
Inuit	50	12 mo ±5	48%[8]	25%[12]	NR	NR	NR	NR	
Cree infants	274	9 mo	26%[8]	20%[13]	NR	No VAD; ↓ serum retinol with anemia; Vit A suppl ↑ Hbg levels	NR	NR	Willows & Gray-Donald 2003

Inuit infants, Nunavik									
	109	2 mo	21%[14] [0% microcytic]	1% ID[13] & anemic	NR	NR	NR	NR	Willows et al 2000
	116	6 mo	47%[14] [4% microcytic]	24% ID[13] & anemic	NR	NR	NR	NR	
	122	12 mo	38%[14] [21% microcytic]	26% ID[13] & anemic	NR	NR	NR	NR	
Inuit, Nunavut	366	3 - >60	NR	NR	80% > 2/3 RDA for iron	>60% less than 2/3 RDA for Vit A	NR	NR	Kuhnlein et al 1996
Yukon, Dene/Métis children	222	10-12	NR	NR	<10% below EAR for iron	>50% below EAR for Vit A, E, Mg, P	NR	NR	Nakano et al 2005

NR = not reported. Abbreviations: ID, iron deficiency; Hbg, hemoglobin; mdn, median; NWT, Northwest Territories; TF, traditional food; VAD, vitamin A deficiency; vit A, vitamin A.

[1] based on the 1989 CDC age-specific criteria
[2] defined as serum ferritin (<12 µg/L)
[3] defined as serum ferritin <15 µg/L and transferrin saturation <16%
[4] defined as serum ferritin (<15 µg/L)
[5] defined as hemoglobin < 115 g/L
[6] 400 households sampled in total
[7] defined as hemoglobin < 120 g/L
[8] defined as hemoglobin < 110 g/L
[9] defined as hemoglobin < 100 g/L
[10] defined as moderately anemic; hemoglobin < 105 g/L
[11] defined as mildly anemic; hemoglobin < 105-109 g/L
[12] defined as serum TfR (<8.5 mg/L)
[13] defined as serum ferritin (<10 µg/L)
[14] defined as > 2 SD below the reference mean

2.5.2 DIETARY INTAKE IN INUIT

The traditional Inuit diet is abundant in bioavailable iron as a result of the reliance on marine mammals, land animals, fish, and the high consumption of liver (Kuhnlein and Receveur, 2007). However, the nutrition transition from traditional foods to market foods involves the replacement of more nutrient-dense traditional foods with processed, high starch, nutrient-poor market foods (Kuhnlein et al., 2004). Also, traditional food use has been shown to be much lower in children and young adults than adults over 40 years of age (Kuhnlein et al., 2004; Kuhnlein and Receveur, 2007), and micronutrient nutrition, including iron, may be inadequate in population subsets not consuming the traditional local diet. Additionally, traditional food is harvested and consumed intermittently, according to season, climate, species migration, and other unpredictable factors. Therefore, if diet and nutritional status is not assessed across seasons, usual intake may be overestimated or underestimated. The extent to which iron intake and iron stores vary seasonally for Inuit has not been reported.

Over the past decade, several dietary surveys have been carried out in the Canadian Arctic, in cooperation with the Indigenous Peoples of the North. A large dietary survey of 44 communities throughout the Yukon, Northwest Territories, and Nunavut, used 24 h recalls and a food frequency questionnaire (traditional food items in past month), assessed in both a high traditional food use season (fall) and a low traditional food use season (winter) in 10 % of households randomly selected for participation (Kuhnlein et al., 2004). From this survey, dietary iron intake was observed to be significantly higher on days when traditional food was consumed compared to days with no traditional food use, after adjustment for community size, age and season (Kuhnlein et al., 2004). Overall, dietary iron intake appeared to be consistently adequate across regions, age (children were not included), and gender (Kuhnlein and Receveur, 2007). In

1996, it was reported that more than 80% of Inuit (n=336) in Qikiqtarjuaq, Baffin Island, consumed more than two-thirds of the dietary recommendation for iron, suggesting that dietary intake was adequate as assessed at the time (Table 2.2) (Kuhnlein et al., 1996). Again, iron intake appeared to be adequate across all ages (3 to >60 years) and sexes. However, this community had the highest rate of wildlife harvesting (by weight per capita) in the Baffin region, and thus may not have been representative of Baffin Island or Nunavut in general, but instead demonstrated the upper end of intake (Kuhnlein et al., 1996). A later study of 222 Yukon and Dene/Métis 10-12 year old children using multiple 24 hour recalls in 2 seasons (late fall/early winter and late summer/early fall), reported that dietary iron intake was likely adequate, based on the estimated average requirement (EAR) (Table 2.2) (Nakano et al., 2005).

2.5.3 IRON LOSS AND MITIGATORS OF IRON METABOLISM

If dietary iron, especially the more bioavailable heme iron, is still adequate in the Inuit diet, the presence of IDA should be investigated as a result of blood loss, malabsorption, parasitosis, and infections, including colonization with *H. pylori*. Occult, gastrointestinal blood loss may be related to an endemic hemorrhagic and erosive gastritis, as suggested by Yip *et al* (1997). This loss of blood could be further aggravated by a high intake of fish oil (n-3 fatty acids), which may alter platelet function and increase bleeding time for clot formation (Yip et al., 1997). High fish oil diets may reduce non-heme iron stores (Miret et al., 2003), although the possible clinical significance of this effect is not known.

Alcohol use in adults may also play a role in nutrient malabsorption. Although data are limited and are expected to vary by region, alcohol consumption among various cultures of Alaskan Natives is reportedly high (Segal, 1999; Hesselbrock et al., 2003) as is chewing tobacco use (Segal and Saylor,

2007). Alcohol abuse is known to damage the gastrointestinal mucosa, leading to impaired digestion and nutrient absorption and consequently, malnutrition (Rajendram and Preedy, 2005). However, excessive alcohol use has also been associated with iron overload and hepatic damage (Ford et al., 1995; Bell et al., 1995). Alcohol may lead to higher iron stores as a result of the iron content of some alcoholic drinks, increased iron absorption (likely through the paracellular route), hepatic inflammation causing iron release and increased ferritin expression, and increased iron turnover for erythropoiesis as a result of folate deficiency (Ioannou et al., 2004). Environmental tobacco exposure is associated with increased rates of respiratory and ear infections, as well as asthma, which may contribute to the anemia of inflammation (Prokhorov et al., 2006). Therefore alcohol and tobacco use cannot be discounted as contributors to anemia.

2.5.4 HELICOBACTOR PYLORI AND IRON METABOLISM

Although chronic infection with *H. pylori* does not appear to produce a hemorrhagic gastritis, the infection may impair iron absorption. The absorption of non-heme iron is highly dependent on gastric acid secretion (an acidic pH <3) and ascorbic acid (either secretory or dietary sources) for the reduction of ferric to ferrous iron and subsequent intestinal uptake. Ascorbic acid also promotes formation of a soluble chelate with ferrous iron for absorption (Annibale et al., 2003). *H. pylori*-induced gastritis reduces gastric acid secretion and is therefore a potential cause of reduced iron absorption and increased risk of IDA (Annibale et al., 2003). Both epidemiological (Queiroz and Luzza, 2006; Cardenas et al., 2006) and clinical evidence (Choe et al., 2001; Choe et al., 1999; and Kearney, 2005) suggest a relationship between IDA and *H. pylori*. Patients with refractory IDA and *H. pylori* infection, have recovered from IDA after eradication of the infection in small, uncontrolled case studies (Annibale et al., 2003; Konno et al.,

2000; Ashorn et al., 2001; Marignani et al., 1997). In fact, a small (n=25) randomized, controlled clinical trial showed that *H. pylori* eradication was effective in treating IDA in children, with or without concomitant oral iron therapy (Choe et al., 1999). These children had *H. pylori* infections with no evidence of gastrointestinal lesions, and treatment with iron alone did not improve hemoglobin levels (Choe et al., 1999). However, it is not known why only some patients with *H. pylori* infection develop IDA (Annibale et al., 2003). Individuals at increased risk of ID in general, are thought to be more likely to develop IDA associated with *H. pylori* infection (DuBois and Kearney, 2005).

The relationship between refractory IDA and *H. pylori* infection may be explained by several hypotheses. *H. pylori* is a bacterium which requires iron as an essential growth factor and is capable of binding and transporting iron across the cell membrane (Annibale et al., 2003). Thus, the bacterium may compete for iron within the GI tract. IDA is also commonly present in chronic inflammatory disorders such as celiac disease (Annibale et al., 2003). In these conditions, IDA results from sequestration of iron in the antral mucosa, as a result of inflammation (Annibale et al., 2003) and inflammation from *H. pylori* infection could result in similar changes. Inflammation may also increase intragastric pH levels, thereby reducing iron absorption (Annibale et al., 2003) (Figure 2.1). Alternately, iron absorption may be indirectly impaired by *H. pylori*-induced changes in ascorbic acid metabolism (Annibale et al., 2003) since ascorbic acid promotes the formation of a soluble ferrous iron chelate and subsequent absorption of ferrous iron (DuBois and Kearney, 2005) and levels of ascorbic acid in gastric secretions have been shown to be depressed in *H. pylori*-infected individuals (DuBois and Kearney, 2005). Decreased gastric acid secretion may be related to atrophy of gastric glands and the mucosa of the fundus, which are characteristic of chronic *H. pylori* infection (DuBois and Kearney, 2005). Furthermore, alterations in gastric acid secretion may affect the absorption of

vitamins B_{12} and E, as well as β-carotene, although little is currently known about these interactions (Annibale et al., 2002).

The *H. pylori* population of the human stomach and the genetic variation among different strains are highly heterogenous (Blaser, 1997). Although many genetic markers have been identified as virulent factors for increased risk of disease, research to date has found no relationship between strain virulence and IDA (Annibale et al., 2003). More research is needed to clarify the association between IDA and chronic *H. pylori* infection, as well as the potential for adaptation given the chronic nature of this infection.

There are very limited data on the epidemiology of *H. pylori* infection in Inuit communities. Low socioeconomic status, overcrowded housing conditions, poor sanitation, and contaminated water supplies are considered risk factors for infection (Sinha et al., 2002) and are pervasive conditions across the Arctic (Jenkins et al., 2003). Bernstein *et al* (1999) reported a 95% positive seroprevalence for *H. pylori* in Wasagamack, a First Nations community in Northern Canada whereas a similar study in two Inuit communities found 50% seroprevalences (McKeown et al., 1999). However, seroprevalences in both studies are considerably higher than the estimated prevalence of 30% in Canada (Hunt et al., 2004). In a follow-up study of children in Wasagamack, 56% of children (n=163) aged 6 weeks to 12 years had positive stool tests for *H. pylori* (Sinha et al., 2002). Notably, although 57% of children were classified as anemic (Hb < 100 g/dL), anemia was not associated with *H. pylori* infection or blood loss and iron status was not assessed (Sinha et al., 2002) contradicting the positive relationship previously described (Christofides et al., 2005). *H. pylori* prevalence of 27% was found in randomly selected umbilical cord plasma samples of Nunavik infants (Table 2.2) (Hodgins et al., 1998) and infection rates are known to increase with age.

2.5.5 VITAMIN A

Vitamin A deficiency is a recognized cause of anemia, although the epidemiology and pathogenesis are not clearly understood (Semba and Bloem, 2002). Various and diverse biological mechanisms of vitamin A deficiency may contribute to anemia, including the modification of hematopoiesis through an altered immunity to infection, leading to the anemia of inflammation (AOI) (Means, 2000) and the modulation of iron metabolism and erythropoiesis (Semba and Bloem, 2002). The hematological profile of a vitamin A-related anemia has been described as hypochromic (or microcyctic and hypochromic), although this has not been fully characterized and is likely amenable to iron status and the presence of infection (Semba and Bloem, 2002).

In studies in areas endemic with nutritional anemia, combined supplementation with vitamin A and iron has been shown to be much more effective than iron or vitamin A alone in the recovery from anemia (Mejia and Chew, 1988; Suharno et al., 1993; Semba and Bloem, 2002). Descriptive studies have also identified both iron and vitamin A as independent risk factors(Gamble et al., 2004) and vitamin A as a primary risk factor for anemia in children living in endemic areas (Thurlow et al., 2005). Carotene-rich traditional foods have been shown to improve vitamin A status and hemoglobin levels in children (Vuong et al., 2002). Investigations on vitamin A and hemoglobin status are mainly from tropical areas in Asia and Africa and have been concisely reviewed by West et al (West Jr et al., 2007).

The Canadian Inuit are thought to be at risk for inadequate vitamin A intake based on dietary surveys (Egeland et al., 2004; Kuhnlein et al., 2007). Traditional Inuit diets include excellent sources of preformed vitamin A, such as liver, fish roe, and blubber (Kuhnlein et al., 2006). However, the nutrition transition from traditional to less nutritious market foods may increase vulnerability to inadequacy. In the dietary survey of Canadian Inuit previously

described, with dietary data collected over a high and low traditional food season, vitamin A inadequacy was found to be of significant concern for young adults. Based on the EAR for retinol activity equivalents, 68% of men and 60% of women (15 to 40 years of age) had an intake below the EAR (Egeland et al., 2004). Among adults over 40 years of age, only 11% of men and 15% of women had intakes below the EAR (Egeland et al., 2004). This pattern reflects the increasing use of traditional food with age among the Canadian Inuit (Kuhnlein et al., 2004). Similarly, vitamin A intake was found to increase with traditional use in Alaskan Natives; however mean or absolute intakes were not reported (Bersamin et al., 2007).

Biochemical evidence of suboptimal vitamin A status among Inuit populations is sparse, with one report of adequate mean plasma vitamin A levels in Alaskan Native urban women (n=74) in 2001 (Nobmann and Lanier, 2001). A survey of vitamin A concentration in umbilical cord blood (n=594) compared infants from Nunavik with First Nations infants, and non-Indigenous infants from Southern Quebec (Dallaire et al., 2003). Nunavik and First Nations infants had significantly lower vitamin A cord blood concentrations than Southern Quebec infants. In addition, 8-12% of Nunavik and First Nations infants had levels indicative of vitamin A deficiency (Dallaire et al., 2003). An earlier study of 135 Aboriginal, and non-Aboriginal mothers in the pre-1999 Northwest Territories also found differences in vitamin A status by race (Godel et al., 1992). Aboriginal mothers had significantly lower (> 2-fold) vitamin A intakes, plasma retinol concentrations, and a greater risk of deficiency in the absence of supplementation. Aboriginal infants in the study also had significantly lower plasma retinol concentrations at birth, although there was no evidence of clinical deficiency (Godel et al., 1992). Circulating retinol concentrations are, of course, only indicative of late-stage deficiency when hepatic retinol stores decline. Indicators to detect subclinical vitamin A deficiency in population studies are not available.

2.5.6 VITAMIN E

The traditional Inuit dietary pattern likely supplied ample vitamin E, based on the high consumption of fatty fish and liver (Kuhnlein et al., 2006) although inadequate vitamin E intake may result from the nutrition transition. While severe deficiency is rare and produces a progressive, neurological decline (Feki et al., 2001), moderate deficiency, which has been associated with malnutrition, lipid malabsorption and anemia, is more common and relatively asymptomatic (Feki et al., 2001). Vitamin E-dependent anemias have been characterized in humans as part of protein-energy malnourished states (Gross and Melhorn, 1972) as well as in premature infants (Melhorn and Gross, 1971a, Melhorn and Gross, 1971b), and have been described as clinically and morphologically due to abnormal and impaired red blood cell maturation (Gross and Melhorn, 1972). However, vitamin E is still considered only a potential erythropoietic factor in humans (Figure 2.1), as a compensatory adaptation during deficiency is thought to circumvent the role of vitamin E in erythropoiesis (Drake and Fitch, 1980). If this compensatory mechanism is not functional (as in protein-energy malnutrition)(Drake and Fitch, 1980), individuals may respond to vitamin E and/or multi-vitamin supplementation.

Vitamin E status is also important during anemia to counteract oxidative stress (Traber and Kamal-Eldin, 2007). Anemia involving RBC destruction results in enhanced levels of circulating iron and consequently, oxidative stress (Traber and Kamal-Eldin, 2007). Poor vitamin E status is commonly associated with anemia and may contribute to the neurological impairments of nutrient deficiency (Traber and Kamal-Eldin, 2007).

A 1975 study found that plasma α-tocopherol levels in Alaskan Natives were comparable to the general U.S. population consuming a mixed diet (Wei Wo and Draper, 1975). A 1989 report also found no difference between serum vitamin E in aboriginal infants versus non-aboriginal infants (n=79), although one

Inuit infant was found to be vitamin E deficient (Godel, 1989). More recently, a dietary survey reported close to 100% inadequacy for vitamin E in Inuit, Yukon, and Dene/Métis adults (Kuhnlein et al., 2006), although the authors acknowledged this figure may be an underestimation of intake, as the stability of α-tocopherol in traditional Inuit foods analyzed was not known. Nonetheless, with limited intake of fresh fruits and vegetables, little use of plant oils, and increasingly limited use of traditional foods, vitamin E nutrition remains a potential issue of concern for anemia in Inuit. Biochemical assessment of vitamin E status is limited, as circulating α-tocopherol, the most commonly used biomarker of vitamin E status, is not representative of dietary intake or whole body stores.

2.5.7 FOLATE

Chronic folate deficiency impairs the formation of RBC, producing immature and atypically large cells (Canada Bureau of Nutritional Sciences, 1975). If the deficiency is severe enough, the hemoglobin content will also be reduced, resulting in macroctyic anemia (Canada Bureau of Nutritional Sciences, 1975). RBC folate reflects tissue stores while serum levels reflect the amount of folate in transport (Canada Bureau of Nutritional Sciences, 1975). Circulating levels fall after several weeks of dietary deficiency, whereas bone marrow (RBC) changes take months to respond (Canada Bureau of Nutritional Sciences, 1975).

Macrocytic anemia may be classified as megaloblastic or non-megaloblastic but only through the examination of the bone marrow (Aslinia et al., 2006). Deficiency of folate or vitamin B_{12} directly impairs erythropoiesis, as a result of their function as a vitamin or cofactor for the normal proliferation and maturation of all cells, including RBC (Aslinia et al., 2006). Under these conditions, DNA synthesis becomes defective, producing larger than normal

erythroblasts (megaloblasts) (Aslinia et al., 2006). The enlarged erythroblasts take on an oval shape containing an immature nucleus (Aslinia et al., 2006), and the marrow responds by increasing production of all myeloid cell lines and erythroid elements, in an attempt to compensate (Aslinia et al., 2006).

Macrocytosis may also result from medication use, non-nutritional hematologic disorders such as leukemia, hypothyroidism, and alcoholism (secondary to nutrient malabsorption), and should be distinguished from primary folate and vitamin B_{12} deficiencies (Aslinia et al., 2006). Sub-clinical or mild cases of folate or B_{12} deficiency may present as macrocytosis without anemia or may not demonstrate hematological changes (Wickramasinghe, 2006). Chronic alcohol abuse commonly results in macrocytosis with normoblastic erythropoiesis (Wickramasinghe, 2006) and is also thought to interfere with folate metabolism through the one-carbon metabolic pathway (Cravo et al., 1996).

Since 1998, most cereal grain products in North America have been fortified with folic acid as a result of a mandatory fortification policies (Ray, 2004). The level of fortification (140 µg/100 g grain) is intended to provide an average daily increase of 100 µg of folic acid to the general population (Liu S et al., 2003), to help meet the RDA of 400 µg dietary folate equivalents (DFE) per day for women over 18 years of age. This policy has resulted in significant improvements in folate status, and has reduced the prevalence of births with neural tube defects (NTD) in US (Choumenkovitch et al., 2001; Choumenkovitch et al., 2002) and Canadian (Ray et al., 2002; Ray, 2004; Liu S et al., 2003) studies.

The primary purpose behind the folic acid fortification policy was to reduce the incidence of pregnancies affected by NTD. In order to reduce the risk of fetal NTD peri-conceptual maternal RBC folate levels should be greater than 400 ng/mL (Reisch and Flynn, 2002). In contrast, the cut-off point for folate deficiency which results in megaloblastic anemia is 140 ng/mL RBC folate (Reisch

and Flynn, 2002). Of note, the optimal level of 400 ng/mL RBC folate can only be achieved through supplementation with folic acid (the synthetic form of folate) and not through the consumption of foods naturally rich in folate (Reisch and Flynn, 2002; Lawrence et al., 2006). The actual amount of folic acid in fortified grain products has been shown to be considerably greater than the mandatory requirements (Bailey, 2004; Choumenkovitch et al., 2002) and has resulted in a projected increase of 200 μg folic acid/day which is twice the predicted increase of the original fortification policy.

Data from the early 1990s suggest that macrocytic anemia and folate deficiency may have been an issue for Inuit children (Moffatt, 1995; Thika et al., 1994). In the Keewatin study previously described (Table 2.2), there was a high prevalence of anemia (11.5%) (Thika et al., 1994). Of the children classified as anemic, 24% had a low mean cell hemoglobin concentration, suggesting hypochromia (possible ID) and 50% showed a high mean cell volume (MCV), pointing toward macrocytosis (Thika et al., 1994). A small subset of the high MCV samples (n=7) was then randomly selected and further analyzed. Three of the seven samples were reported to have very low serum folate levels (Moffatt, 1995). In the last Canadian nutrition survey (1975) reporting serum folic acid levels in Inuit populations (Canada Bureau of Nutritional Sciences, 1975) approximately 80% of Inuit were found to be at high risk of deficiency (defined as serum folate < 2.5 ng/mL) and serum folate levels were much lower in the Inuit survey than the national sample (Canada Bureau of Nutritional Sciences, 1975). The majority of participants were categorized at moderate or high risk of folate deficiency, based on serum folate values (Canada Bureau of Nutritional Sciences, 1975). More recent surveys with biochemical data are not available; however, evidence of clinical cases of folate deficiency and macrocytic anemia in Inuit children, who responded to folic acid supplementation suggests that subclinical deficiency was likely at the time (1995) (Moffatt, 1995).

Since the implementation of the national folic acid fortification program (1998), one would expect dietary folate intake and folate status to improve. However, it must be considered whether this national fortification has reached all segments of the population. For example, several years after national mandatory folic acid fortification was implemented differences in serum folate levels in pregnant Californian women were found to be related to maternal race and ethnicity as well as age and BMI (Lawrence et al., 2006). Races including Black, Hispanic, and Asian; younger age; and classification as overweight or obese were independently related to a serum folate level in the lowest quartile (Lawrence et al., 2006). This issue is a special concern with communities in the Arctic due to geographic isolation and food availability. Traditionally, folate sources in the Inuit diet were provided by organ meats and wild plants(Moffatt, 1995), however, consumption of traditional foods has declined (Kuhnlein et al., 2004). A small cross-sectional study (n=74) of Native Alaskan women in 1996-97 reported low intake of traditional foods and low RBC folate levels (< 200 ng/mL) in 32% (mean=276 ng/mL; range=68 to 857 ng.mL) of the women studied (Nobmann and Lanier, 2001). In a large survey of 44 Arctic communities, mean folate intakes of Yukon, Dene/Métis, and Inuit adults, were reported in the range of 303 ± 10 to 319 ± 13 ug DFE, based on dietary data collected in both a low and high traditional food use season (Kuhnlein et al., 2004). This amount is close to the EAR for adults (320 ug DFE) and therefore not likely to produce anemia. However, more vulnerable groups within these populations such as infants and children may still be at risk for folate deficiency if folate fortified foods are not regularly consumed.

2.5.8 OTHER NUTRIENTS ESSENTIAL IN ERYTHROPOIESIS

Erythropoiesis and heme synthesis are complex processes dependent on many nutrients in addition to adequate iron, including vitamins B_6 and riboflavin (Fishman et al., 2000), in addition to vitamins A, B_{12}, C, folate, and possibly E, as noted above. Supplementation trials with riboflavin have produced an enhanced hematological response to iron when dietary iron intakes are low (Powers, 2003), similar to what has previously been reported in vitamin A trials. There is evidence from animal and cell culture work suggesting that riboflavin deficiency impairs iron mobilization and transport, as reduced flavins appear to participate in the mobilization of ferritin iron in gastrointestinal and other tissues (Powers, 2003). Riboflavin deficiencies are common in less developed countries when diets are primarily rice and lacking in meat, dairy, and fresh fruits and vegetables (Fishman et al., 2000). Under these conditions, riboflavin status may contribute to a significant percentage of cases of anemia. Based on the Arctic dietary intake survey previously described, Inuit experienced a moderate prevalence of adequacy (40-57% adequate) of riboflavin (Kuhnlein et al., 2007), but further work is needed to confirm this finding.

Vitamin C is a known enhancer of non-heme iron absorption and therefore may improve the hematological response to iron supplementation when dietary iron intake is low. Vitamin C may also have indirect effects on folate metabolism (Fishman et al., 2000). However, a direct action of vitamin C in erythropoiesis remains unclear and human trials have reported equivocal findings (Fishman et al., 2000). Vitamin C intake in the Canadian Arctic was reported to be highly variable with 0-99% adequacy based on the EAR (Kuhnlein et al., 2007). Across three Arctic cultures, elder men were most at risk for inadequacy (Kuhnlein et al., 2007).

Thiamin, niacin and pantothenic acid have also been implicated in anemia development or treatment within experimental studies and/or through

anecdotal evidence (Fishman et al., 2000). However, data from supplementation trials in malnourished populations is not available to confirm these roles. Vitamin B_6 may be effective in treating sideroblastic and sickle cell anemia when B_6 status is low, but a similar role in nutritional anemia is unclear (Fishman et al., 2000). Copper and zinc deficiencies are known to produce hematological disturbances including anemia. However, clinical deficiencies of vitamin B_6, copper, and zinc are rare and not suspected in the Arctic based on Inuit dietary surveys that demonstrate regular meat, organ, fish and seafood consumption (Kuhnlein et al., 2007).

Beyond the issue of anemia, there is also concern for inadequate micronutrient intake based on current dietary patterns in the North and supplement use is a consideration. However, less than 5% of more than 2000 Inuit, Yukon First Nations, and Dene/Métis adults surveyed reported supplement use (Kuhnlein et al., 2007). Supplement use was more commonly reported (25% of respondents) among Dene/Métis and Yukon children but information on type of supplement and dose was lacking (Nakano et al., 2005). Thus little information exists on supplement use in the North, including type as well as frequency of use. Considering both diet and supplement use, poor micronutrient status may be a contributing factor in the high rate of anemia and infections among Inuit.

2.5.9 ANEMIA OF INFLAMMATION

Infection is considered a main cause of anemia in children and may be related to a higher rate of erythrocyte destruction, impaired release of storage iron, and insufficient production or response to erythropoietin (Abshire, 1996). AOI presents as a mild to moderate anemia and is characterized by low serum iron concentrations, low to normal serum transferrin, and normal to high serum ferritin concentrations (Ganz, 2006). The chronic state of inflammation results in

higher circulating levels of interleukin-6 and other inflammatory cytokines, which up-regulate hepcidin synthesis by hepatocytes (Ganz, 2006). Hepcidin interacts with the iron exporter ferroportin to block the release of cellular iron from enterocytes, hepatocytes, and macrophages, resulting in hypoferremia and anemia (Ganz, 2006; Atanasiu et al., 2007).

If relatively mild, AOI should not compromise long term health, as occurs with IDA (Willows and Gray-Donald, 2004). However, distinguishing AOI from IDA is difficult, as iron metabolism is modified in both conditions and when present together, they can create a more severe anemia than either condition alone (Willows and Gray-Donald, 2004; Walter et al., 1997). It is important to differentiate between AOI and IDA prior to treatment, as iron supplementation in iron-sufficient children may impair growth (Dewey et al., 2002). Thus, from a public health perspective, recommendations to increase dietary iron, rather than supplemental iron, may be more appropriate when infection is a confounding factor in anemia (Willows and Gray-Donald, 2004).

Inuit children are reported to experience higher rates of bacterial and viral infections and illness than non-Aboriginal children (Jenkins et al., 2003). Infections of particular concern include respiratory tract, otitis media, gastrointestinal, and tuberculosis (Jenkins et al., 2003). Researchers have speculated that poor micronutrient intake, especially inadequate vitamin A nutrition, may be a contributing factor to this problem, although tobacco exposure and socioeconomic factors such as housing conditions likely play a role in some conditions (Jenkins et al., 2003). Adults may also suffer from chronic infections including *H. pylori* and parasitosis. Thus, evaluation of anemia in the North should also assess state of inflammation, which can impact upon hemoglobin levels as well as iron metabolism.

2.5.10 PARASITES

Malnutrition induced by parasitic infections is a significant public health issue in less developed countries and regions. Parasites can both cause and aggravate malnutrition, as well as impair developmental outcomes (Stephenson et al., 2000), although risk of anemia is parasite species-specific. Malnutrition and parasitosis also tend to occur simultaneously in the same populations making it difficult to determine cause and effect relationships. Nematode infections in particular, including roundworm, hookworm, and whipworm, are especially damaging in the presence of protein-energy malnutrition, IDA, vitamin A deficiency, or goiter (Stephenson, 1994).

Trichinellosis is an important public health issue in the Arctic (MacLean et al., 1992; MacLean et al., 1989). *Trichinella spiralis* is a parasitic roundworm that invades the intestinal mucosa, producing larvae which enter the circulation and penetrate striated skeletal myocytes (Kociecka, 2000). *T. spiralis* has been isolated in many arctic food species including polar bear and marine mammals (MacLean et al., 1992). Reported trichinellosis cases in Alaska were 36 times more prevalent than in the general U.S. population on a per capita basis from 1970-85 (MacLean et al., 1989). While the incidence of this infection has been on the decline over the past few decades, several outbreaks of human infection in the Canadian Arctic were reported in the literature between 1982 and 1987 and all were linked to previous consumption of raw polar bear or walrus meat (MacLean et al., 1989). Although *Trichinella* infection is a health issue in the North, it is not typically associated with blood loss (Kociecka, 2000). The acute stage of infection involves gastrointestinal changes that may limit nutrient absorption and morphological changes to the mucosa may persist (Kociecka, 2000). Other nematodes such as hookworm feed on blood and cause intestinal micro-hemorrhage and have been clearly linked to IDA (Persson et al., 2000). The

impact and etiology of trichinellosis on micronutrient status is less certain and should be further investigated.

Fish tapeworms from the cestode *Diphyllobothrium* also infect humans in Arctic regions (Curtis and Bylund, 1991). Specifically, *D. latum* has been associated with pernicious anemia, although it is more common in sub-Arctic regions than the far North (Curtis and Bylund, 1991). *D. latum* associated anemia was documented in Finland and thought to involve a genetic predisposition as well as general malnutrition (Curtis and Bylund, 1991). However, the last reported case with co-occurring anemia was in the 1970s (Curtis and Bylund, 1991). Other species of *Diphyllobothrium*, including *D. ursi* which is more common in northern Canada and Alaska are not known to cause anemia (Curtis and Bylund, 1991). In summary, current data on the prevalence and variety of parasitosis among Inuit are lacking, but must be considered as potentially contributing to malnutrition and anemia.

2.6 CONCLUSIONS

Based upon recent dietary surveys and cross-sectional studies from communities throughout the Arctic, anemia appears to be a public health concern, especially for Inuit women and young children. However, reliable estimates do not exist for the prevalence of anemia for Inuit throughout Alaska and Canada.

It cannot be assumed that IDA constitutes the majority of anemia cases in the Arctic, especially among adults. There is concern for other underlying nutritional deficiencies including vitamins A, E, C, and folate, which may complicate the etiology of anemia. Thus, measures of hemoglobin concentration should be complemented with a full biochemical nutritional status assessment analysis as well as mean cell volume to differentiate macrocytosis from

microcytosis, when possible. In addition to nutritional status, active infections, parasitosis and chronic gastrointestinal *H. pylori* colonization should also be considered. Multiple micronutrient deficiencies may contribute to infection and inflammation, complicating anemia diagnosis. The etiology of anemia in the North is likely to be complex and multifactorial. This complexity can further complicate diagnosis, such as when macrocytosis is present at the same time as microcytosis. Cause(s) of anemia need to be fully understood for effective health promotion programs that deal with this preventable disorder.

2.7 ADDITIONAL RECENT LITERATURE

The prevalence of anemia (43 %) and iron deficiency anemia (21 %) for Inuit women of Nunavik (n=466) was published in 2011 (Plante et al., 2011), using data collected in the 2004 Inuit Health Survey for Nunavik. Among anemic over 50 years of age, 42 % suffered from AOI while 44 % of cases were of unknown etiology. Notably, almost all women (99 %) had normal values for serum retinol, serum vitamin B_{12}, and serum folate. Elevated blood lead concentrations (>0.48 µmol/L) were present in 7.5 % but there was no correlation between blood lead and hemoglobin. Hemoglobin and iron biomarkers were not measured for Inuit men in the Nunavik survey.

The Nunavut Inuit Child Health Survey, 2007-2008, occurred concurrently with the IPY Inuit Health Survey, 2007-2008. Children aged 3-5 years (n=388) were assessed for hemoglobin, serum ferritin, and C-reactive protein, with anemia observed among 16.8 % and IDA among 5.4 % (Pacey et al., 2010). Diet was assessed during a high traditional food use season (fall) using a food frequency questionnaire (past month) and 24 hour recall (with a second, non-consecutive recall obtained from 19.8 % of children) (Johnson-Down and Egeland, 2010). Almost all children (>99 %) had iron intakes above the EAR. Children treated in the past year for an ear infection and those residing in

crowded homes were more likely to have anemia from other causes (not IDA). A borderline significant interaction was observed between TF consumption and food insecurity, whereby % anemia was higher among children from food insecure households who consumed no TF (Egeland et al., 2011c).

Analyses from the IPY Inuit Health Survey, 2007-2008, have reported a high rate of food insecurity (62. 6 %), which was associated with higher RBC trans-fatty acid %, and lower hemoglobin and serum ferritin concentrations, and lower energy-adjusted micronutrient intakes (Egeland et al., 2011b). TF consumption, conversely, was associated with higher vitamin D status, n3-PUFA status and serum ferritin (Egeland et al., 2011b). A comparative analysis of dietary intakes in 2007-2008 with surveys in 1998-1999 also revealed a decline in TF consumption among Inuit and a relationship between higher BMI and consumption of high fat market foods. These findings suggest that dietary transition continues among Inuit and raises concern about diet quality (Sheikh et al., 2011).

A subset analyses from the IPY Inuit Health Survey, 2007-2008, reported a weak association between serum ferritin and the activity of desaturase 5, suggesting fatty acid synthesis may be impaired in iron deficiency (Zhou et al., 2011). This nutrient interaction could have implications for the n3-PUFA status of Inuit as a result of the nutrition transition.

Bridge 1

From the review of the literature, there is little known about current iron status and anemia prevalence among Inuit adults. Evidence suggests that ID has historically been highest among young Inuit women while anemia appears to increase with age for Inuit men. However, with the exception of Alaska and Nunavik, surveys have been limited to convenience samples with small sample sizes, and most have failed to account for the influence of inflammation on nutritional biomarkers. Furthermore, the Alaska Native population includes the Inupiat and Yup'ik (Inuit) as well as other distinct Aboriginal groups, and therefore is not representative of Inuit alone. In Canada, anemia among First Nations has been less common and ID more prevalent in comparison to Inuit (Valberg et al., 1979). Given the ongoing nutrition transition away from traditional Inuit foods, which are known to be rich sources of micronutrients (Kuhnlein et al., 1991; Kuhnlein et al., 2006), it is prudent to re-examine the public health significance of ID and anemia for Canadian Inuit.

Authours have previously dismissed the significance of anemia for Inuit by suggesting low hemoglobin concentrations may be a physiological adaptation to the cold climate and/or the traditional diet (Milman et al., 2001b; Valberg et al., 1979), not necessarily representing a public health concern. However, this hypothesis has not been investigated. The Inuit population has experienced a rapid dietary and lifestyle transition over recent decades, with implications for both overnutrition and undernutrition. The 2007-2008 International Polar Year Inuit Health Survey (Egeland et al., 2011a) is a cross-sectional survey designed to estimate population prevalence of selected health indicators within accepted errors of margin and to analyze determinants of health by multivariate modeling. Sampling was stratified by community, with randomization of households within communities. The household response rate was 68 % overall and included 2550 eligible adults ≥18 years of age (60.9 % female). Biological, dietary, anthropometric, and socio-demographic data collected from the 2007-2008 Inuit

Health Survey was provided to allow us to design this study and test the hypotheses of this dissertation.

While there are advantages to modeling determinants of health for an entire population and investigating subsequent interactions, it was decided to separately analyze the iron status of men and women from the Inuit Health Survey, given that iron requirements (Institute of Medicine of the National Academies, 2006) and iron stores (Zacharski et al., 2000) vary significantly by sex and age. Also, from preliminary analyses distinct relationships among iron status and other variables for both sexes emerged which dictated data presentation. Finally, there was a lack of power to investigate three-way interactions among sex and other variables in multivariate regression models, necessitating the use of separate models for each sex. Given that sex is a major determinant of serum ferritin, a regression model for the entire population would have had to include 3-way terms such as sex * age * food insecurity or sex * LC-PUFA * food insecurity.

CHAPTER 3: MANUSCRIPT 2

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Traditional food is correlated with iron stores in Canadian Inuit men

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3.1 ABSTRACT

Accelerated loss of traditional lifestyles may place Inuit at risk of iron depletion, given that anemia has been observed among Arctic men. The objectives were to determine the prevalence of anemia, storage iron depletion and iron overload; and to identify correlates of iron status in Canadian Inuit men. In a cross-sectional survey of 994 men in the International Polar Year Inuit Health Survey, 2007-08, hemoglobin, serum ferritin (SF), soluble transferrin receptor (on a subset), CRP, RBC fatty acid composition, and *Helicobacter pylori* serology were measured in venous blood drawn from fasting men. Anthropometric, dietary, sociodemographic, and health data were collected. Dietary and non-dietary correlates of iron status were assessed with multiple linear and logistic models. For men with CRP≤10 mg/L (*n*=804) 6.5 % had depleted, 19.8 % had low, and 10.3 % had elevated iron stores. Anemia was moderately prevalent (16.1 %) but iron deficiency anemia less common (2.4 %). There was a low probability of dietary iron inadequacy (2.4 % < Estimated Average Requirement) and excess iron intakes (10.7 % >Tolerable Upper Intake Level). Food-insecure men and those without a household hunter had a higher risk of low or depleted iron stores. Adiposity, TF intake, long-chain RBC PUFA status, and inflammation were positively associated with SF and food insecurity, smoking, and *H. pylori* seropositivity were negatively associated SF. Despite a moderate prevalence of anemia, iron stores are largely adequate in this population, although lower than expected based on iron intake. The regulation of iron metabolism in this population and the high prevalence of anemia in older men warrants further investigation.

3.2 INTRODUCTION

Iron deficiency (ID) and anemia are rare among men residing outside of areas where malaria, *Shistosoma*, and hookworm are endemic. Over the past several decades higher than expected rates of ID and anemia have been observed among Arctic Indigenous men, despite a traditional diet rich in animal-source foods and lack of parasitic explanation. Prevalence rates, however, vary considerably by Arctic region. Surveys of Alaska Natives (n=2024) from 1988-89 found that up to 10 % of men were ID and 15 % anemic (Petersen et al., 1996). The 1976 Nutrition Canada Survey (n=346) observed a substantial proportion of Inuit men (24-38 %) with low transferrin saturation (<20 %) and a high rate of anemia (28-48 %) (Canada Bureau of Nutritional Sciences, 1975). However, a sub-analyses on this sample reported ≤1 % of men with depleted iron stores (serum ferritin (SF) <15 µg/L) (Valberg et al., 1979). The 1993-94 Greenland Inuit survey (n=224) reported low rates of both ID and anemia (<4 %) but up to 32 % of men in a traditional community had elevated iron stores (Milman et al., 2001a, Milman et al., 2001b). In the Alaskan and Canadian surveys anemia rates tended to increase with age while ID decreased with age.

Changing lifestyle and dietary patterns among Arctic Indigenous Peoples include less reliance upon traditional food (TF), a well documented characteristic of the nutrition transition. For Inuit, this transition exists most strongly in younger Inuit while high TF use continues predominantly among elders (Kuhnlein et al., 2004; Kuhnlein and Receveur, 2007).Traditional Inuit foods are largely animal-based and excellent sources of bioavailable iron (Kuhnlein and Receveur, 2007). Whether reduced intakes of TF may be responsible for low iron status indices or if other endemic factors may contribute to ID and anemia among Inuit men have not been thoroughly investigated.

Adequacy of dietary iron intake among Arctic Indigenous Peoples has been demonstrated (Kuhnlein et al., 2008; Petersen et al., 1996) but is in contradiction with previous biochemical surveys (Petersen et al., 1996; Canada

Bureau of Nutritional Sciences, 1975). However, among Inuit preschoolers, high dietary iron intake was recently observed with low rates of iron deficiency anemia (IDA) and ID (Johnson-Down and Egeland, 2010; Pacey et al., 2010). In Greenland, 98 % of Inuit men had serum ferritin (SF) ≥20 μg/L but there was regional variation (Milman et al., 2001b). The most traditional Greenlandic community had a higher median SF (118 μg/L) than the most westernized community (92 μg/L) and SF was reasonably correlated (R_S=0.26, *P*=0.01) with TF intake (Milman et al., 2001b). Therefore, accelerated loss of traditional lifestyles may place Inuit at heightened risk of iron depletion.

Other factors endemic to the Arctic that might relate to iron status include a high prevalence of household food insecurity as observed in homes with children (69.6 %) in the Canadian territory of Nunavut (Egeland et al., 2010b); where there are barriers in TF acquisition and access to healthy market foods (Chan et al., 2006). Additionally, *Helicobacter pylori* infection is associated with decreased SF and/or increased risk of IDA in some populations (Cardenas et al., 2006; Muhsen et al., 2009; Ciacci et al., 2004; Milman et al., 1998) likely through multiple mechanisms including bacterial iron utilization, decreased gastric acidity leading to impaired iron absorption, and increased iron losses through gastric microbleeding (Cardenas et al., 2006). *H. pylori* infection may, therefore, impair iron status of Inuit which appears modest despite potentially abundant heme iron intake (Jamieson and Kuhnlein, 2008). Finally, inflammation impacts nutritional status indicators including SF. Inuit are at increased risk for tuberculosis, pneumonia, hepatitis, and food-borne pathogens such as *Trichinella* compared to national risk statistics (Bjerregaard et al., 2004). Inadequate housing sanitation, water supply, and overcrowded conditions are thought to contribute to the high rates of infectious disease for Inuit (Canadian Tuberculosis Committee, 2007). Previous biochemical surveys have not accounted for the impact of infections and inflammation and may therefore have underestimated the prevalence of ID in the past.

The objectives of this study were to determine the prevalence of anemia, depletion of iron stores, and iron overload; and to identify correlates of iron status in a representative sample of Canadian Inuit men from Inuvialuit Settlement Region (ISR), Nunavut Territory, and Nunatsiavut of Northern Labrador.

3.3 METHODS

3.3.1. Survey Design. A cross-sectional Inuit health survey of adults was conducted in the summer and fall of 2007-08 in 36 Arctic communities across ISR, Nunavut, and Nunatsiavut, as previously described (Egeland et al., 2011a). The sample was stratified by community with households randomly selected from each community. Of the 2796 Inuit households approached, 1901 households (68 %) agreed to participate, with a total of 2595 adult participants, of whom 38.5 % were male. Fasting venous blood samples, anthropometric measurements, a 24-hour dietary recall, a semi-quantitative food frequency questionnaire, and 5 general questionnaires were administered either on-land or upon the Canadian Coast Guard Ship Amundsen. Venous blood samples were obtained from 880 (88.2 %) male participants of whom 852 men had both a SF and a high-sensitivity C-reactive protein (hs-CRP) test result. Iron status for Inuit women is reported separately because of the magnitude of the dataset and different inter-relationships among variables which become obscured when combined into one dataset. Ethical approval for the study was obtained from the McGill University Faculty of Medicine Institutional Review Board and appropriate territorial research licenses were acquired. The survey was guided through a participatory process (Egeland et al., 2011a).

3.3.2. Clinical assessment. Weight and body composition were measured using bioelectrical impedance analysis (Tanita TBF-300GS, Arlington Heights, IL, USA).

Height was measured with a portable stadiometer (Road Rod 214, Seca, Maryland) to the nearest millimetre. Normal weight, overweight and obesity were defined by the WHO classification system (World Health Organization, 2000). At-risk percent body fat was defined as >20 %, >22 %, and >25 % for ages 18-39, 40-59, and ≥60 y, respectively. Fasting venous blood was collected in SST^{TM} vacutainer tubes with clot activator and polymer gel for serum separation (Becton Dickinson, Franklin Lakes, USA) or EDTA-coated vacutainers for plasma and whole blood hematology (Becton Dickinson, Franklin Lakes, USA). Hemoglobin measures were obtained from venous blood drops or blood drops from a finger prick using the azidemethemoglobin method with HemoCue^{TM} 201+ portable photometer (HemoCue, Inc., Lake Forest, California), due to survey logistics. Hemoglobin values were adjusted for cigarette smoking according to WHO protocols (Nestel, 2002). Prevalence estimates of anemia were based on venous samples only and classified according to the WHO 130 g/L cut-off for adult men (Nestel, 2002). IDA was defined as anemia + serum ferritin <15 µg/L or ferritin 15-50 µg/L + hs-CRP>10 mg/L.

3.3.3 Laboratory analyses. Iron status was determined in serum by ferritin, hs-CRP and, for a subsample (n=387), soluble transferrin receptor (sTfR). SF was measured at McGill University using an automated chemiluminescence assay (Liaison Ferritin; Diasorin, Italy) with a detection limit of 0.5 µg/L. Hs-CRP was measured using an auto-analyzer (Beckman Coulter, Brea, CA, USA) with a 0.2 mg/L limit of detection at the Montreal General Hospital. Due to high cost of the assay, sTfR concentration was analyzed as a secondary marker of iron status on a subsample by ELISA (R&D Systems, Minneapolis, USA) at McGill University on a Synergy HT microplate reader (BioTek; Winooski, CT, USA). The subsample included participants from all 3 regions but only samples collected in 2008, as sTfR is stable for 1 year at -80^{o} C, according to the manufacturer. The limit of

detection for the assay was 0.225 mg/L. Iron status was evaluated by amount of storage iron as well as functional tissue deficiency. STfR>2.75 mg/L was considered iron deficient erythropoeisis, as suggested by the manufacturer. Depleted iron stores was defined by SF<15 µg/L or SF=15-50 µg/L in the presence of acute inflammation (hs-CRP ≥10 mg/L). Low iron stores was defined as SF ≤32 µg/L and >14.9 µg/L in the absence of acute inflammation as SF>32 µg/L reflects the presence of stainable iron in the marrow and is considered an iron-replete state (Milman et al., 2001b). SF>200 µg/L in the absence of acute inflammation was used to define elevated iron stores in order to compare to other studies of Inuit populations (Milman et al., 2001b), with age-appropriate cut-offs for iron overload utilized by NHANES (Gibson, 2005) also determined. Immunoenzymatic methods (ELISA) were used to detect IgG antibodies against *H. pylori* in serum (Calbiotech; Spring Valley,CA, USA) at the Montreal General Hospital. Fatty acid composition was analyzed on red blood cell (RBC) membranes (Lipid Analytical Laboratories Inc., University of Guelph Research Park, Guelph, ON), with lipid extraction based on the methodology of Folch *et al* (Folch et al., 1957).The fatty acid methyl esters were prepared by standard techniques (Morrison and Smith, 1964) and analyzed on a Varian 3400 gas-liquid chromatograph (Palo Alto, CA, USA) with a 60-metre DB-23 capillary column (0.32 diameter). Total EPA and DHA were expressed as % of total fatty acids and will hereafter be referred to as LC-PUFA (long chain-PUFA).

3.3.4. Dietary assessment. Dietary intake data were collected by trained interviewers using a single 24 hour recall with a four stage, multi-pass approach (Gibson, 2005). Portion sizes were estimated with a graduated, three-dimensional food model kit (Santé Québec). Recall data were entered into CANDAT software (Godin London Inc,. London, Ontario, Canada) and nutrient analyses obtained from the 2007b Canadian Nutrient File (CNF), a database

containing foods not available on the CNF, recipes, information from food labels and data from an indigenous food nutrient file developed by the Centre for Indigenous Peoples' Nutrition and Environment (CINE), and an additional database of imputed values for nutrients missing in the CNF housed at the School of Dietetics and Human Nutrition (McGill University). There were no missing values for the foods and nutrients included in the analysis. Recall data were available for 805 male participants after 25 recalls were excluded due to incompleteness. Dietary iron adequacy was assessed by the Estimated Average Requirement (EAR) cut-point method using SIDE software (Iowa State University) in which within-subject variation estimates for iron intake were obtained from previous CINE dietary surveys with Canadian Inuit populations (Kuhnlein et al., 2004). Nutrient coefficients of variation were available for Inuit <40 and ≥40 y of age. Median nutrient intakes were analyzed for men from the 24-hour recall and compared to the EAR of select nutrients. Eighteen year olds (n=18) were excluded from this intake analysis due to different DRI requirements for their age group. Nutritional supplement and medication use were documented in a questionnaire administered by a nurse, for which participants were asked to bring containers of supplements and medicines taken to their appointment for accurate recording. Supplement content was not included in nutrient analysis of the 24 hour recalls as most supplement users could not recall the brand or amount of supplement taken. TF frequency data were collected and available for 805 male participants. Frequency of TF use was recorded for in-season and off-season consumption of each item over the past 12 months. Seasons were determined according to regional wildlife harvest calendars and intakes adjusted to frequency per month (assuming 30.4 days per month).

3.3.5. Questionnaires. Questionnaires for sociodemographic, health and household characteristics were adapted from Greenlandic and Nunavik (Canada)

Inuit Health Surveys (Anctil, 2008) and the Aboriginal Peoples Survey (Statistics Canada, 2001) and through consultations with regional steering committees and key informants. The household questionnaire included a version of the 18-item USDA Household Food Security Survey Module (Nord et al., 2006); details of the questionnaire and classification of household food security are described elsewhere (Egeland et al., 2010b). A household food security score was dichotomized into secure or insecure for analyses. Marital status was dichotomized into single (including widowed, divorced, or separated) and married (including common-law marriage). Alcohol use was dichotomized by whether alcohol was consumed in the past year or not. Current smoking behaviour was assessed as yes or no and further quantified by cigarettes per day. Acetylsalicyclic acid (ASA) use was dichotomized into either daily users or infrequent and non-users to investigate chronic ASA use as a correlate of iron status.

3.3.6. Calculations and statistics. SF and sTfR concentrations were $\log_{10}$ transformed to improve normality of the respective distributions. Weighted prevalence estimates of iron status are given with 95% CIs. Sampling weights reflected the proportion of participating men using Statistics Canada's Census data of age-appropriate Inuit men by community. Age categories were based on the DRI recommendations for iron intake (Institute of Medicine Food and Nutrition Board, 2001), although age groups 51-70 and ≥70 y were combined due to small sample size among the elderly. Independent determinants of $\log_{10}$SF were examined in a multivariable linear mixed regression model, with household and community as random effects. Variables known *a priori* or suspected to be related to iron status were selected and evaluated. The model was based on 803 men with SF available and hs-CRP≤10 mg/L. Sample size limited interaction testing to only 2x2 interactions between main effects, but none were found.

Independent determinants of elevated iron stores vs good iron stores and low or depleted iron stores vs good iron stores were assessed with multivariable logistic models. Two by two interactions were investigated but none observed. Model assumptions (normality of residuals and homoscedasticity) were confirmed graphically with standard procedures. All analyses were performed in STATA (version 11; StataCorp LP, College Station, TX). *P* values were all two-sided and significance was set at $P \leq 0.05$.

3.4 RESULTS

3.4.1. Study population. Mean age of the study population was 42 ± 15 y (range: 18-89 y), with 33 % overweight and 27 % obese by WHO criteria. Underweight was almost nonexistent (0.4% or 3 men). Sixty-eight percent currently smoked, with a median of 12 cigarettes/d (IQR: 7-17). Daily use of ASA (80-325 mg) and prescription or non-prescription medication use was reported by 4.9 % and 36.3 %, respectively. High blood pressure, diabetes mellitus, and high cholesterol were self-reported in 23 %, 6 %, and 10 % of men respectively, and a low hs-CRP concentration (hs-CRP<3 mg/L) was observed in 75 %. The majority of the sample (69 %) were classified as married. Eighty % of men reported having an active hunter in the home and 58 % consumed TF on the day prior to the survey, representing 9.8 % of energy intake. Sixteen men (1.8 %) reported taking an iron-containing supplement and were included in analyses because of lack of difference in SF between supplement users and non-users.

3.4.2. Iron Status and Anemia. Prevalence of anemia was mild in men ≤50 y, but high among men >50 y (Table 3.1). SF increased with age (Figure 3.1), whereas sTfR was low in all age groups (Table 3.2). Of note, median SF was 50.4 µg/L when excluding hs-CRP>3 mg/L, 55.9 µg/L when excluding hs-CRP>10 mg/L and 58.3 µg/L with no exclusion criteria. Most men had adequate iron stores

accompanied by low rates of IDA and low rates of iron deficient erythropoiesis. Overall 63.5 % were classified as iron replete (SF=32-200 µg/L). Low or depleted iron stores were common (40.5 %) among young men (18-30 y) but few were categorized with iron depletion. Prevalence of elevated iron stores was moderately high in men over 50 y (19.2 %). Severe iron overload (SF>700 µg/L) was only present in 2 men. When using age-appropriate cut-offs for iron overload (Gibson, 2005) prevalence rates dropped to 1.6 % (18-30 y), 4.2 % (31-50 y) and 5.2 % (≥51 y).

Median dietary iron intake (adjusted for within-subject variation and unadjusted values) exceeded both the EAR and RDA (Table 3.2). Median intakes of vitamin C, calcium, and vitamin A were <EAR. Tea and coffee were consumed by 44.0 % and 4.1 %, respectively, of men on the day prior to the survey. Median intakes from carbohydrate, protein and fat accounted for 45.5 %, 19.9 % and 31.6 % of energy, respectively. Across all age-groups there was a low probability of iron inadequacy, with 2.4 % of adjusted intakes below the age-specific EAR. A small proportion of the sample (10.7 %) had an adjusted iron intake above the DRI upper limit (45 mg/d). Daily frequency of consumption of TF species was highest for game, followed by marine mammals, fish, and birds. Liver consumption was infrequent (Table 3.2). In order of contribution, the top five sources of iron for men 19-50 y were: TF meats (28 %), market food (MF) meats (12 %), mixed foods with meat (12 %), breads (9 %), and cereals (4 %). For men over 50 y the greatest iron sources were: TF meats (48 %), MF meats (11 %), breads (10 %), bannock (6 %), and cereals (5 %).

3.4.3. Correlates of iron status. Correlations of SF concentrations with dietary variables and biomarkers are shown in Table 3.2. SF was positively correlated with % energy as TF, TF meat, dietary iron from TF, hs-CRP, LC-PUFA, Hb, and frequency of TF intake (including game and birds). SF was negatively associated with sTfR concentrations.

In a multivariate-adjusted model (Table 3.3) the positive predictors of SF were % body fat, elevated hs-CRP (3-10 mg/L), TF on the previous day, and LC-PUFA. LC-PUFA status also correlated with frequency of consuming marine mammals (rho=0.31; *P*<0.0001) and fish (rho=0.16) (*P*<0.0001). Smoking, food insecurity, single marital status and *H. pylori* infection were negatively associated with SF. Daily ASA use and tea on the previous day were marginally significant in the model (P≤0.1). The model explained 28 % of the variation (adjusted R^2=0.28) in $\log_{10}$SF. In a multivariate-adjusted logistic model (Pseudo-R^2=0.12) food insecurity and having no hunter in the home were associated with increased risk of low or depleted iron stores in comparison with iron replete stores (SF 32-200 µg/L) (Table 3.4). At-risk body fat % and elevated hs-CRP were associated with reduced risks of low or depleted iron stores. TF on the previous day and tea on the previous day were marginally (P≤0.1) significant in the model. Risk of elevated iron stores was evaluated in another multivaritate-adjusted logistic model (Pseudo-R^2=0.23). Risk increased with increasing age and at-risk % body fat compared to the risk associated with replete iron stores (Table 3.5). Food insecurity, smoking, and *H. pylori* infection were associated with reduced risks of elevated iron stores. Single marital status, daily ASA use, tea on the previous day, and lack of a hunter at home were marginally (P≤0.1) associated with a lower risk.

3.5 Discussion

This is the first representative survey to report prevalence estimates of anemia and depleted iron stores for Inuit men in Canada. Anemia was moderately prevalent with the highest rates observed among those over 50 y. Although low iron stores were common, iron depletion and IDA were infrequent and corresponded with a low prevalence of dietary iron inadequacy. If good iron status is defined by the minimal amount of iron stores to meet requirements

(SF=15 μg/L), the DRI criteria, then over 93 % of Inuit men would have adequate iron stores. Indeed, there is no reported benefit of having excess iron stores (Institute of Medicine Food and Nutrition Board, 2001) and SF>50 μg/L is correlated with increased risk of vascular disease (Zacharski et al., 2000). Nonetheless, iron status is lower than would be expected from a diet with abundant heme iron. Limited iron storage may be an evolutionary adaption to prevent infectious disease (Denic and Agarwal, 2007) or an adaptation to a traditional diet rich in heme iron.

Anemia estimates are consistent with previous studies in Canada (Valberg et al., 1979) and Alaska (Petersen et al., 1996) but higher than the Greenland rate (3.5 %) reported in 1993-94 (Milman et al., 2001a). Of note, anemia estimates were consistently higher among older men and mainly occurred in those with adequate iron stores, suggesting a non-dietary iron etiology. Prevalence of iron depletion reported here is similar to previous Alaska Native surveys (4-10 %) (Petersen et al., 1996) but higher then reported by a previous Canadian survey (<1 %) (Valberg et al., 1979) and the Greenlandic Inuit survey (0-4 %) (Milman et al., 2001b). Similarly, median SF (Table 3.2) was considerably lower in the current study then observed in the Greenlandic communities (92-118 μg/L) but higher than Alaska Native estimates (38 μg/L). It is unknown at this time if these differences reflect differences in TF use, failure to assess the impact of inflammation on iron status, other risk factors, or temporal changes associated with the nutrition transition. However, adjusting for hs-CRP, had little impact on iron status assessment for this sample.

SF increased progressively with age in Inuit men (Figure 3.1), similar to observations among Inuit and Alaska Native populations (Petersen et al., 1996; Milman et al., 1992). This is in contrast to Caucasian men who show a consistent plateau in SF by 30-35 y that is maintained until ≥70 y (Zacharski et al., 2000; Milman et al., 1992; Pan and Jackson, 2008). This pattern of accumulation of iron stores for Inuit has been attributed to the lifelong intake of an iron-rich diet

rather than a potential genetic difference in iron absorption (Milman et al., 1992). Milman *et al*. were able to show a stronger correlation between age and SF in a traditional community with higher TF use compared to a more westernized community in Greenland (Milman et al., 2001b). Similarly, age and SF were correlated in Nunavut but not in the less traditional regions of ISR and Nunatsiavut (data not shown). We have also shown SF to increase with TF use on the previous day as well as with % LC-PUFA in RBC (a marker of TF use) independent of other confounders (Table 3.3), suggesting that continued TF use may explain iron status differences.

To our knowledge no studies have investigated determinants of iron status based on SF in Inuit men. SF, an acute-phase protein, is known to increase with adiposity either because of iron overload or chronic inflammation (Zafon et al., 2010; McClung and Karl, 2009) and may explain the positive association observed between excess adiposity and risk of iron depletion. Whether this relationship is a reflection of how the biomarkers change in parallel or of true iron stores requires further studies. Nonetheless, the majority of participants (75%) had hs-CRP values <3 mg/L suggesting that the high prevalence of obesity (27 %) did not confound the assessment of iron status.

TF was the most important dietary source of iron for men of all ages. TF use and access (a hunter at home) were also related to SF and adequate iron stores, demonstrating the importance of these nutrient-dense foods. In particular, the proportion of RBC as LC-PUFA remained positively associated with SF after multivariate adjustment. Consumption of marine mammals and fish are predictors of RBC LC-PUFA in our study population, as also noted for Inuit in Northern Quebec (Lucas et al., 2010). Therefore consumption of animal-source foods including traditional meats and fish may explain the relationship between LC-PUFA and SF. Marine mammals, second only to cereals and pasta as an iron source for Alaska Natives (Johnson et al., 2009), are rich sources of iron, ranging from 17 mg/100 g raw food (walrus meat) to 57 mg/100 g dried food (beluga

meat) (CINE, 2005). In comparison, raw ground beef contains 1.8 mg iron/100 g (Health Canada, 2010). Therefore, LC-PUFA sea dietary sources may contribute to iron status for Inuit.

Food insecurity was an independent determinant of extremes of iron stores (insecure with low or depleted and secure with elevated iron stores) and SF concentrations. Food insecurity can result in consumption of less desirable foods, sporadic meal skipping, or not having food for a whole day (Eicher-Miller et al., 2009). Thus, it is not unexpected to find food insecurity associated with lower SF, as has been reported in U.S. children (Park et al., 2009) and adolescents (Eicher-Miller et al., 2009). In Inuit preschoolers from 16 communities in Nunavut, no differences were observed in ID or IDA prevalence rates between children from child food secure and insecure households, although, 99 % of the children had consumed TF in the past month (Johnson-Down and Egeland, 2010; Pacey et al., 2010). However, adults appear to moderate their intakes during periods of household food insecurity in order to protect children (Egeland et al., 2010b), which may explain this association in adults.

H. pylori seropositivity was associated with a lower SF than seronegativity after multivariate adjustment and a reduced risk of elevated iron stores. No relationship was found with risk of low or depleted iron stores. It should be noted that seropositivity was common in the population (73 %), limiting the number of the non-infected population for statistical comparisons. In addition, there are limitations with the seropositive test which reflects any past antigen exposure rather than current infection, leading to misclassification of infection status and attenuation of the association. Nonetheless, our findings support other observations that *H. pylori* infection may contribute to lower SF but this does not necessarily lead to iron depletion and deficiency (DuBois and Kearney, 2005). Twenty percent of men with depleted iron stores were seronegative, suggesting alternate etiologies.

Iron overload through diet is virtually impossible due to down-regulation of non-heme iron absorption as body stores increase (NIN, 2002). Toxicity is mainly related to inappropriate iron supplementation or the genetic defect causing hereditary hemochromatosis, which is most common in males of Northern European descent (NIN, 2002). The prevalence of hemochromatosis among Inuit is unknown; and Canadian Inuit may include mixed Inuit and Northern European ancestry. Our logistic model demonstrated a 10-fold increased risk of elevated iron stores for a 10 year increase in age. Adiposity was the only other positive predictor of elevated iron stores. TF use did not impact risk of high iron in our study and use of supplements was rare. Overall prevalence of iron overload was low in our sample, and far more common in surveys of Greenlandic Inuit (Milman et al., 2001b, Milman et al., 1992), possibly due to a greater prevalence of Inuit with mixed Danish and Inuit heritage in Greenland than in our study population, higher TF use and dietary iron intake at the time of the Greenland survey, or a lack of exclusion of participants with inflammation.

There are several important limitations to the study. Blood was collected for one season only (late Summer/early Fall) and therefore the biochemical assessment of iron status may not accurately reflect dietary intake and TF availability throughout the entire year as examined using the food frequency questionnaire. Inflammation status was defined by hs-CRP alone and may have underestimated the prevalence of chronic inflammation in the population. Measurement of sTfR on the entire sample, or a second and third indicator of iron status would have enhanced the ability to classify participants as ID and IDA. Alcohol intake as assessed (dichotomized into users and non-users) was not related to iron status in exploratory analyses.

In conclusion, iron depletion does not explain the moderate anemia rate for Inuit men in Canada. Iron stores are largely adequate in this population, although lower than expected based on dietary iron intake. Further research is

warranted to understand the regulation of iron metabolism in this population. These data are based on the first representative survey of Inuit men in Inuvialuit Settlement Region, Nunavut and Nunatsiavut. Although previous Inuit surveys have reported IDA and iron overload in Inuit men, these surveys did not account for inflammation or measure functional ID. In terms of modifiable determinants: TF use, TF access and food insecurity were important correlates of adequate iron stores and TF was the greatest contributor of dietary iron. As traditional lifestyles in the North are increasingly pressured, continued access to micronutrient-rich TF is needed to ensure the health and well-being of Inuit and Northern peoples during this period of transition.

Figure 3.1. Serum ferritin concentrations in Inuit men by age-group and hs-CRP status: International Polar Year Inuit Health Survey, 2007-08. Values are geometric means (95% CI). For unadjusted ferritin, *n* are shown on the X axis. Data points without a common letter differ between age-groups. No statistical differences were observed between age-groups for different hs-CRP status.

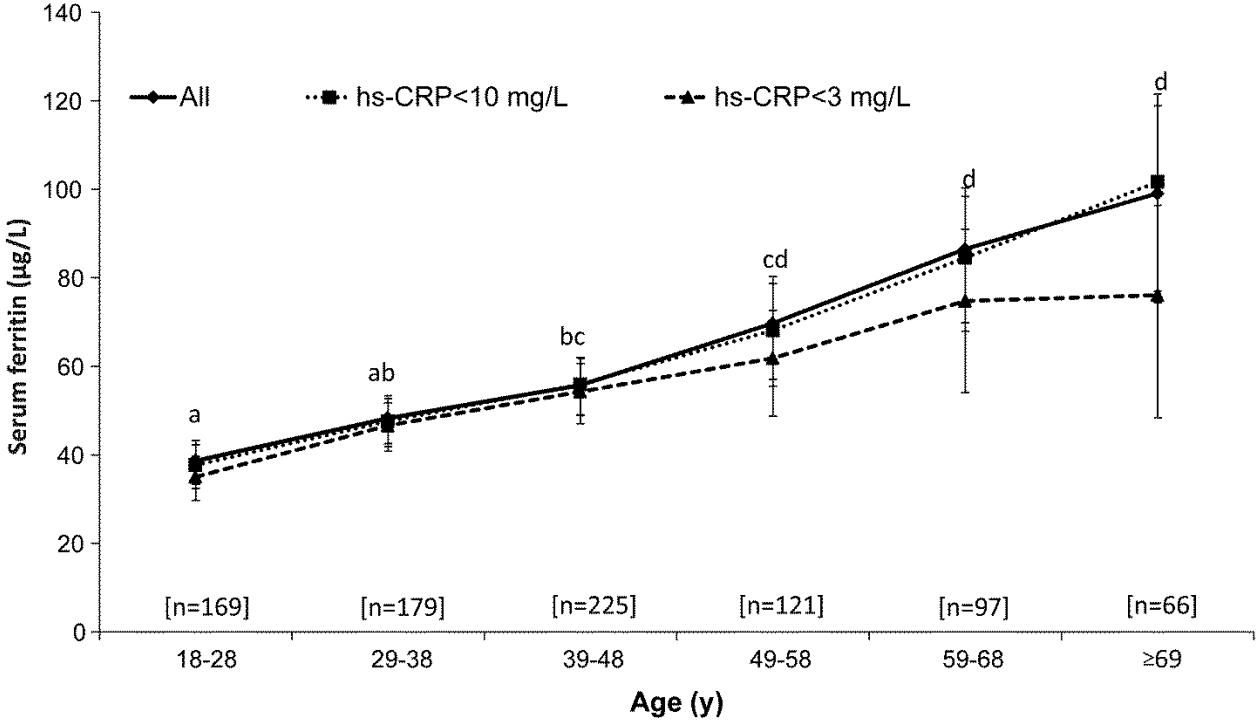

Table 3.1. Weighted prevalence of iron status and anemia among Inuit men: International Polar Year Inuit Health Survey, 2007-08.[1]

Age, y	Anemia[2]		Depleted iron stores		Low iron stores		Elevated iron stores		Iron deficiency anemia		Iron deficient erythropoiesis[3]	
	n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
18-30	99	6.4 (3.0-13.0)	196	9.9 (5.6-16.8)	196	30.7 (23.5-38.9)	196	1.6 (0.5-5.1)	96	2.7 (0.9-8.0)	81	2.7 (0.7-9.8)
31-50	220	10.6 (6.7-16.2)	386	6.3 (4.1-9.6)	386	22.7 (17.9-28.3)	386	8.4 (5.1-13.5)	213	3.2 (1.4-7.5)	180	1.1 (0.3-4.9)
≥51	135	30.3 (22.1-40.0)	232	4.2 (2.0-8.4)	232	7.7 (5.0-11.7)	232	19.2 (12.9-27.6)	119	1.1 (0.2-4.6)	126	0.6 (0.1-4.1)
Total	454	16.1 (12.5-20.6)	814	6.5 (4.8-8.7)	814	19.8 (16.7-23.2)	814	10.3 (7.5-13.9)	428	2.4 (1.3-4.5)	387	1.2 (0.5-3.0)

[1] Where anemia = hemoglobin<130 g/L; depleted iron stores = serum ferritin <15 µg/L or ferritin 15-50 µg/L + CRP>10 mg/L; low iron stores = ferritin 15-32 µg/L + CRP≤10 mg/L; elevated iron stores = ferritin>200 µg/L + CRP≤10 mg/L; iron deficiency anemia = depleted iron stores + anemic; iron deficient erythropoiesis = serum soluble transferrin receptor (sTfR)>2.75 mg/L.

[2] Analyses on a subset with venous blood sampling for hemoglobin determination.

[3] Analyses on a subset with soluble transferrin receptor measurements.

Table 3.2. Correlations between dietary variables and serum ferritin for Inuit men: International Polar Year Inuit Health Survey, 2007-08.

Dietary intake on the previous day[1], *unit*	*n*		Spearman's rho
Energy, *MJ*	739	9.06 (6.04-12.8)	<0.01
Traditional food, *% energy*	739	9.8 (0.0-34)	0.15***
Traditional food meat, *g/d*	739	147 (0-421)	0.14***
All meat, *g/d*	739	353 (164-653)	0.07*
Unadjusted dietary iron, *mg/d*	739	16.6 (10.1-28.2)	0.05
Adjusted dietary iron, *mg/d*	739	20.2 (13.7-31.6)	0.07
Dietary iron from traditional food, *mg/d*	739	1.9 (0.0-16)	0.15***
Dietary iron from non-traditional meats, *mg/d*	739	0.5 (0.0-2.6)	0.01
Heme iron, *mg/d*	739	6.3 (1.7-17.1)	0.14***
Vitamin C, *mg/d*	739	61.8 (13.5-172.3)	0.06
Calcium, *mg/d*	739	441 (249-734)	-0.03
Vitamin A, *µg RAE/d*	739	417 (145-870)	0.05
Tea, *mL/d*	739	0 (0-500)	0.02
Dietary intake, *n/d*			
All traditional food	738	0.74 (0.32-1.63)	0.09*

Marine mammals	738	0.07 (0.01-0.29)	0.03
Game	738	0.29 (0.08-0.70)	0.11**
Fish	738	0.11 (0.02-0.33)	-0.012
Birds	738	0.02 (0.01-0.10)	0.15***
Liver, all species	738	0.01 (0-0.10)	0.04
Iron status and other indicators			
RBC LC-PUFA (% of total FA)	800	3.7 (2.1-5.7)	0.17***
Serum hs-C-reactive protein (mg/L)	803	1.2 (0.5-2.9)	0.28***
Hemoglobin (g/L)	776	142 (131-152)	0.14***
Serum soluble transferrin receptor (mg/L)	366	1.27 (1.11-1.46)	-0.14**
Serum ferritin (μg/L)	803	55.9 (30.4-95.6)	-

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

[1]Values are median (IQR), n=803.

Table 3.3. Multiple linear regression coefficients for serum ferritin[1] based upon non-dietary and dietary determinants as independent variables among Inuit men: International Polar Year Inuit Health Survey, 2007-08.[2-4]

	Univariate Coefficient	SE	Multivariate Coefficient	SE
Constant	1.755***	0.022	1.534***	0.077
Age, *y*	0.008***	<0.001	0.002	0.001
Body fat, *%*	0.018***	0.001	0.010***	0.002
Smoker (1=yes, 0=no)	-0.231***	0.028	-0.102**	0.031
Food insecure (1=yes, 0=no)	-0.174***	0.029	-0.104**	0.030
H. pylori (1=yes, 0=no)	-0.105**	0.031	-0.064*	0.030
Single marital status (1=yes, 0=no)	-0.159***	0.029	-0.064*	0.030
Daily ASA use (1=yes, 0=no)	0.060	0.067	-0.135	0.063
hs-CRP (1=3-10 mg/L, 0=<3 mg/L)	0.213***	0.032	0.138***	0.034
TF on previous day (1=yes, 0=no)	0.143***	0.028	0.083**	0.028
RBC *n*3-PUFA, *% of total fatty acids*	0.033***	0.005	0.014*	0.006
Tea on previous day (1=yes, 0=no)	0.048	0.030	-0.050	0.029

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

[1] Analyses conducted on $\log_{10}$(serum ferritin, µg/L), n=614.

[2] The within-dwelling and within-community variance components were 0.03 and <0.01, respectively.

[3] Participants with hs-CRP>10 mg/L were excluded to remove the effect of inflammation on SF.

[4] All variables presented were evaluated together in one model.

Table 3.4. Logistic regression coefficients, Odds Ratios (OR and 95% CI) with low or depleted iron stores as the dependent variable among Inuit men: International Polar Year Inuit Health Survey, 2007-08.[1-3]

	Univariate OR	Multivariate OR	95 % CI	
Age, *y*	0.964***	0.982	0.963	1.002
% Body fat >cut-off (1=yes, 0=no)	0.189***	0.251***	0.120	0.527
Food insecure (1=yes, 0=no)	3.014**	2.331*	1.171	4.640
hs-CRP[2] (1=3-10 mg/L, 0=<3 mg/L)	-1.529**	0.330**	0.138	0.790
Hunter in home (1=no, 0=yes)	1.695	2.060*	1.032	4.111
TF on previous day (1=yes, 0=no)	0.581*	0.634	0.366	1.100
Tea on previous day (1=yes, 0=no)	1.154	1.540	0.903	2.628

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

[1]The within-dwelling variance component was 2.0.

[2] Men with hs-CRP>10 mg/L and men with elevated iron stores were excluded from the model, *n*=622.

[3] For highly prevalent outcomes, the odds ratios will tend to exaggerate the true relative risk.

Table 3.5. Logistic regression coefficients, Odds Ratios (OR and 95% CI) with elevated iron stores as the dependent variable among Inuit men: International Polar Year Inuit Health Survey, 2007-08.[1]

	Univariate OR	Multivariate OR	95 % CI	
Age, *y*	1.044***	1.049***	1.021	1.079
% Body fat >cut-off (1=yes, 0=no)	6.608***	3.260*	1.187	8.949
Smoker (1=yes, 0=no)	0.199***	0.303**	0.145	0.635
Food insecure (1=yes, 0=no)	0.358***	0.441*	0.219	0.888
H pylori (1=yes, 0=no)	0.562*	0.419*	0.204	0.858
Single marital status (1=yes, 0=no)	0.481*	0.466	0.169	1.288
Daily ASA use (1=yes, 0=no)	1.158	0.218	0.042	1.130
Hunter in home (1=no, 0=yes)	0.404	0.321	0.084	1.236
Tea on previous day (1=yes, 0=no)	1.235	0.558	0.262	1.187

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

[1] Men with hs-CRP>10 mg/L and men with low or depleted iron stores were excluded from the model, *n*=435.

Bridge 2

Although both iron overload (Milman et al., 2001a) and iron depletion (Petersen et al., 1996) have been observed among Inuit men in the past, it is clear from our second manuscript that iron status of Canadian Inuit men is lower than might be expected based on dietary iron intake. This is especially true, considering that median heme iron intake alone was higher than the EAR for iron. This finding concurs with Alaska surveys (Petersen et al., 1996) and earlier Canadian surveys (Valberg et al., 1979; Canada Bureau of Nutritional Sciences, 1975), but not data from Greenland (Milman et al., 2001a). However, it is difficult to make regional comparisons given the differences in survey methodologies and the considerable number of years between surveys. Lack of control for the effect of inflammation on serum ferritin in previous surveys may have biased results, as the health transition experienced by Inuit may also include changes in communicable and noncommunicable disease patterns.

Notably, iron stores did not follow the typical pattern of accumulation with age, as observed in other populations (Zacharski et al., 2000). Men, after adolescence, and women, after menopause, experience a 4-6 fold rise in serum ferritin, although a physiological requirement for this increase is not known (Zacharski et al., 2000). Serum ferritin peaks at age 30-35 in Caucasian men (Milman et al., 1992) whereas Inuit men continually accumulate iron stores with age. Additionally, it is rare to see a population of men with ferritin<50 µg/L (Cook et al., 1976; Zacharski et al., 2000; Custer et al., 1995), as was the case with almost half of the men in the present study. In fact, lower iron stores, as observed in athletes and frequent blood donors, may be more compatible with health, given the adverse consequences of excess iron storage. Our data, in agreement with studies from Greenland, support a relationship between iron stores, traditional food use, and older age. Although the Inuit diet contains

minimal iron inhibitors, it is possible that selective pressure towards reduced risk of infectious disease has led to a down-regulation of iron absorption from a traditional diet highly bioavailable in iron. Such adaptations could hinder iron absorption from non-heme food sources, increasingly relied upon from market foods. Indeed, the association of food insecurity and lack of a hunter at home with low iron stores suggests that access to nutrient-dense food may be involved. The regulation of iron metabolism in this population is of interest, given the increasing recognition of iron overload as risk factor for chronic disease. Furthermore, iron depletion does not explain the prevalence of anemia observed in men and anemia is a public health issue for older Inuit men, requiring further investigation beyond iron status. Given the modest, although adequate, iron stores of Inuit men, a thorough examination of iron status among women was warranted, with particular attention to the role of iron depletion in anemia for Inuit women according to age-groups.

CHAPTER 4: MANUSCRIPT 3

n3-fatty acid status as a marker of traditional food intake is associated with lower risk of iron depletion among food insecure Canadian Inuit women

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4.1 ABSTRACT

High rates of iron deficiency and anemia are common among Inuit and Arctic women but representative data on iron status and relevant determinants are lacking. The objectives were to determine the prevalence of anemia and depletion of iron stores, then to identify correlates of iron status in non-pregnant Canadian Inuit women. In a cross-sectional survey of 1550 women in the International Polar Year Inuit Health Survey, 2007-08, hemoglobin, serum ferritin, soluble transferrin receptor (on a subset), C-reactive protein (CRP), red blood cell (RBC) fatty acid composition, and *Helicobacter pylori* serology were analyzed on fasting venous blood. Sociodemographic, anthropometric, dietary, and health data were collected. Correlates of iron status were assessed with multivariate linear and logistic models. Anemia was observed in 21.7 % and iron deficiency anemia (IDA) in 11.1 % of women, with prevalence of dietary iron inadequacy at 15.6 % among premenopausal women. For women with CRP≤10 mg/L (n=1260) 29.4 % had depleted and 32.2 % had low iron stores. Among food insecure women, higher long-chain PUFA status (a marker of traditional food [TF] intake) was associated with reduced risk of iron depletion. TF meats were the greatest contributors of dietary iron for Inuit women. Despite largely adequate dietary iron (and heme iron) intakes, prevalence rates for low and depleted iron stores are high. Interventions to improve the iron status of premenopausal Inuit women are needed and should address modifiable risk factors including food insecurity, better quality market food and access to TF.

4.2 INTRODUCTION

Iron deficiency (ID) is the most common nutritional deficiency in the world and one of the leading 15 contributors to the global burden of disease (Lynch, 2007). Adequacy of dietary iron intake has been observed among Arctic Indigenous Peoples (Kuhnlein et al., 2008; Petersen et al., 1996; Canada Bureau of Nutritional Sciences, 1975) but is inconsistent with surveys demonstrating moderate to high rates of both ID (22-39 %) and anemia (10-42 %) for Canadian Inuit (Plante et al., 2007; Canada Bureau of Nutritional Sciences, 1975; Valberg et al., 1979a) and Alaska Native (Petersen et al., 1996) women. Among Canadian Inuit preschoolers, high dietary iron intake was observed and corresponded with low rates of ID and iron deficiency anemia (IDA) (Johnson-Down and Egeland, 2010; Pacey et al., 2011). However, high rates of ID and IDA have been reported among Inuit infants (Christofides et al., 2005; Hodgins et al., 1998; Willows and Gray-Donald, 2000), suggesting that iron status may be discordant across age groups. Adequate iron stores are especially important for women of child-bearing age to ensure healthy pregnancies and fetal development. Post-menopause, ID may indicate chronic blood loss or a more serious underlying condition.

Arctic Indigenous Peoples have experienced significant changes in lifestyle and dietary patterns over recent decades. Animal-source foods, central to the traditional Inuit diet, are rich sources of bioavailable iron (Kuhnlein and Receveur, 2007). Less reliance upon traditional food (TF), a well documented feature of the nutrition transition, is most evident in younger Inuit (Kuhnlein et al., 2004; Kuhnlein and Receveur, 2007), and therefore of particular relevance to women of child bearing years. Whether reduced TF consumption contributes to low iron status indices among Inuit women or if other endemic factors may account for iron depletion has not recently been thoroughly investigated. However, data from a 1993-94 Greenlandic Inuit survey (n=224) suggested that iron depletion was over twice as common and iron overload one-tenth as

prevalent among women in a westernized community compared to a more traditional community (Milman et al., 2001b). Therefore declining use of TF may place Inuit women at increased risk of ID and anemia.

The extent to which IDA can currently explain anemia prevalence for adult Inuit women, given the ongoing nutrition transition, requires clarification. In the Northwest Territories, Canada (1972) and Greenland (1993-94), IDA rates were <4 % among women despite anemia rates of 10-18 % (Valberg et al., 1979b, Milman et al., 2001a). More recently, in Nunavik (2004), 21 % of women were classified with IDA, with 43 % anemic overall (Plante et al., 2011). Other factors endemic to the Arctic and with particular relevance to iron status may include *Helicobacter pylori* infection, food insecurity, infections and inflammation, as described in the companion paper (Jamieson et al., 2012). Although Inuit women consume less TF (g/d) than Inuit men in general (Kuhnlein and Receveur, 2007), access to iron-rich TF is likely to be an important source of dietary iron for women with higher iron requirements.

The objectives of this study were to determine the prevalence of anemia and depletion of iron stores, then to identify correlates of iron status in Canadian Inuit women of Inuvialuit Settlement Region (ISR), Nunavut Territory, and Nunatsiavut of Northern Labrador.

4.3 METHODS

4.3.1. Survey Design. A cross-sectional Inuit health survey of adults residing in randomly selected households was carried out between August to September, 2007; and August to October, 2008 in 36 Arctic communities across ISR, Nunavut, and Nunatsiavut (Egeland et al., 2011a). All non-pregnant household members 18 years and older from selected houses were eligible for participation. Of the 2796 Inuit households approached, 1901 households (68 %) agreed to participate, with a total of 2595 adult participants, of whom 61.5 % were

women. Forty-five women were excluded from current analyses due to possible pregnancy or lactation. A 24-hour recall, semi-quantitative food frequency questionnaire (FFQ), 5 general questionnaires, a fasting venous blood sample, and anthropometric measurements were obtained either on-land or utilizing the Canadian Coast Guard Ship Amundsen. Venous blood samples were obtained from 1403 (87.9 %) female participants of whom 1309 women had both a SF and high-sensitivity C-reactive protein (hs-CRP) test result and met the inclusion criteria. Appropriate regional research licenses were obtained and ethical approval for the survey was granted from the McGill University Faculty of Medicine Institutional Review Board as previously described. Prevalence and correlates of iron status in Canadian Inuit men were reported separately in a companion paper (Jamieson et al., 2012) because of different inter-relationships among variables and the magnitude of the dataset. TF use and access to a hunter were important correlates of iron stores for Inuit men, although iron status did not explain the moderate prevalence of anemia observed.

4.3.2. Clinical assessment. Weight and body composition were measured using bioelectrical impedance analysis (Tanita TBF-300GS, Arlington Heights, IL, USA) and height was measured with a portable stadiometer (Road Rod 214, Seca, Maryland). The WHO classification system was used to characterize normal weight, overweight and obesity (WHO (World Health Organization), 2000). Percent body fat was classified according to the manufacturer's age-appropriate healthy body fat ranges. For women, an at-risk body fat percentage was defined as >33 %, >34 %, and >36 % for ages 18-39, 40-59, and 60-99, respectively. Participants were requested to fast at least 8 hours overnight, prior to morning venous blood collection. Samples obtained in the morning were processed into serum, plasma or red blood cell (RBC) fractions and frozen at -80^{o} within 4-8 hours. . Hemoglobin was measured on venous (or capillary) blood drops with a

HemoCue™ 201+ portable photometer (HemoCue, Inc., Lake Forest, California), and adjusted for cigarette smoking (Nestel, 2002). Prevalence estimates of anemia were derived from venous samples only and categorized according to the WHO 120 g/L cut-off for non-pregnant women (Nestel, 2002). Iron deficiency anemia (IDA) was defined as anemia + serum ferritin <15 µg/L or ferritin 15-50 µg/L + hs-CRP>10 mg/L.

4.3.3. Laboratory analyses. Serum samples were analyzed for ferritin (automated chemiluminescence assay; Liaison Ferritin;Diasorin, Italy), hs-CRP (auto-analyzer; Beckman Coulter, Brea, CA, USA), and soluble transferrin receptor (sTfR) concentration on a subsample (n=652, ELISA assay; R&D Systems, Minneapolis, USA).Iron status was evaluated by amount of storage iron as well as functional tissue deficiency. Serum sTfR>2.75 mg/L was defined as iron deficient erythropoiesis (IDE), as suggested by the manufacturer. Depleted iron stores was defined by SF<15 µg/L or SF=15-50 µg/L in the presence of acute inflammation (hs-CRP ≥10 mg/L). Low iron stores was defined as SF ≤32 µg/L and >14.9 µg/L in the absence of acute inflammation as SF>32 µg/L is considered an iron-replete state, indicating the presence of stainable iron in the marrow (Milman et al., 2001b). SF>200 µg/L in the absence of acute inflammation was used to define elevated iron stores in order to compare across studies of Inuit populations (Milman et al., 2001b) with sex and age-appropriate cut-offs for iron overload (Gibson, 2005a) also determined. Immunoenzymatic methods (ELISA) were used to detect IgG antibodies against *H. pylori* in serum (Calbiotech; Spring Valley,CA, USA). RBC membranes were analyzed for fatty acid composition (Lipid Analytical Laboratories Inc., University of Guelph Research Park, Guelph, ON), with lipid extraction based on the methodology of Folch *et al* (Folch et al., 1957). The fatty acids methyl esters were prepared by standard techniques (Morrison and Smith, 1964) and analyzed on a Varian 3400 gas-liquid chromatograph (Palo Alto, CA,

USA) with a 60-metre DB-23 capillary column (0.32 diameter). Total EPA and DHA were expressed as % of total fatty acids and hereafter referred to as LC-PUFA (long chain-PUFA). Complete assay details have been previously reported (Jamieson et al., 2012).

4.3.4. Dietary assessment. Dietary assessment was conducted by trained interviewers using a single 24 hour recall with a four stage, multi-pass approach (Gibson, 2005b) and a 42-item semi-quantitative food frequency questionnaire. Portion sizes were estimated with a graduated, three-dimensional food model kit (Santé Québec). Recall data were entered into CANDAT software (Godin London Inc,. London, Ontario, Canada) and nutrient analyses obtained from the 2007b Canadian Nutrient File (CNF) and additional databases as described previously. There were no missing nutrient values in the analysis. Recall data were available for 1248 female participants after 25 recalls were excluded due to incompleteness. Dietary iron adequacy was assessed by the Estimated Average Requirement (EAR) probability method (pre-menopausal women) or the cut-point method (post-menopausal women) using the SIDE method (Carriquiry, 1998) and SIDE software (Iowa State University) in which within-subject variation estimates for iron intake were obtained from dietary surveys with Canadian Inuit populations (Kuhnlein et al., 2004). Median nutrient intakes were analyzed for women using 24-hour recall data and compared to the EAR of select nutrients. Twenty-nine 18 year olds (with different DRI requirements for their age group) were excluded from this intake analysis. Nutritional supplement and medication use was recorded by a nurse. Supplement content was not included in nutrient analysis of the 24 hour recalls as the majority of supplement users could not recall the brand or amount of supplement taken. TF frequency data were available for 1229 female participants. Frequency of TF use over the past 12 months was documented for in-season and off-season consumption of each

item. Seasons were established according to locally developed regional wildlife harvest calendars and intakes adjusted to frequency per month (assuming 30.4 days per month).

4.3.5. Questionnaires. Questionnaires for sociodemographic, health and household characteristics were adapted from Greenlandic and Nunavik (Canada) Inuit Health Surveys (Anctil, 2008) and the Aboriginal Peoples Survey (Statistics Canada, 2001), as previously described. The household questionnaire included a version of the 18-item USDA Household Food Security Survey Module (Nord et al., 2006), with details of the questionnaire and classification of household food security described elsewhere (Egeland et al., 2010b). A household food security score was dichotomized into secure or insecure and applied to each adult household member for analyses. Current smoking status was assessed as yes or no and quantified by cigarettes per day. Alcohol use was categorized by assessing whether alcohol was consumed in the past year or not to distinguish non-consumers from consumers. Menopausal status was self-reported and defined by absence of a regular menstrual period or age ≥50 y if menstrual status not reported. Women were asked about contraceptive use to account for known effects of hormonal contraceptives (estrogen and/or progesterone) and intrauterine device use on iron status (Beard and Han, 2009).

4.3.6. Calculations and statistics. Weighted prevalence estimates (reflecting the proportion of participating women using Statistics Canada census data of age-appropriate Inuit women by community) of iron status and anemia are provided with 95% CIs. Age categories for prevalence estimates were based on the DRI recommendations for iron intake (Institute of Medicine Food and Nutrition Board, 2001), although age-groups 51-70 and ≥70 were combined due to small

sample size among the elderly. Independent determinants of $\log_{10}$SF were examined in a multivariable linear regression model. *A priori* selection of variables known or suspected to be related to iron status were evaluated. Age was not a determinant of SF once menopausal status entered the model and was therefore removed. The model was based on 1040 women with SF available and a hs-CRP≤10 mg/L to reduce invalid SF results due to inflammation. Low-grade inflammation (hs-CRP 3-10 mg/L) was included as a control variable. Household, community and region were included as random effects in a mixed model. Sample size limited interaction testing to only 2x2 interactions between main effects. Independent determinants of depleted iron stores vs good iron stores were assessed with a multivariable logistic model, with community included as a random effect to improve model fit. Variable selection was as described above and model specification was verified with STATA linktest. Standard model diagnostics were performed. Analyses were conducted in STATA (version 11; StataCorp LP, College Station, TX) using two-sided *P* values, and with significance set at $P \leq 0.05$.

4.4 RESULTS

4.4.1. Study population. Mean age ± SD of the study population was 42 ± 15 y (range: 18-90 y), with 25 % of women overweight and 43 % obese using WHO criteria. Underweight was infrequent (1 %). Seventy-one percent of women currently smoked, with a median 10 cigarettes/day (IQR: 6-12). Prescription or non-prescription medication use was reported by 43 % of women, oral or hormonal contraceptives were used by 11 % of premenopausal women and intrauterine device by 4 %. In all, 70 % of women were classified as premenopausal. High blood pressure, diabetes mellitus, and high cholesterol were self-reported in 28 %, 7 %, and 12 % of women respectively, and a low hs-CRP concentration (<3 mg/L) was observed in 71 % of female participants. Sixty-

two women reported taking an iron-containing supplement and were included in analyses because of lack of difference in SF between supplement users and non-users. TF consumption was reported by 57.3 % of women on the day prior to the survey, representing 6.8 % of energy intake. The majority of the sample (61 %) was classified as married and 66% reported living with an active hunter in the home.

4.4.2. Iron status and anemia. Prevalence of anemia was moderate and similar across all age-groups (Table 4.1). IDA accounted for approximately half of the anemia cases, with the majority of IDA cases observed in premenopausal women. There was little evidence of iron deficient erythropoiesis (Table 4.1) but depleted iron stores and low iron stores were common (62.0 % combined; Table 4.1). Only 35.9 % of women were classified as iron replete (SF 32-200 µg/L). Elevated iron stores were rare and severe iron overload (SF>700 µg/L) was absent. When using age-appropriate cut-offs for iron overload (Gibson, 2005a) rates were 0.3 % (18-30 y), 0.9 % (31-50 y) and 4.6 % (≥51 y). SF concentration was low and stable in women until after age 48 (Figure 4.1) when it was more than 2-fold higher, whereas sTfR was low in all age-groups (Table 4.2). Adjusting for CRP<10 mg/L and CRP<3 did not appreciably alter SF concentrations or prevalence of iron depletion.

Prevalence of dietary iron inadequacy ranged from 15.6 % (pre-menopausal women) to 0.6 % (post-menopausal). A small portion of the sample (4.1 %) had adjusted iron intakes above the upper limit DRI (45 mg/d). Median iron intakes exceeded both the EAR and RDA for post-menopausal women but were between the EAR and RDA for pre-menopausal women (Table 4.2). Median intakes of vitamin C (64.8 mg/d) were >EAR and <RDA for pre-menopausal women, but <EAR for post-menopausal women (43.1 mg/d). Median calcium and vitamin A intakes were <EAR for all women. Median intakes from carbohydrate, protein and fat (as % energy) were 45.2 %, 17.3 % and 29.4 %, respectively. Tea

and coffee were consumed by 43.8 % and 3.7 %, respectively, of women on the previous day. Daily frequency of consumption of TF species was highest for game, followed by fish, marine mammals, birds and liver (all species) (Table 4.2). In order of contribution, the top five sources of iron for women 19-50 y were: TF meats (23 %), baked products (13 %), market food (MF) meats (13 %), breads (10 %), and mixed foods with meat (9 %). For women over 50 y the greatest iron sources were: TF meats (44 %), baked products (12 %), bannock (8 %), breads (7 %), and market food (MF) meats (6 %).

4.4.3. Correlates of iron status. Associations between SF concentrations, dietary variables and biomarkers are presented in Table 4.2. SF was positively associated with % energy as TF, TF meat, adjusted iron intake, iron from TF, heme iron, tea, frequency of marine mammal, land mammal, avian species, and liver intakes, RBC % LC-PUFA, hs-CRP, and Hb concentrations. SF was negatively associated with sTfR, energy and calcium intake.

In a multivariate-adjusted model (Table 4.3) postmenopausal status, % body fat, elevated hs-CRP (3-10 mg/L), LC-PUFA status and oral contraceptive use were positively associated with SF. Food insecurity was negatively associated with SF and *H pylori* seropositivity was marginally (*P*=0.1) related to SF (negative). The model explained 39 % of the variation (adjusted R^2=0.39) in $\log_{10}$SF. In a multivaritate-adjusted logistic model (Pseudo R^2=0.19) postmenopausal status, at-risk % body fat, elevated hs-CRP, and oral contraceptive use were independently associated with reduced risks of depleted iron stores, respectively (Table 4.4). RBC LC-PUFA status was associated with a lower risk of depleted iron stores but only in food insecure women.

4.5 DISCUSSION

Rates of anemia among post-menopausal women (25 %) and ID among pre-menopausal women (38 %) in this sample population are high. Half of the anemia cases observed were associated with depleted iron stores, although this is likely an overestimate of IDA as functional ID was rare, even among premenopausal women (3.0 %). Prevalence of both anemia and IDA are higher than reported in Greenland (9 % and <1 %, respectively) in 1993-94 (Milman et al., 2001a) but lower than observed rates in Nunavik (43% and 21 %, respectively) in 2004 (Plante et al., 2011). These differences may represent regional differences and/or differences in analytical methods among surveys.

Low rates of functional ID corresponded with a moderate prevalence of dietary iron inadequacy. Similar national rates of iron inadequacy were reported among 19-30 y (16 %) and 31-50 y (19 %) Canadian women (Health Canada, 2009) as Inuit women of the same age (16 % and 17 %, respectively) although median iron intakes have not been reported from the Canadian Community Health Survey. The Third National Health and Nutrition Examination Survey, 1988-1994, found that 30-51 year old women consumed 13 ± 4 mg iron/d, 15 % appeared to have inadequate iron intake, and 16 % had SF<15 µg/L (Institute of Medicine Food and Nutrition Board, 2001). Depleted iron stores among all women was comparable to the 2004 Inuit Health Survey for Inuit women of Nunavik (34.8 %) (Plante et al., 2007) and Alaska Native women (28-39 %) surveyed in 1988-89 (Petersen et al., 1996). Rates of iron depletion were higher and median SF was considerably lower in our survey than the 1993-94 Greenlandic Inuit health survey (40-69 µg/L; n=115 women) (Milman et al., 2001b). SF may have been overestimated in the Greenland survey as inflammation was not assessed. In the present study, SF concentrations and prevalence of iron depletion did not vary greatly when adjusted for hs-CRP (Figure 4.1). Infection rates could, of course, vary by region and over time. In comparison to U.S. prevalence rates (NHANES 2003-06) of iron deficiency (15.7 ±

0.8 %) for women of child-bearing age, based on a multi-parameter ferritin model (Cogswell et al., 2009), our rates of iron depletion are 2.5-fold higher for the same age group. However, these surveys are not directly comparable as the criteria for iron depletion used in the present study may be a more sensitive indicator (WHO et al., 2001). Nonetheless, rates of ID are much higher in this population than North American samples consuming similar iron intakes, with proportionately less heme iron (7-10 %)(Institute of Medicine Food and Nutrition Board, 2001).

In comparison to young Inuit men (18-50 y), premenopausal women in this sample had higher rates of anemia, IDA, IDE, and much higher rates of iron depletion (5-fold). Post-menopausal women and men > 50 y had similar rates of anemia, IDA, IDE, and iron depletion. It is not unusual to observe little or no correlation between SF concentrations and dietary iron intake in population surveys (Asakura et al., 2009; Samuelson et al., 1996); however, this is often reported when non-heme iron is the primary source of dietary iron. Iron from meat sources has been shown to be more closely correlated with SF than iron from plant sources (Spodaryk, 1999). In this sample of women, SF was modestly but significantly correlated with % energy as TF, TF meat servings, heme iron, and adjusted iron intakes, as well as frequency of liver, sea, land, and avian TF species consumption. There was no correlation between SF and iron from non-traditional meats suggesting that TF species are greater contributors of dietary iron. In a related paper, we note that the % of energy from protein is significantly lower on days when TF is not consumed (Egeland et al., 2011b) and this together with the lower iron content of market food may help explain the lack of an association between non-traditional meat consumption and SF in our data. Notably, for women, SF was more strongly correlated with dietary variables as well as RBC LC-PUFA, sTfR, and Hb than observed among Inuit men. Although, men consume more energy and dietary iron than women, heme iron sources

likely have more impact on iron status for women because of higher iron requirements.

Despite numerous reports of high rates of ID among Arctic Indigenous women no studies, to the best of our knowledge, have investigated determinants of iron status or SF for Inuit women. Post-menopausal status was the strongest predictor of SF and reduced risk of iron depletion in multivariate models, reflecting the rise in SF after menopause associated with lower iron requirements. SF, an acute-phase protein, is also elevated during acute and chronic inflammatory conditions, including the state of obesity (Zafon et al., 2010; McClung and Karl, 2009). Therefore, higher SF and lower rates of iron depletion are not surprising among the at-risk % body fat participants. Further investigation is needed to determine whether this relationship reflects analogous changes in these biomarkers or true differences in iron stores during obesity. Nonetheless, most participants (71%) had hs-CRP values <3 mg/L suggesting that the high prevalence of obesity (43 %) did not confound iron status assessment. In addition, low-grade inflammation (hs-CRP= 3-10 mg/L) and oral contraceptives, which decrease menstrual losses, were both associated with higher SF and less risk of iron depletion after multivariate adjustment. *H pylori* seropositivity was marginally associated with lower SF but not risk of iron depletion, supporting previous evidence that *H pylori* infection does not fully explain ID among Arctic populations (Parkinson et al., 2000).

As observed among Inuit men (Jamieson et al., 2012), food insecurity and RBC LC-PUFA proportions were opposing correlates of iron status. For women, however, an important interaction was observed. RBC % LC-PUFA was associated with a lower risk of iron depletion among food insecure women only. This interaction demonstrates the importance of TF intake (specifically marine mammals and fish) for nutritional health of food insecure women, especially when quality market foods not consumed in abundance. Similarly, in a related paper, food insecurity and nutrition transition combined to affect a wide range

of nutrient intakes and biomarkers of nutritional status among Inuit (Egeland et al., 2011b). Marine species (including Arctic char, beluga, narwhal, seal, salmon, and whitefish) were second only to caribou as the top species of TF consumed in this sample (Sheikh et al., 2011).

Given the adequacy of dietary iron intake and the large contribution of heme iron and TF meats to dietary iron intake, the high rates of iron depletion are curious. TF of Inuit contains many animal-source foods rich in iron, with marine mammal meats, in particular, being especially high sources of dietary iron (Jamieson et al., 2012). Iron in marine mammals and fish (including non-heme iron) is well absorbed because of active substances, termed 'meat factors', present in animal tissues. The traditional Inuit diet also contains few iron absorption inhibitors such as calcium and phytate (fibre) (Kuhnlein et al., 2007; Kuhnlein et al., 2008). In this population, coffee consumption was infrequent and calcium intakes were well below the EAR. Tea consumption was common, especially among older individuals, and was positively associated with SF, but the association disappeared after adjusting for age and other confounders. Vitamin C intake was limited, which may reduce non-heme iron bioavailability for Inuit women. Low vitamin A intakes may also contribute to iron sequestration and limited iron availability for erythropoiesis. Iron absorption rates and regulation of iron absorption for Inuit should be further investigated in light of the recent and rapid nutrition transition for Arctic peoples. There may be evolutionary adaptations to a diet rich in heme iron, which limits iron absorption and accumulation for Inuit.

High iron stores and iron overload were 5-fold lower among Inuit women than men in the survey. Notably, prevalence was much lower than previously reported for Greenlandic traditional communities (Milman et al., 2001b) and Greenlandic hunter families (Milman et al., 1992), where moderate and severe iron overload was present in 7.9 % and 2.6 % of women, respectively. However,

sample size was modest and inflammation was not assessed in these studies which may have contributed to elevated SF.

Limitations of this study include a lack of repeat dietary recalls on the sample. In order to estimate usual intake of iron, within person variability from a previous Inuit dietary survey were relied upon. The coefficient of variation for iron intake may have changed over time. Also, there were no available data on TNF-alpha or related markers of chronic inflammation. Inflammation status assessed by hs-CRP likely underestimated the impact of inflammation in the population. Nutritional status biomarkers may not reflect status throughout the entire year, as only one season was assessed (late summer/early fall) and TF intakes vary with season.

In conclusion, ID is a concern for young Canadian Inuit women whereas anemia without ID is prevalent post-menopause. The relatively low prevalence of dietary iron inadequacy supports an alternate etiology for iron depletion, which was much more common. Inuit women may have increased dietary iron needs in light of endemic *H. pylori* infection, inadequate micronutrient intakes including vitamins A and C, and the nutrition transition toward increased reliance on non-heme iron sources combined with less reliable access to iron-rich TF. LC-PUFA status, a marker of TF intake, was associated with a lower risk of ID among food insecure women, confirming the importance of TF for health when quality market food is limited in diets. As reliance on market foods with low nutrient density persists in the Arctic, micronutrient nutrition can be expected to decline. Interventions to improve the iron status of premenopausal Inuit women are needed and should address modifiable risk factors including food security, use of better quality market food and access and consumption of TF.

Figure 4.1. Serum ferritin concentrations in Inuit women by age-group and hs-CRP status: International Polar Year Inuit Health Survey, 2007-08. Values are geometric means (95% CI). For unadjusted ferritin, *n* are shown on the X axis. Data points without a common letter differ between age-groups. No statistical differences were observed between age-groups for different hs-CRP status.

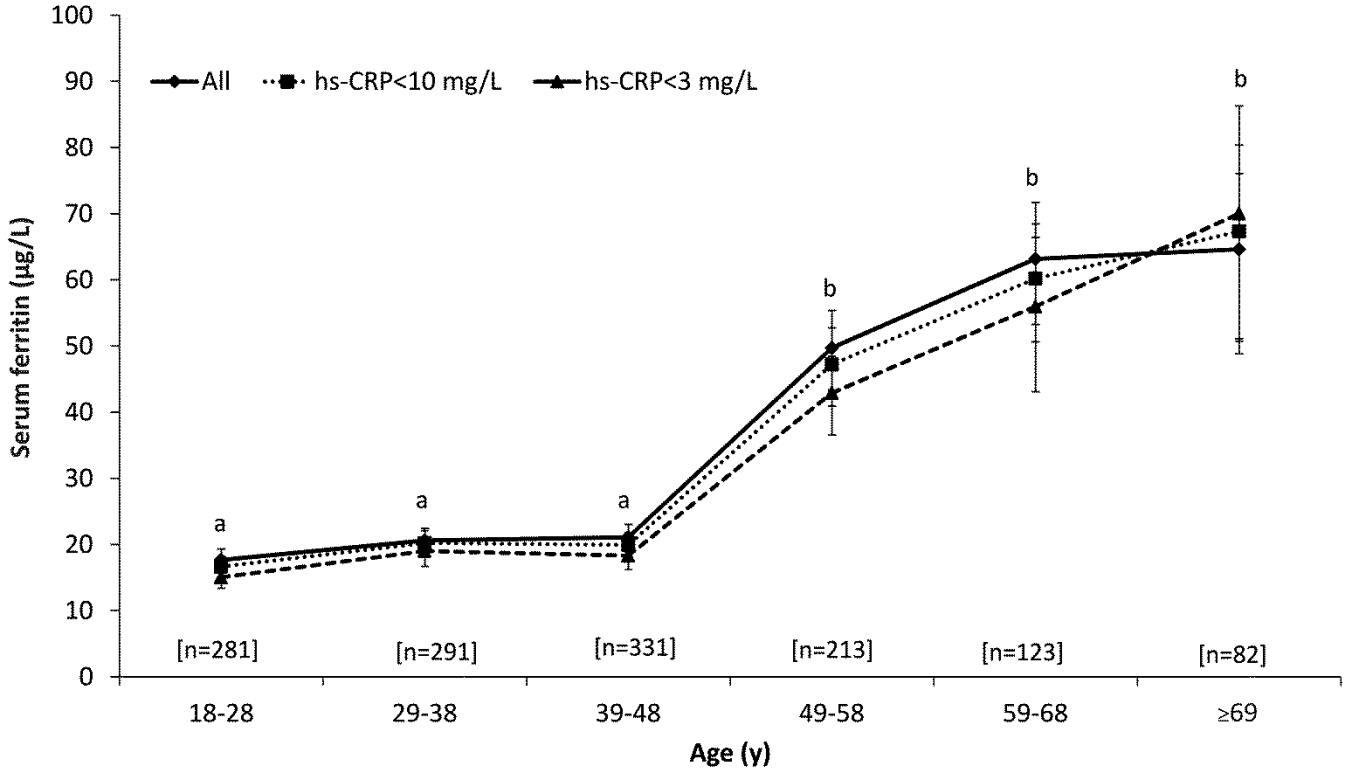

Table 4.1. Weighted prevalence of iron status and anemia among Inuit women: International Polar Year Inuit Health Survey, 2007-08.[1]

Age, y	Anemia[2]		Depleted iron stores		Low iron stores		Elevated iron stores		Iron deficiency anemia		Iron deficient erythropoiesis[3]	
	n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
18-30	169	17.9 (12.7-24.6)	333	40.3 (34.3-46.6)	333	38.9 (32.7-45.4)	333	-	166	11.7 (7.4-18.2)	153	1.0 (0.1-6.9)
31-50	326	21.3 (16.7-26.8)	583	37.0 (32.7-41.6)	583	36.7 (32.3-41.2)	583	0.9 (0.3-3.0)	316	15.4 (11.5-20.3)	294	6.2 (3.3-11.4)
≥51	202	24.9 (18.1-33.3)	344	9.2 (6.3-13.2)	344	20.0 (15.6-25.3)	344	5.9 (3.4-10.1)	182	3.9 (1.5-9.6)	186	0.7 (0.2-3.0)
Total	697	21.7 (18.3-25.5)	1260	29.4 (26.7-32.3)	1260	32.2 (29.3-35.2)	1260	2.2 (1.3-3.6)	664	11.1 (8.7-14.0)	633	3.3 (1.9-5.8)

[1] Where anemia = hemoglobin<120 g/L; depleted iron stores = serum ferritin <15 µg/L or ferritin 15-50 µg/L + CRP>10 mg/L; low iron stores = ferritin 15-32 µg/L + CRP≤10 mg/L; elevated iron stores = ferritin>200 µg/L + CRP≤10 mg/L; iron deficiency anemia = depleted iron stores + anemic; iron deficient erythropoiesis = serum soluble transferrin receptor (sTfR)>2.75 mg/L.

[2] Analyses on a subset with venous blood sampling for hemoglobin determination.

[3] Analyses on a subset with soluble serum transferrin receptor measurements.

Table 4.2. Correlations between dietary variables and serum ferritin for Inuit women: International Polar Year Inuit Health Survey, 2007-08.

Dietary intake on the previous day[1], unit	*n*		Spearman's rho
Energy, MJ	1123	7.56 (5.43-10.4)	-0.09**
Traditional food, % energy	1123	6.8 (0-24)	0.20***
Traditional food meat, g/d	1123	81.6 (0-277)	0.15***
All meat, g/d	1123	235 (128-420)	0.05
Unadjusted dietary iron, mg/d	1123	12.8 (8.2-20.3)	0.08
Adjusted dietary iron, mg/d	1123	15.5 (10.6-22.7)	0.09**
Dietary iron from traditional food, mg/d	1123	1.1 (0-9.0)	0.19***
Dietary iron from non-traditional meats, mg/d	1123	0.5 (0-2.0)	-0.06
Heme iron, mg/d	1123	4.0 (1.2-11)	0.17***
Vitamin C, mg/d	1123	59.0 (15.5-163)	-0.04
Calcium, mg/d	1123	388 (238-610)	-0.06*
Vitamin A, µg RAE/d	1123	353 (161-684)	0.04
Tea, mL/d	1123	0 (0-500)	0.16***

Dietary intake, n/d			
All traditional food	1105	0.80 (0.31-1.57)	0.13***
Marine mammals	1105	0.08 (0.01-0.31)	0.13***
Game	1105	0.27 (0.07-0.77)	0.07*
Fish	1105	0.11 (0.02-0.29)	0.07
Birds	1105	0.01 (0-0.04)	0.23***
Liver, all species	1105	0 (0-0.02)	0.14***
Iron status and other indicators			
RBC LC-PUFA, % of total FA	1197	3.67 (1.91-5.76)	0.38***
Serum hs-C-reactive protein, mg/L	1210	1.3 (0.4-3.4)	0.39***
Hemoglobin, g/L	1149	128 (119-136)	0.30***
Serum soluble transferrin receptor, mg/L	575	1.33 (1.12-1.63)	-0.41***
Serum ferritin, µg/L	1210	24.5 (14.0-51.0)	-

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

[1]Values are median (IQR), n=803.

Table 4.3. Multiple linear regression coefficients for serum ferritin[1] based upon non-dietary and dietary determinants as independent variables among Inuit women: International Polar Year Inuit Health Survey, 2007-08.[2-4]

	Univariate Coefficient	SE	Multivariate Coefficient[4]	SE
Constant	1.417***	0.044	0.877***	0.055
Post-menopause (1=yes, 0=no)	0.480***	0.022	0.353***	0.026
Body fat, *%*	0.014***	0.001	0.009***	0.001
hs-CRP (1=3-10 mg/L, 0=<3 mg/L)	0.215***	0.028	0.098***	0.026
Food insecure (1=yes, 0=no)	-0.121***	0.026	-0.052*	0.023
RBC *n*3-PUFA, *% of total fatty acids*	0.070***	0.004	0.036***	0.005
Oral contraceptive use (1=yes, 0=no)	-0.088***	0.040	0.097**	0.034
H pylori positive (1=yes, 0=no)	-0.044	0.027	-0.037	0.023

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

[1]Analyses conducted on $\log_{10}$(serum ferritin, µg/L), n=1040.

[2] The within-dwelling, within-community, and within-region variance components were 0.020, 0.001 and 0.003, respectively.

[3]Participants with hs-CRP>10 mg/L were excluded to remove the effect of inflammation on serum ferritin.

[4]All variables presented were evaluated together in one model.

Table 4.4. Logistic regression coefficients, Odds Ratios (OR and 95% CI) with low or depleted iron stores as the dependent variable among Inuit women: International Polar Year Inuit Health Survey, 2007-08.[1-3]

	Univariate OR	Multivariate OR[4]	95 % CI
Post-menopausal (1=yes, 0=no)	0.140***	0.120***	0.069 – 0.207
% Body fat >cut-off(1=yes, 0=no)	0.374***	0.414***	0.297 – 0.578
hs-CRP[2] (1=3-10 mg/L, 0=<3 mg/L)	0.334***	0.571*	0.369 – 0.884
RBC *n*3-PUFA, *% of total fatty acids*	0.763***	0.896	0.793 – 1.012
Oral contraceptive use (1=yes, 0=no)	0.907	0.426*	0.262 – 0.693
Food insecure (1=yes, 0=no)	1.421*	1.371	0.977 – 1.923
Food insecure * RBC *n*3-PUFA interaction	0.881*	0.842*	0.721 – 0.984

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

[1]The within-dwelling variance component was 0.1

[2] Women with hs-CRP>10 mg/L and women with elevated iron stores were excluded from the model, *n*=1062.

[3] For highly prevalent outcomes, the odds ratios will tend to exaggerate the true relative risk.

Bridge 3

Iron status among women was more closely tied to traditional food intake and a marker of TF intake (LC-PUFA), compared to men. ID and IDA were more than 4-fold higher among Inuit women than Inuit men. In contrast to men, patterns of accumulation of iron stores with age were similar to observations in the U.S. population with a pronounced rise in serum ferritin after menopause (Zacharski et al., 2000), although median serum ferritin was at least 2-fold lower for Inuit women in all age-groups. Dietary iron inadequacy (16 %) was lower than iron depletion rates (38 %), suggesting that iron absorption may be inefficient among premenopausal women, as well as was shown for men, in this population, despite ample heme iron in the diet. Bioavailability may be confounded by low intakes of vitamins A and C, nutrients important for iron absorption and transport, and pervasive *H. pylori* infection that may compete for iron in the gastrointestinal tract and modify the relationship between ascorbic acid and ferric iron reduction.

Due to remoteness and the exorbitant costs associated with accessing Arctic populations, surveys of Inuit have relied upon serum ferritin and hemoglobin for diagnosis of IDA. Depleted iron stores (ferritin<15 µg/L) represents the first stage of iron deficiency, followed by iron deficient erythropoiesis, and finally a decrease in hemoglobin resulting in IDA (Gibson, 2005). Therefore, this criterion for IDA (low ferritin + low hemoglobin) likely overestimates the extent of anemia due to ID, as we have shown with the sTfR subset analysis. Consequently, even among pre-menopausal women, ID is not likely the only contributing factor to anemia.

After 50 years of age, rates of anemia were similar between men and women, and largely unaccounted for by iron status. Anemia rates among Inuit adults are of a moderate public health concern (>20 %) and, thus, the etiology requires further clarification. To better understand the anemia observed, we

identified dietary and non-dietary correlates of hemoglobin concentrations and examined predictors of anemia unexplained by ID for both Inuit men and women.

CHAPTER 5: MANUSCRIPT 4

Mild anemia prevalent among Inuit men and postmenopausal women is associated with characteristics of a more traditional lifestyle

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5.1 Abstract

Objective: To identify correlates of hemoglobin (Hb) and anemia unexplained by iron deficiency (UA) in Canadian Inuit adults.

Design: A cross-sectional survey assessed diet, demographic information, anthropometrics, fasting Hb, ferritin, soluble transferrin receptor (on a subset), and high-sensitivity C-reactive protein (hs-CRP) in serum, red blood cell (RBC) fatty acid composition, blood lead, and antibodies to *Helicobacter pylori*.

Setting: Thirty-six Inuit communities in Inuvialuit Settlement Region (ISR), Nunavut Territory, and Nunatsiavut of Northern Labrador, Canada.

Subjects: Non-pregnant, Inuit adults (*n* 2550), ≥18 years from randomly selected households.

Results: Hb concentrations were lower and UA cases higher in men after 50 years of age. Rate of anemia was constant among women but changed from primarily iron deficiency anemia in pre-menopause to primarily UA post-menopause. Adiposity (% fat) was associated with a reduced risk while hs-CRP and low education levels were associated with increased risk of UA. For men, % RBC EPA and elevated blood lead were also associated with increased risk of UA.

Conclusions: Age patterns and regional variation of anemia suggest that ethnicity-related physiological differences cannot explain anemia prevalence for Inuit. High RBC EPA status, inflammation and infections, and lower education levels, characteristics associated with a more traditional lifestyle, may explain the prevalence of anemia in this population. The clinical significance of UA for older Inuit adults requires further investigation.

5.2 Introduction

Anemia, a disorder commonly observed in tropical nations with grain-based diets, has long been a concern in the Arctic, despite a traditional diet rich in animal-source foods. The 2007-08 Inuit Health Survey reported 24.9 % and 30.3 % anemia prevalence rates among women and men over 50 years of age, according to WHO cut-offs (WHO, 2004). In adults 18-50 y, 20.2 % of women and 9.3 % of men were anemic, with iron deficiency anemia (IDA) observed in 14.1 % and 3.1 %, respectively. IDA was observed in only 3.9 % and 1.1 % of women and men over 50 y (Jamieson et al., 2012a, Jamieson et al., 2012b), respectively, indicating that iron status did not explain the prevalence of anemia in this age-group.

Women are typically disproportionately affected by anemia until 75 years of age, when the prevalence becomes 5 % higher among men (Guralnik et al., 2005). Therefore, the comparable rates of anemia among the Inuit men and women and higher prevalence among those > 50 y, suggests an environmental or pathological etiology. Potential causes or contributing factors to anemia for Inuit include micronutrient deficiencies, poverty, chronic inflammation, *Helicobacter pylori* infection, lead exposure, chronic blood loss, and impaired iron absorption and/or utilization (Jamieson and Kuhnlein, 2008). Studies by the Centre for Disease Control in a similar geographical and cultural region, Alaska, found no parasitic or genetic cause of anemia in the Native population (Scott et al., 1955; Petersen et al., 1996; Hitchcock, 1950; Gessner, 2009). Alternately, it has been hypothesized that Inuit may have physiologically lower hemoglobin (Hb) concentrations (Milman et al., 2001b; Valberg et al., 1979), as has been observed among individuals of African descent. However, this hypothesis has yet to be

experimentally tested and does not concur with the lower prevalence rate of anemia in younger Inuit adults.

The available literature on anemia among Arctic Indigenous Peoples is limited by a lack of control for inflammation on biomarkers and may not reflect the current socio-environmental conditions of Inuit who are experiencing a rapid social, cultural, and dietary transition. Further losses of traditional lifestyle and dietary patterns (Kuhnlein et al., 2004) and pervasive food insecurity (Egeland et al., 2010b) may place Inuit at increased risk of anemia. A deeper understanding of the factors related to anemia in Inuit is needed to inform health policy, given the considerable morbidity and mortality associated with anemia among older adults (Roy, 2011). The objectives of this study were to identify correlates of Hb and anemia unexplained by iron deficiency (UA) in Inuit adults of Inuvialuit Settlement Region (ISR), Nunavut Territory, and Nunatsiavut of Northern Labrador, Canada.

5.3 Experimental methods

5.3.1. Setting. This research is part of the Inuit Health Survey conducted in 36 Arctic communities within ISR, Nunavut, and Nunatsiavut. Details of study design are described elsewhere (Jamieson et al., 2011a, Jamieson et al., 2011b). Briefly, sampling was stratified by community with randomization of households within communities. The household response rate was 68 % overall and included 2550 eligible adults ≥18 years of age (60.9 % female). Individual informed consent was obtained, as well as ethical approval through the McGill University Faculty of Medicine Institutional Review Board.

5.3.2. Clinical assessment. Nurses collected anthropometric measures, % adiposity using bioelectrical impedance analysis (Tanita TBF-300GS, Arlington Heights, IL, USA), and a fasting venous blood sample. Due to logistical restraints half of the morning blood draws were conducted on the research vessel and half were conducted on-land in participants' homes. This design allowed for afternoon data collection on-board without prolonged fasting times and unnecessary discomfort of participants. Normal weight, overweight and obesity were defined by the WHO classification system (WHO (World Health Organization), 2000) and normal waist circumference (<102 cm) according to Health Canada criteria (Health Canada, 2003). Percent body fat was classified according to the Tanita age-appropriate healthy body fat ranges (Jamieson et al., 2011a, Jamieson et al., 2011b).

5.3.3. Biochemical assessment. Hb measures were obtained from venous blood drops (morning participants) or blood drops from a finger prick (afternoon participants) using the azidemethemoglobin method with HemoCue^{TM} 201+ portable photometer (HemoCue, Inc., Lake Forest, California). Quality control (in low, medium, and high ranges) was tested daily to ensure accuracy. Prevalence estimates of anemia were based on venous samples only and classified according to the WHO cut-offs for adult men and women, after adjustment for cigarette smoking (Nestel, 2002). Serum ferritin (SF) was measured with an automated chemiluminescence assay (Liaison Ferritin; Diasorin, Italy) and high-sensitivity CRP (hs-CRP) with an auto-analyzer (Beckman Coulter, Brea, CA, USA). Cut-offs of >3 mg/L and >10 mg/L were used for elevated and acute inflammation, repectively. Due to high cost of the assay and limited serum, sTfR concentration was analyzed as a secondary marker of iron status on a subsample (n=1039) by ELISA (R&D Systems, Minneapolis, USA). Iron deficiency without anemia (IDWA) was defined as normal Hb + serum ferritin <15 µg/L or ferritin 15-50 µg/L if

CRP>10 mg/L; iron deficiency anemia (IDA) was defined as anemia + serum ferritin <15 µg/L or ferritin 15-50 µg/L + CRP>10 mg/L; and anemia unexplained by iron deficiency (UA) defined as anemia + serum ferritin ≥15 µg/L. A sTfR:SF index > 1.5 was considered evidence of IDA and a sTfR:SF index <1.5 evidence of anemia of chronic inflammation (AOI). Body iron stores were calculated as: body iron (mg/kg)=-[log(sTfR/SF ratio) – 2.8229]/0.1207 (Cook et al., 2003), after adjustment of the R&D sTfR assay (1.5 x R & D sTfR + 0.35 mg/L) to equivalent units to the Flowers sTfR assay (Flowers et al., 1989). A <0 mg/kg cutoff is suggested for defining iron deficiency, with positive values representing iron stores and negative values signifying tissue iron deficiency (Mei et al., 2011).

Whole blood was analyzed for lead content by inductively coupled plasma mass spectrometry (ICP-MS; DRC II (M-572)) at the Institut national de santé publique Laboratoire de Toxicologie (Québec City, Québec, Canada). The detection limit was 0.95 µg/L and precision was ≤3.4 %. Blood lead concentrations ≥100 µg/L were considered elevated and ≥600 µg/L evidence of overt toxicity (Center for Disease Control and Prevention, 2002). Fatty acid composition was analyzed on red blood cell membranes (Lipid Analytical Laboratories Inc., University of Guelph Research Park, Guelph, ON), as previously described (Jamieson et al., 2011a). Total eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were expressed as % of total fatty acids and will hereafter be referred to as LC-PUFA (long chain-polyunsaturated fatty acids), when combined (EPA+DHA). Immunoenzymatic methods (ELISA) were used to detect IgG antibodies against *H. pylori* (Calbiotech; Spring Valley,CA, USA), *Toxocara, Trichinella, Echinococcus* (IVD Research Inc; Carlsbad, CA, USA), and *Toxoplasma* (Abbott Axsym; Abbot Park, IL, USA) in serum. Number of total seropositive tests were compiled into a composite variable and dichotomized into 0 or ≥1 positive result. Serum 25-hydroxy vitamin D [25-(OH)D] was

measured using LIAISON 25(OH)D assay (DiaSorin), as described elsewhere (El Hayek et al., 2011). A cut-off of 50 nmol/L was used to describe low vitamin D status (Mason, 2011; Institute of Medicine Food and Nutrition Board, 2011). Erythrocyte (RBC) folate was measured on a randomly selected subsample (n=232) of women (<40 years of age) at Nutrasource Diagnostics (University of Guelph, ON), with a competitive chemiluminescent immunoassay (Siemens ADVIA Centaur; Deerfield, IL, USA).The cut-off for inadequate folate stores was <305 nmol/L (Senti and Pilch, 1985) and <363 nmol/L for low stores (Institute of Medicine Food and Nutrition Board, 2000).

5.3.4. Dietary assessment. Dietary intake data were collected by trained interviewers using a single 24 hour recall with a four stage, multi-pass approach (Gibson, 2005). Portion sizes were estimated with a graduated, three-dimensional food model kit (Santé Québec). Recall data were entered into CANDAT software (Godin London Inc,. London, Ontario, Canada) and nutrient analyses obtained from the 2007b Canadian Nutrient File (CNF), and additional databases described elsewhere (Jamieson et al., 2011a). There were no missing values for the foods and nutrients included in the analysis. Recall data were available for 2053 participants after 25 recalls were excluded due to incompleteness. Nutritional supplement and medication use were documented in a questionnaire administered by a nurse. Traditional food (TF) frequency data, obtained from a semi-quantitative food frequency questionnaire (FFQ), were available for 2052 participants. Frequency of TF use was recorded for in-season and off-season consumption of each item over the past 12 months. Seasons were determined according to regional wildlife harvest calendars and intakes adjusted to frequency per month (assuming 30.4 days per month) and expressed as frequency of intake per day. Analyses were conducted on frequency of all TF

items as well as categories of marine mammals, game, fish, birds, bird eggs, and seafood.

5.3.5. Non-dietary variables. Questionnaires for sociodemographic, health and household characteristics were adapted from Greenlandic and Nunavik (Canada) Inuit Health Surveys (Anctil, 2008) and the Aboriginal Peoples Survey (Statistics Canada, 2001) and through consultations with regional steering committees and key informants. The household questionnaire included a version of the 18-item USDA Household Food Security Survey Module (Nord et al., 2006); details of the questionnaire and classification of household food security are described elsewhere (Egeland et al., 2010b). A household food security score was dichotomized into secure or insecure for analyses. Marital status was dichotomized into single (including widowed, divorced, or separated) and married (including common-law marriage). Education level was assessed by number of school grades completed and dichotomized into ≤elementary school (grade 6) or ›elementary school.

5.3.6. Statistical analysis. Smoking-adjusted mean Hb concentrations by age decile were analyzed by one-way ANOVA and reported in Figure 5.1. Weighted prevalence estimates of IDWA, IDA, and UA were estimated from venous blood samples and presented in Figure 5.2. Sampling weights reflected the proportion of participating adults using Statistics Canada's Census data of age-appropriate Inuit by community. Age-adjusted partial correlation coefficients for FFQ variables and biomarkers with Hb are presented in Table 5.1.

Independent determinants of Hb were assessed in a multivariate linear model using *a priori* selection of variables known or suspected to be related to Hb (Table 5.2). Model assumptions were confirmed graphically and standard diagnostic tests performed. Independent determinants of UA vs. non-anemic were assessed in multivariable logistic model, after exclusion of all IDWA and IDA participants (Table 5.3). Model specification was verified with STATA linktest and region was included as a random effect in a mixed model. Sample size limited interaction testing to only 2x2 interactions between main effects. Age and sex adjusted correlates of Hb and UA were compared across regions in Table 5.4, using linear regression. All analyses were performed in STATA (version 11; StataCorp LP, College Station, TX). *P* values were all two-sided and significance was set at $P \leq 0.05$, with the exception of interaction terms ($P \leq 0.1$).

5.4 Results

5.4.1. Study Population. Mean age ± SD of the study population was 42 ± 15 years (range: 18-90 years), with 28 % of participants overweight and 36 % obese by WHO criteria. Underweight was almost nonexistent (0.8 %). Seventy percent of the sample currently smoked, with a median of 10 cigarettes/day (IQR: 6-13) and prescription or non-prescription medication use was reported by 40 %. High blood pressure, diabetes mellitus, and high cholesterol were self-reported in 26 %, 7 %, and 11 % respectively, and a low hs-CRP concentration (<3 mg/L) was observed in 73 % of the sample. Five % of participants, 39-89 years of age, reported taking a daily dose (80-325 mg) of acetylsalycilic acid (ASA) and a further 4 % reported regular use of other non-steroidal anti-inflammatory drugs (NSAID). However, these participants did not differ in Hb concentrations from age-adjusted non-ASA or NSAID users and were therefore included in analyses.

5.4.2. Anemia Prevalence. Hb concentrations were lower and rates of anemia higher in men after approximately 50 years of age, but both remained relatively stable among women across age groups (Figures 5.1 & 5.2). The majority of cases (75 %) among those over 50 y were observed among 51-70 y olds. For men, iron depletion was associated with only 18 % of anemia cases while 82 % of cases were unexplained by iron stores. Over a third of men with UA (37 %) had elevated hs-CRP. Iron depletion was associated with most cases of anemia among premenopausal women, although this is likely an overestimate of IDA based on a one-parameter model for classifying iron deficiency (Jamieson et al., 2011b). Nonetheless, iron depletion among premenopausal women with and without accompanying anemia was pervasive. For women with UA, 17 % of cases also had an elevated hs-CRP. Among men, 96 % of anemia cases were mild (Hb 100-129 g/L) and none were severe (Hb<70 g/L). For women, 87.5 % of anemia cases were mild (Hb 100-119 g/L) and none severe (Hb<70 g/L).

In a subset analysis of men and women with anemia, who also had sTfR and SF measured (n=211), 94 % of men and 89 % of women were categorized with AOI (sTfR index<1.5). IDA (sTfR index>1.5) with or without accompanying inflammation was observed in 6 % of men and 11 % of women with anemia. Using the total body iron calculation on the subset sample, 1.6 % of men (95 % CI: 0.7-3.6 %) and 6.1 % of women (95 %CI: 4.1-8.7) had no body iron stores (<0 mg iron/kg).

5.4.3. Anemia, other causes. Lead exposure was investigated as a potential cause of anemia. Median blood lead concentration was higher among men than women and elevated blood lead (EBL) may have contributed to low Hb among 16 men and 13 women but lead concentrations were only moderately elevated

(100-250 µg/L) in these cases. No participants had evidence of overt lead toxicity. Blood lead was negatively associated with Hb among men (Table 5.1), as was EBL and Hb after multivariate adjustment (Table 5.2). RBC folate was investigated as a potential cause of anemia on a subsample of women of child-bearing age (n=232). Although 26 % of women sampled were categorized as anemic, no women were found to have inadequate folate stores or low stores that would be associated with macrocytic anemia. Parasitic infections including *Toxoplasmosis, Trichinella, Toxocara, Echinococcus,* and *H. pylori* were also assessed as potential contributing factors for anemia but no individual relationships were evident. Antibody response to *H. pylori* did not vary (*P*=0.189) among anemic (71 % positive) or non-anemic (74 % positive) participants. However, as a composite variable, the number of seropositive test results was related to Hb concentration (Tables 5.1 and 5.2). Low serum 25(OH)D was investigated as a contributing factor in UA and number of seropositive test results, but no relationship was evident. Low vitamin D status was observed in 80.3 % of 18-30 y, 50.3 % of 31-50 y, and 11. 9 % of 51+ years of age. Also of note, for men with an inflammatory state (hs-CRP>3 mg/L) anemia was more prevalent (34.2 % vs. 18 %; P<0.001), but there was no difference in anemia for women with and without inflammation (26.7 % vs. 25.8 %; *P*=0.738).

5.4.4. Dietary intake. Median dietary intakes were analyzed for men and women as groups using 24-hour recall derived nutrient intakes considered relevant to anemia. Forty-four 18 year olds were excluded from this analysis due to different DRI requirements for their age group. Median intakes of men and women exceeded both the EAR and RDA for vitamin B_{12} (4.9 and 3.6 µg/d, respectively) and riboflavin (2.5 and 1.9 mg/d, respectively). Median intakes of folate (318 and 275 mg DFE/d), however, were less than the EAR for both men and women, respectively. Median iron, vitamin C and calcium intakes, as well as

macronutrient distributions were reported elsewhere (Jamieson et al., 2011a, Jamieson et al., 2011b), with insufficient micronutrient intakes observed (median<EAR) for vitamin C, vitamin A and calcium, but not iron.

5.4.5. Correlates of haemoglobin. Frequency of TF intake was not associated with age-adjusted Hb concentrations. However, Hb was moderately correlated with SF concentration in both men and women and negatively correlated with sTfR in women. For men, Hb was negatively, although weakly, correlated with % RBC LC-PUFA, % RBC EPA, blood lead, and serum 25(OH)D (Table 5.1). In a multivariate-adjusted linear model (adjusted R^2=0.30) SF, % adiposity, and frequency of TF intake were positively and ≤ elementary school education was negatively associated with Hb (Table 5.2). For participants over 50 y, hs-CRP and ≥1 seropositive test were also negatively associated with Hb. For men, % EPA and EBL were negatively associated with Hb.

5.4.6. Multivariate correlates of unexplained anemia. In a multivaritate-adjusted logistic model (Pseudo R^2=0.09) % adiposity was independently associated with a reduced risk while hs-CRP and ≤ elementary school education were associated with increased risk of UA (Table 5.3). For men, % RBC EPA and EBL were also associated with increased risk of UA. Having ≥1 seropositive test was marginally associated with higher risk among those over 50 y.

5.4.7. Regional variations in unexplained anemia and correlates. There were regional differences in anemia prevalence, with the highest rate observed in the more traditional region of Nunavut (26.7 %), an intermediary rate in Nunatsiavut

(21.1 %) , and the lowest rate in ISR (13.4 %), which paralleled RBC EPA proportions, frequency of TF intake, and blood lead concentrations (Table 5.4). Despite similar concentrations of hs-CRP and serum 25(OH)D across regions, Nunavut participants had a higher number of seropositive test results than the other regions. In Nunavut, the higher RBC EPA proportions corresponded to greater frequency of marine mammal intakes. In Nunatsiavut, high RBC DHA % and intermediate EPA % were reflected by higher frequency of fish intake but less marine mammal consumption.

5.5. DISCUSSION

Anemia has been observed among Inuit adults for decades but the etiology never fully explained. While iron deficiency can explain much of the anemia observed in pre-menopausal women, men > 50 y and post-menopausal women have moderate rates of anemia despite adequate iron stores. This population is unique in that anemia disproportionately affects men instead of women (Tussing-Humphreys and Braunschweig, 2011) before 75 years of age. Consequently, this is not simply an anemia of aging, as most cases were observed among 51-70 y olds and Hb declined specifically in men after 50 years of age (Figure 5.1). Although the definitive cause of the UA could not be defined, our study provides important clues that can direct further investigation. Our data suggest that there is regional variation in anemia and that multiple factors may contribute to anemia among older Inuit including inflammation, infections, and low socioeconomic status, as well as RBC EPA status and EBL in men.

Previously, the 1976 Nutrition Canada Survey for Inuit (n=184) and 1988-89 survey of Alaska Natives (n=2021) reported moderate anemia rates, with 2-fold higher prevalence in older adults(Canada Bureau of Nutritional Sciences,

1975; Petersen et al., 1996; Valberg et al., 1979), a pattern also observed in this sample. More recently, high anemia prevalence rates of 38% (18-50 y) and 60 % (>50 y) was reported among Inuit women from Nunavik, Canada (Plante et al., 2007). In our sample, there were regional differences in anemia prevalence, with the highest rates observed in the more traditional region of Nunavut and an intermediary rate in Nunatsiavut, which corresponded to regional differences in RBC EPA % and blood lead levels (Table 5.4). These patterns, in combination with the age pattern of cases, suggest that low Hb concentrations among Inuit cannot be explained by physiological differences. Similarly, anemia prevalence was highest in a more traditional Alaskan region compared to other regions among both Alaska Native and non-Native children, suggesting an environmental determinant might be involved(Gessner, 2009). Prevalence of anemia for Inuit, therefore, appears to be highest in more traditional regions, which may be related to many factors including dietary patterns, health care access, infectious disease exposure, and/or lower socioeconomic status. Indeed, a low level of education was independently associated with lower Hb and increased risk of UA, after multivariate adjustment in the present study.

Anemia in older populations is generally due to micronutrient deficiencies, medication or disease-related changes in micronutrient bioavailability, blood loss, impaired renal function and chronic illness or inflammation (Tussing-Humphreys and Braunschweig, 2011). Among the elderly, one-third of anemias are idiopathic or unexplained (Guralnik et al., 2005). Chronic blood loss results in IDA, and therefore would not explain the high rates of UA observed here. AOI is not easily measured due to the lack of a single unequivocal marker of inflammation and non-specificity of the indicators. Thus, AOI may include anemias of various pathophysiological causes (Guralnik et al., 2005). Although *H. pylori* seropositivity on its own was not related to anemia risk, the associations of hs-CRP and the composite index of seropositive tests

with risk of UA, as well as the low sTfR:SF index in this population, supports a role for inflammation in the UA cases. Similarly, AOI was reported to explain 42 % of the anemia among post-menopausal Nunavik women in 2004, with 14 % due to IDA and 44 % remaining unexplained (Plante et al., 2011).

Iron intake and iron stores did not appear to contribute to anemia among older adults. Vitamin B_{12} intake appeared adequate and is supported by observations of adequate serum B_{12} in Nunavik women (Plante et al., 2007). Folate intakes were low but RBC folate, at least among young women, were not in a range associated with macrocytic anemia. Given the current consumption of bannock and wheat flour bread (Kuhnlein et al., 2008), severe folate deficiency would not be suspected. Vitamin A intakes < EAR were common although reliable estimates of vitamin A intake require many days of dietary recalls. Low vitamin A status can contribute to infectious disease incidence and impair iron mobilization and transport for erythropoiesis (West Jr. et al., 2007). However, a 1998-99 dietary survey of Canadian Inuit observed low probably of inadequacy among Inuit > 40 y and high probability of inadequacy among those ≤40 y old, reflecting the ongoing nutrition transition (Egeland et al., 2004). Therefore it is unlikely that low vitamin A status could explain the UA among older adults. Similarly, 25(OH)D increased with age so that few adults with UA also had low vitamin D status. Thus, although low vitamin D status may be associated with increased risk of infections and a pro-inflammatory state(Mason, 2011), it does not appear to be a contributing factor to UA for Inuit. Although it should be noted that the cross-sectional nature of the study may have limited this assessment.

The primary source of lead for Inuit is thought to be through bird and game tissues contaminated with lead shot (Bjerregaard et al., 2004; Scheuhammer et al., 1998; Smith and Rea, 1995), and this was reflected in

dietary correlations with Hb for men (Table 5.1). However, age was shown to be the most important predictor of blood lead for Inuit in a recent analysis, with little association to dietary sources (Fontaine et al., 2008). In general, blood lead levels have declined among Inuit over time in accordance with national bans on lead in gasoline and lead shot, although concentrations are still higher for Inuit than non-Aboriginal comparison groups (Fontaine et al., 2008). The reason for higher blood among Nunavut participants is not clear and should be further investigated. Isotopic studies have identified the activity of hunting as a potential route of lead exposure through inhalation and/or hand-to-mouth activities, which could help explain the gender difference in blood concentrations (Tsuji et al., 2008). EBL in the present study was independently associated with lower Hb and increased risk of UA for men. There are toxic effects of lead on several enzymes involved in heme synthesis at concentrations as low as 50-100 µg/L (Papanikolaou et al., 2005), although overt anemia occurs only with acute toxity (≥600 µg/L). Therefore, given the moderate blood concentrations in this population lead is not likely an important cause of anemia. It is difficult to separate the effects of TF components in a cross-sectional survey, given that markers of TF use were correlated with blood lead levels and TF is a source of both micronutrients and contaminants. Furthermore, lead release from bone loss associated with aging may be a contributing source of exposure as well.

It is interesting that % EPA, but not % DHA in RBC, was associated with lower Hb and increased risk of UA, despite positive associations between LC-PUFA and SF (Jamieson et al., 2011a, Jamieson et al., 2011b). There is biological plausibility for an effect of EPA on Hb concentrations, as animal studies have observed increased RBC oxidative stress, accelerated RBC turnover, and increased RBC vitamin E requirement with a highly unsaturated fish-oil rich diet (Miret et al., 2003). However, EPA and LC-PUFA RBC proportions were not exceptionally high in this sample, even among older adults. In theory, high RBC

LC-PUFA concentrations would be expected to have an anti-inflammatory effect, however a potential effect on RBC stability cannot be ruled out. Although, not measured in this survey, vitamin E intakes were previously a concern for adequacy among Inuit adults (Kuhnlein et al., 2008). It is possible that vitamin E status is insufficient to maintain RBC stability at higher EPA proportions and should be further investigated. Absence of this association in women in the present study may be explained by less frequent marine mammal consumption and 8 % lower RBC EPA proportions compared to men, despite similar DHA and LC-PUFA status (Table 5.1).

Despite the negative association between %RBC EPA and Hb, daily frequency of TF intake was positively associated with Hb concentrations after multivariate adjustment. While frequency of marine mammals (rich sources of EPA and DHA (Innis and Kuhnlein, 1987)) alone is negatively associated with Hb, the overall index (marine mammals, game, fish, birds, bird eggs, and seafood) remained positive. Inuit TF are mainly animal source-foods, rich in micronutrients. On days when TF is consumed, Inuit have been shown to consume more protein iron, zinc, copper, vitamins A, B_6, E and riboflavin (Kuhnlein et al., 2004), all nutrients necessary for erythropoiesis.

Inuit in a healthy adiposity category appear to be at increased risk for UA compared to Inuit in an 'at-risk' adiposity category, even after controlling for age and hs-CRP. In contrast, higher BMI categories were associated with anemia in post-menopausal US women (Thomson et al., 2011). The chronic inflammation of obesity is associated with higher hepcidin levels and lower iron status resulting in an anemia with characteristics of both IDA and AOI (Thomson et al., 2011). Although Inuit are not immune from the metabolic consequences of obesity

(Egeland et al., 2011a), it is likely that obese Inuit differ from obese Americans, especially in terms of dietary patterns. Overweight and obese Inuit adults have been shown to consume higher % energy as TF than those in the healthy BMI category and TF consumption has been associated with higher micronutrient intakes (Kuhnlein et al., 2004).

There are several important limitations to the study. Inflammation status was defined by hs-CRP alone and therefore may have underestimated chronic inflammation in the population. Logistical restraints of the survey limited dietary assessment to a single 24 recall hour (and FFQ). Field-based measurements necessitated the use of a portable hemoglobinometer, rather than an automated complete blood count, which would have aided in anemia classification using RBC size and number counts. Data on renal function was not available, although renal disease in the general population is not expected. Finally, alternate cut-offs for anemia may be appropriate for older adults but have yet to be established based on functional outcomes.

Anemia is a public health concern among Inuit men and women >50 y, and IDA is a significant concern among pre-menopausal women. Although a definitive cause of anemia among older adults could not be established our study provides important factors that may contribute to UA for Inuit including inflammation, infections, and low socioeconomic status, as well as % RBC EPA and EBL in men. The negative association between Hb and % RBC EPA in men suggests a possible effect on RBC stability and turnover that should be further investigated. Yet despite the negative effect of EPA, TF intake overall remained positively associated with Hb, likely due to the micronutrient-rich content of these foods. For older Inuit, the contribution of micronutrient deficiencies to

anemia prevalence should be further considered, given the high rates of nutrient intakes <EAR and ongoing nutrition transition. The regional and age distributions of anemia observed indicate that the prevalence cannot likely be explained by physiological differences, but appears to be related to characteristics of a more traditional lifestyle. Given the associated morbidity and mortality of anemia in older adults, and the uncertainty around appropriate age and ethnicity based anemia cut-offs, determining the clinical significance of this mild but prevalent anemia is important for the health and well-being of older Inuit adults.

Figure 5.1. Mean hemoglobin (95 % CI) by age among men (n=846) and women (n=1286): International Polar Year Inuit Health Survey, 2007-08.

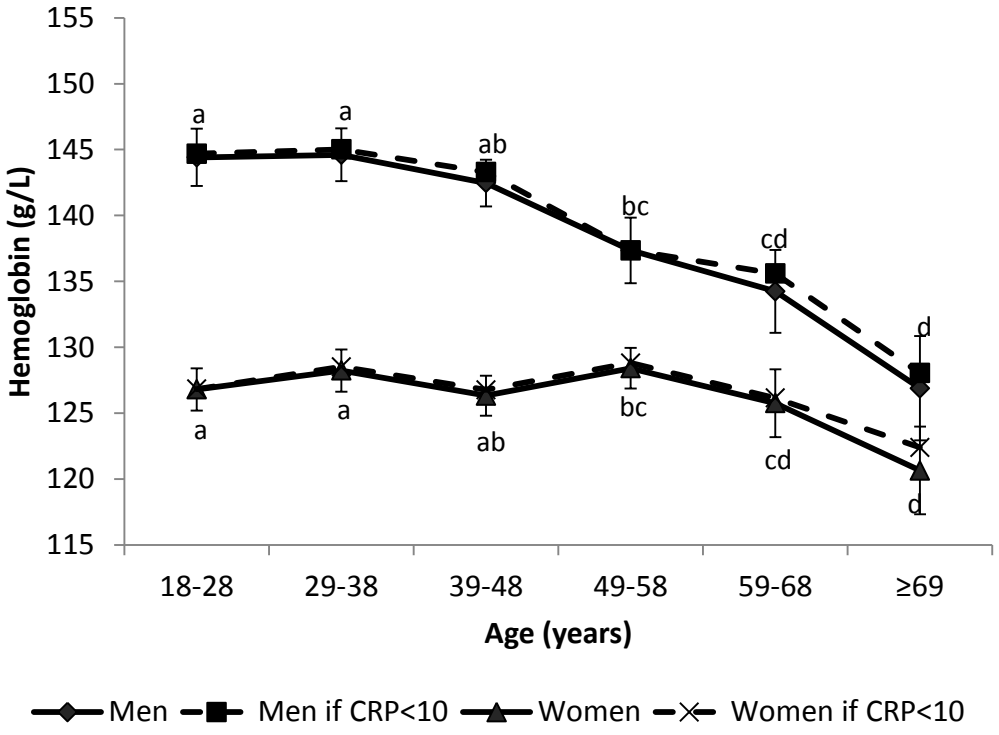
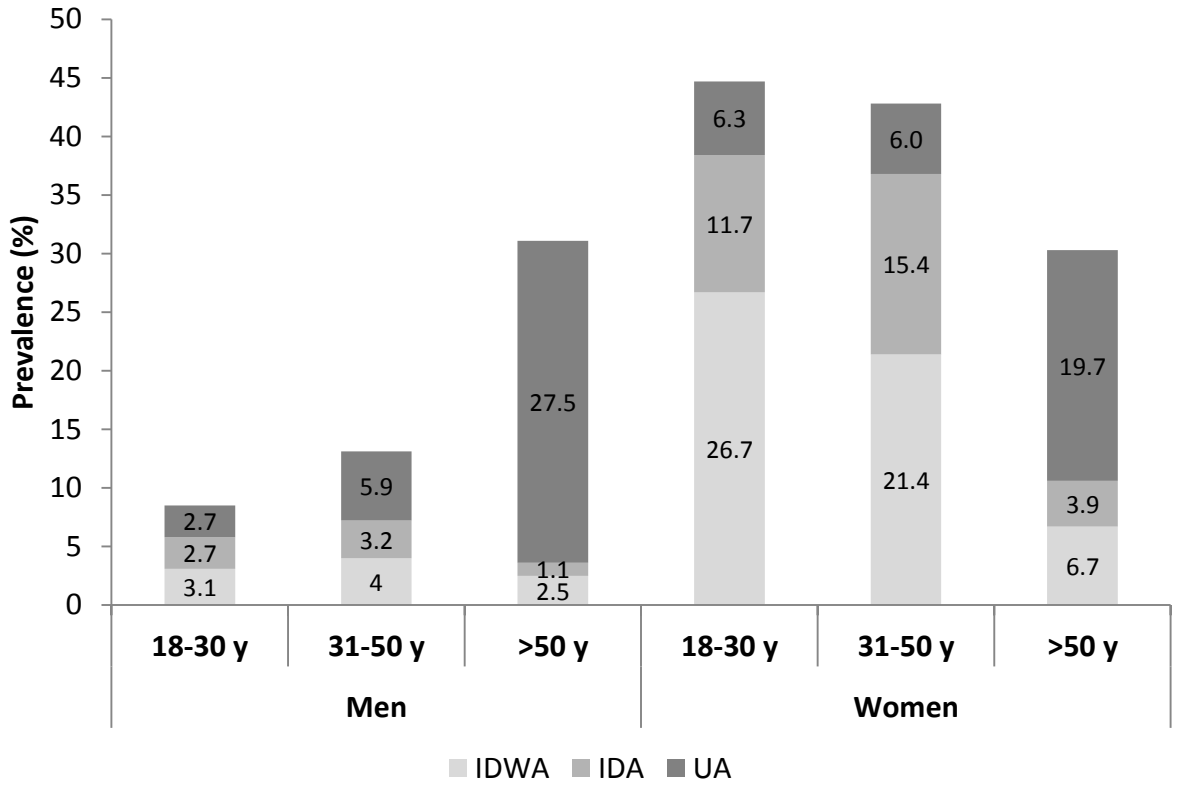


Figure 5.2. Weighted prevalence of iron depletion, iron deficiency anemia and unexplained anemia among men and women: International Polar Year Inuit Health Survey, 2007-08.[1]



[1] Analyses on a subsample (n=1092) with venous blood sampling for hemoglobin (Hb) determination where anemia=Hb<120 g/L (women) or Hb<130 g/L (men). Iron deficiency without anemia (IDWA) was defined as normal hemoglobin + serum ferritin <15 µg/L or ferritin 15-50 µg/L if hs-CRP>10 mg/L; Iron deficiency anemia (IDA) was defined as anemia + serum ferritin <15 µg/L or ferritin 15-50 µg/L + hs-CRP>10 mg/L; and unexplained anemia (UA) defined as anemia + serum ferritin ≥15 µg/L.

Table 5.1. Age-adjusted correlations between dietary and biochemical indicators with hemoglobin: International Polar Year Inuit Health Survey, 2007-08.

	Men			Women		
Dietary intake, *n/d*	*n*	median (IQR)	r_{age}	*n*	Median (IQR)	r_{age}
All traditional food species	792	1.02 (0.45-1.93)	0.05	1195	0.80 (0.31-1.57)	-0.02
Marine mammals	792	0.16 (0.03-0.66)	-0.02	1195	0.08 (0.01-0.31)	-0.03
Game	792	0.35 (0.10-0.91)	0.09*	1195	0.27 (0.07-0.77)	-0.01
Fish	792	0.14 (0.03-0.43)	0.04	1195	0.11 (0.02-0.29)	-0.02
Birds	792	<0.01 (0-0.06)	0.09*	1195	<0.01 (0-0.04)	0.03
Bird eggs	792	<0.01 (0-0.02)	-0.01	1195	0 (0-<0.01)	-0.04
Seafood	792	0 (0-<0.01)	0.04	1195	0 (0-<0.01)	-0.01
Energy as traditional food, *%*	792	9.8 (0-34)	-0.01	1195	6.8 (0-25)	-0.03
Biomarkers						
Serum ferritin, *µg/L*	776	55.9 (30.4-95.6)	0.26***	1149	24.5 (14-51)	0.35***
Serum soluble transferrin receptor, *mg/L*	372	1.3 (1.1-1.5)	0.05	595	1.3 (1.1-1.6)	-0.26***
Body iron stores, *mg/kg*	370	10.6 (8.4-12.4)	0.25***	593	7.0 (4.8-9.7)	0.35***
Serum hs-C-reactive protein, *mg/L*	824	1.2 (0.5-2.9)	-0.04	1241	1.3 (0.4-3.4)	0.04
RBC LC-PUFA, *% of total FA*	822	3.7 (2.1-5.7)	-0.09**	1237	3.7 (1.9-5.8)	-0.03
RBC EPA, *% of total FA*	822	1.2 (0.7-2.1)	-0.15***	1237	1.0 (0.6-2.1)	-0.04

RBC DHA, *% of total FA*	822	2.4 (1.4-3.6)	-0.03	1237	2.4 (1.2-3.7)	-0.04
Blood lead, *µg/L*	811	26 (6.8-49)	-0.22***	1222	15 (4.3-35)	-0.05
Serum 25(OH)D, *nmol/L*	824	55.2 (34.7-79.4)	-0.11**	1241	51.7 (30.8-78.6)	0.03
Seropositive tests, *n*	745	1 (1-2)	-0.13***	1125	1 (1-1)	-0.03
Hemoglobin, *g/L*	846	142 (131-152)		1286	128 (119-136)	

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 5.2. Multiple linear regression coefficients for hemoglobin[1] based upon non-dietary and dietary determinants as independent variables among Inuit men: International Polar Year Inuit Health Survey, 2007-08.[1-2]

	Multivariate Coefficient	SE	P
Constant	116.891	2.055	<0.001
Age-group (1=≥51 y, 0=≤50 y)	-1.227	1.805	0.497
Sex (1=male, 0=female)	18.395	0.885	<0.001
Adiposity (%)	0.296	0.040	<0.001
hs-CRP (mg/L)	-0.173	0.222	0.435
hs-CRP x age-group interaction	-0.760	0.356	0.032
Serum ferritin (µg/L)	0.026	0.005	<0.001
RBC EPA (%)	1.138	0.378	0.003
RBA EPA (%) x sex interaction	-2.861	0.497	<0.001
Elevated blood lead (1=yes, 0=no)	3.301	2.383	0.166
Elevated blood lead x sex interaction	-4.575	3.209	0.154
TF, daily frequency (FFQ)	0.756	0.373	0.043
≥1 seropositive tests (1=yes, 0=no)	0.149	0.978	0.879
Age-group x ≥1 seropositive tests interaction	-4.720	1.938	0.015
≤ Elementary school education (1=yes, 0=no)	-2.702	0.940	0.004

[1]Analyses conducted on *n*=1653; where all variables presented were evaluated together in one model.

[2] The within-region variance component for the model was 5.90.

Table 5.3. Logistic regression coefficients, Odds Ratios (OR and 95% CI) with unexplained anemia as the dependent variable among Inuit adults: International Polar Year Inuit Health Survey, 2007-08.[1-3]

	Multivariate OR	95 % CI	P
Age-group (1=≥51 y, 0=≤50 y)	1.527	0.672-3.473	0.326
Sex (1=men, 0=women)	0.521	0.346-0.785	0.003
Adiposity, 5 *%*	0.745	0.660-0.800	<0.001
hs-CRP, *mg/L*	1.097	1.019-1.181	0.011
RBC EPA, *% of total fatty acids*	0.874	0.736-1.038	0.464
EPA * sex interaction	1.293	1.052-1.591	0.029
Elevated blood lead (1=yes, 0=no)	0.289	0.079-1.062	0.034
Sex * elevated blood lead interaction	4.776	1.047-21.783	0.022
Traditional food, *n/d*	0.921	0.778-1.08	0.306
≥1 seropositive tests (1=yes, 0=no)	0.900	0.535-1.514	0.701
Age-group * ≥1 seropositive tests interaction	2.141	0.895-5.120	0.109
≤ Elementary school education (1=yes, 0=no)	1.683	1.145-2.474	0.013

[1]The within-region variance component was 0.13. All variables were evaluated together in the same logisitic mixed model.

[2] For highly prevalent outcomes, the odds ratio will tend to exaggerate the true relative risk (n=292 unexplained anemia cases).

[3] Participants with iron depletion or iron deficiency anemia were excluded from the model (final model n=1337).

Table 5.4. Regional comparisons in correlates of unexplained anemia among Inuit adults, adjusted for age and sex: International Polar Year Inuit Health Survey, 2007-08.[1]

		n	Nunavut (74 %)	ISR (14 %)	Nunatsiavut (12 %)	P
Hemoglobin, *g/L*	Male	846	139 ± 1	147 ± 1	146 ± 1	<0.0001
	Female	1286	126 ± 1	131 ± 1	128 ± 1	<0.0001
RBC EPA, *% of total fatty acids*		2159	1.26 (1.22-1.31)	0.54 (0.49-0.59)	0.98 (0.89-1.07)	<0.0001
RBC DHA, *% of total fatty acids*		2159	1.87 (1.78-1.97)	0.55 (0.49-0.62)	3.41 (3.01-3.85)	<0.0001
Serum 25(OH)D, *µmol/L*		2207	49.4 (48.2-50.6)	49.9 (47.1-52.9)	52.4 (49.3-55.6)	0.2088
Adiposity, %		2128	27.2 (26.7-27.6)	31.5 (30.2-32.8)	30.4 (29.2-31.7)	<0.0001
Serum hs-CRP, *mg/L*		2164	1.31 (1.23-1.39)	1.37 (1.18-1.59)	1.52 (1.30-1.77)	0.2145
Blood lead, *ug/L*		2132	21.8 (20.7-23.1)	4.3 (3.8-5.0)	3.1 (2.7-3.6)	<0.0001
Traditional food, *n/d*		2037	0.74 (0.70-0.78)	0.57 (0.50-0.65)	0.67 (0.58-0.76)	0.0016
Marine mammals, *n/d*		2037	0.13 (0.12-0.15)	0.09 (0.07-0.11)	0.04 (0.03-0.05)	<0.0001
Game, *n/d*		2037	0.23 (0.22-0.25)	0.21 (0.17-0.25)	0.19 (0.16-0.23)	0.1270
Fish, *n/d*		2037	0.10 (0.09-0.11)	0.12 (0.10-0.14)	0.16 (0.13-0.20)	<0.0001
Birds, *n/d*		2037	0.02 (0.02-0.02)	0.03 (0.02-0.03)	0.06 (0.05-0.06)	<0.0001

Bird eggs, *n/d*	2037	<0.01 (<0.01-0.01)	0.01 (<0.01-0.01)	0.02 (0.01-0.02)	<0.0001
Seafood, *n/d*	2037	<0.01 (<0.01-0.01)	0.02 (0.01-0.02)	0.01 (0.01-0.01)	0.0742
Seropositive tests, *n*	1964	1.35 (1.32-1.37)	1.03 (0.98-1.08)	1.05 (0.99-1.11)	<0.0001

[1]Values are geometric means, adjusted for age and sex for all variables, except hemoglobin (adjusted for age)

CHAPTER 6: OVERALL SUMMARY AND CONCLUSIONS

6.1 Iron deficiency and anemia assessment

Anemia, with and without ID, among Inuit has been sporadically documented since 1955 (Scott et al., 1955), but never fully explained. Given the considerable regional differences observed across the circumpolar nations, the ongoing nutrition transition, and that the last Canadian survey to include Inuit took place in 1970-72, a re-assessment of anemia and iron status for Canadian Inuit was necessary. This thesis research has established, using the first representative sample of Inuit men and women, moderate to high prevalence of anemia and ID (in pre-menopausal women) among Inuit adults of ISR, Nunavut, and Nunatsiavut, with limited concurrence of ID and anemia in men and post-menopausal women. It is the first survey with Inuit adults to demonstrate the effect of acute inflammation on iron status markers and to compare iron deficient erythropoiesis (utilizing sTfR and SF) with the conventional criteria of IDA (low SF and low Hb) in this population (Appendix G).

The prevalence of elevated (20.7 %) or high (6.7 %) CRP levels, in this population, was lower than rates observed in developing countries (Thurnham and Northrop-Clewes, 2007) and did not have a great effect on median SF (an acute phase positive protein) or estimates of ID, although participants with high CRP levels were excluded from prevalence estimates. Similarly, the effect on Hb (an acute phase negative protein) was minimal and was not corrected for in prevalence estimates, as is standard practice. However, a significant proportion of UA cases were associated with elevated hs-CRP in men (37 %) and women (17 %) and a sTfR:SF ratio <1.5 in most cases suggested AOI.

The measurement of sTfR in the subset, confirmed that despite observing low or depleted iron stores in a large proportion of the population, functional iron deficiency was uncommon among men (1.6 %) and mild in women (6.1 %).

In this subset (n=941), the estimate for IDA in women using the SF and Hb criteria (9.7 %; 95 %CI: 7.4-12.6) was 1.5-fold higher than the rate estimated from the absence of body iron stores (R:F body iron) (6.1 %; 95 %CI: 4.2-8.7) (Appendix G). Estimates in men were similar with both models, given the very low prevalence of IDA in men and therefore wide confidence intervals. This second model (R:F body iron) may be preferable in population studies as it does not rely on Hb (an indicator with low sensitivity and low specificity for ID) as a measure of the severity of ID and is thought to provide a more accurate estimate of the true population prevalence of nutritional ID (Lynch, 2011b). It also has the advantage of avoiding the use of as many as 5 different assays (including Hb) to determine iron status and severity with the conventional multi-parameter model.

Regardless of the iron indicators used the prevalence of ID and IDA among pre-menopausal women was a concern (Manuscript 3; Table 4.1) and did not match the relatively high prevalence of dietary iron adequacy observed. Pre-menopausal women also had median intakes of vitamin A, folate, and calcium below the EAR and vitamin C intake below the RDA, suggesting low diet quality (Appendix E). ID and IDA can impair the immune response, compromising resistance to infection and increase the risk of pre-term births and low birth weight during pregnancies (Cooper et al., 2006). ID and IDA in young children inhibits cognitive development and has been linked with misbehaviour and poor school performance (Cooper et al., 2006; Gleason and Scrimshaw, 2007). Despite traditional diets with many animal source foods and median heme iron intakes of >3 mg/d, young Inuit women are still susceptible to ID. The consumption of TF (as indicated by higher RBC LC-PUFA proportions) was associated with a reduced risk of ID among food insecure women (Manuscript 3; Table 4.4), suggesting that intakes of marine mammals were an important source of dietary iron for these women and that access to TF is important for nutritional health.

The significance of low but adequate iron stores among Inuit men is also of interest, in light of the recent attention on high iron stores as a risk factor for chronic disease (Ahluwalia et al., 2010). Iron stores for both men and women are lower than might be expected when considering dietary iron (especially heme iron) intakes, suggesting either impaired absorption or increased excretion. Given the limited consumption of dietary iron inhibitors in the diet (such as calcium, coffee and fibre), and that iron absorption rates should be high in the presence of low iron stores, bioavailability should not be an issue. However, iron utilization by *H. pylori* and an altered gastric environment associated with gastric inflammation could help explain this finding. This hypothesis is supported by the negative association of *H. pylori* and SF in our multivariate models. Non-heme iron absorption may also be low in older adults given the low median vitamin C intakes observed (Appendix E). Systemic inflammation, associated with frequent infections or chronic conditions can also reduce gastrointestinal heme and non-heme iron absorption through hepcidin induction and down-regulation of the iron exporter protein, ferroportin (Oates, 2007). Alternatively, increased urinary iron excretion has been observed in rats fed a highly unsaturated fish oil diet (Perez-Granados et al., 1995), and a selective advantage for lower iron stores may have evolved among Inuit as an adaptation to infectious disease (Denic and Agarwal, 2007). Hypoferremia during the acute phase inflammatory response is thought to be a defensive mechanism to limit iron availability for pathogen growth. Future studies should examine the regulation of iron metabolism in this population, given the high n3-PUFA consumption, increased risk of infectious disease, and recent dietary transition. Although Inuit do not have higher prevalences of hemoglobinopathies or thalassemias (Langlois et al., 2008), polymorphisms in iron transporters such as the heme transporter ABCG2 (Leimanis & Georges, 2007) or divalent metal transporter-1 (DMT1) should be investigated as potential explanations of low iron absorption in this population.

6.2 Physiological differences in hemoglobin concentrations do not explain anemia rates

Authors of the 1993-94 Greenland Inuit Health Survey reported that physiological differences might explain why Inuit aged 19-82 y (n=234) had lower hemoglobin concentrations than a Caucasian Danish sample (n=2804) aged 30-60 y (Milman et al., 2001b). This conclusion has since been cited in several publications as support for establishing appropriate anemia cut-offs for different races. There are several important limitations of the Greenlandic survey, however, that should first be considered. Firstly, hemoglobin values were not reported to be weighted or age adjusted and the Greenlandic sample contained older participants than the Danish sample. Secondly, the Greenlandic participants were apparently healthy but the presence of infectious or inflammatory conditions were not assessed, and therefore AOI may have been undetected. Thirdly, the Inuit sample was obtained from the capital (Nuuk), a smaller town (Ilulissat) and more traditional settlements (Uummannaq district), while the Danish sample was obtained from Copenhagen County, a largely metropolitan area. Thus, there were likely differences in socioeconomic status and lifestyle factors between these populations that could be expected to impact health disparities. Finally, the sample size obtained in Greenland was relatively small (n=234 or 0.4 %) and while randomly selected, may not have been representative of the population at the time (approximately 56 000 (Government of Greenland, 2011)).

If a physiologically lower hemoglobin concentration could explain a high prevalence of anemia among Canadian Inuit then one would expect to see a similar rate of anemia across different age-groups. This was clearly not the case among men as Hb declined and anemia rates rose with age (Manuscript 4; Figure 5.1). A similar trend is also observed among women when comparing rates of UA across age-groups (Manuscript 4; Figure 5.2). Hemoglobin alone or total cases of

anemia among women is influenced by the higher rates of IDA in pre-menopausal women, masking the non-iron related cases of anemia across ages. Cases of UA increased by 3 to 4-fold from 31-50 to >50 years of age for both men and women and by 10-fold for men 18-30 years, compared to men >50 years of age.

In addition to differences in Hb distributions by age, there are important regional differences that also support an environmental cause of UA (Manuscript 4; Table 5.4). Age-adjusted Hb concentrations are lower in Nunavut than the other two regions. Nunavut participants had higher TF intakes (most evident in marine mammal consumption), higher proportions of RBC EPA, 7-fold higher blood lead concentrations, and more seropositive antibody test results for past infections. These factors are associated with a more traditional lifestyle and were independently correlated with risk of UA. Importantly, these findings support a recent study in Alaska that showed anemia in both Aboriginal and non-Aboriginal children to be most prevalent in a more traditional region of rural Alaska implicating the environment in the etiology (Gessner, 2009).

Interestingly, rates of anemia among Nunavik Inuit women (38 %, 18-50 y; 60 %; >50 y) in 2004 (n=466) were 2 to 2.5-fold higher than in the present study (Plante et al., 2011), despite very similar median SF values (24.8 µg/L in Nunavik, 24.5 µg/L presently) and rates of acute inflammation (CRP>10 mg/L: 6.6 % in Nunavik, 7.6 % presently). Whether these differences can be explained by differences in survey methodology or true regional differences is not known. The Nunavik survey measured Hb on venous blood using an automated counter whereas the present survey utilized a portable photometer (Hemocue^{TM}), which has been shown to have high sensitivity and high specificity and produce comparable prevalence estimates to the gold standard method when used with venous blood collections (Sari et al., 2001; Neufeld et al., 2002). Although TF as a % of energy was similar between the two surveys (16 % in 2004 and 17 %

presently), RBC EPA+DHA was higher in the Nunavik survey (7 % of total fatty acids) than the present survey (4 % of total). Nunavik Inuit were observed to consume fish, seafood and caribou most commonly of all TF species (Blanchet, 2008), although RBC n3-PUFA correlated most strongly with marine mammal consumption rather than fish consumption (Lucas et al., 2010). Therefore, high n-3 PUFA intakes may have also contributed to the UA in the Nunavik population as well, although we did not see a relationship among Inuit women, who had marginally lower EPA proportions than men in the present study. Unfortunately n3-PUFA status from the Nunavik surey was not reported by sex (Lucas et al., 2010) and therefore we cannot compare sex differences with our survey. Elevated blood lead (>100 µg/L) was reported in 7.5 % of Nunavik women but was not associated with anemia, concurring with the present findings among women. Anemia and iron status of Nunavik men was not assessed.

6.3 Double burden of malnutrition

This population is characterised by both undernutrition (ID and anemia) and emerging obesity (Chateau-Degat et al., 2011). For Inuit, being in a healthy % adiposity category was independently associated with less risk of ID and anemia and the same relationship was also observed when waist circumference and BMI were used in place of % adiposity. While the effect of inflammation on SF (an acute phase positive protein) may explain this relationship with ID, it does not explain the risk of anemia.

There is some evidence relating overweight and obesity among Inuit with higher TF intake (and therefore higher micronutrient intake) (Kuhnlein et al., 2004), which may explain this paradoxical relationship. In this sample, those classified as overweight, obese, or at-risk adiposity were more frequent consumers of TF and frequency of TF intake and dietary iron were correlated

after adjustment for age (rho=0.13; *P*<0.0001), confirming previous work. This relationship is interesting in light of the current interest in the ID and anemia associated with obesity in other populations, including NHANES surveys (McClung and Karl, 2009). The mechanistic link between ID and obesity is not fully understood but proposed to include blood volume expansion together with hepcidin-induced down-regulation of iron absorption (Tussing-Humphreys and Braunschweig, 2011). Future studies should consider the role of body composition and inflammation in IDA and anemia of other causes for Inuit. It is possible that higher n3-PUFA status may counter the inflammatory processes typically observed in overweight and obesity.

6.4 Traditional Inuit foods and iron status

To date, no study has reported correlates of ID or anemia among Inuit adults. In the second and third manuscripts of this thesis the prevalence of ID and correlates of ID for men and women were examined. Independent correlates of iron status for men included LC-PUFA, TF on the previous day (positive), food insecurity, smoking, *H pylori* seropositivity, and single marital status (negative). For women, independent correlates of iron status were menopause, oral contraceptive use, LC-PUFA (positive) and food insecurity (negative), with higher LC-PUFA status being associated with less risk of iron depletion among food insecure women. These data demonstrate the importance of TF access for nutritional status and the implications of food insecurity, which is pervasive (Egeland et al., 2010b), for Inuit men and women.

Dietary analyses confirmed the importance of TF as a source of dietary iron. Traditional meats were the greatest sources of iron for all age-groups and both sexes (Appendix F). For women, traditional meats supplied 2 to 3-fold more iron than the next greatest source (baked products). For men, traditional meats

provided 2 to 4-fold more iron than the next greatest dietary source (market food meats). In addition, tradition meat intake and % energy as traditional food were significantly correlated with SF concentrations in both men and women. Frequency of intake of game and bird species correlated with SF in men and frequency of intake of birds, game, marine mammals and liver correlated with SF in women.

Hemoglobin (manuscript 4), was also positively associated with TF use, but negatively associated with low education levels, ≥1 seropositive test, hs-CRP, RBC % EPA, and EBL. It is clear from our analysis that correlates of ID and anemia for Inuit are quite distinct. Notably, the disparate relationships between RBC LC-PUFA (and EPA), SF and Hb are of interest. The Inuit diet is rich in animal-source foods including caribou, which was reported to be consumed by 31.7 % of the study population (Sheikh et al., 2011). Consumption of fish and various marine mammals was less common (1.0 – 13.3 %) but marine mammals are rich in heme iron and appear to be a significant iron source in the diet of Arctic Peoples (Johnson et al., 2009) They are also distinct in having approximately equal composition of EPA and DHA, whereas fish species are much more abundant in DHA (Innis and Kuhnlein, 1987). Although a negative effect of highly unsaturated fish oil on iron status has been observed experimentally (Perez-Granados et al., 1995), this is not supported by our data. The negative association between EPA and Hb appears not to be mediated through iron absorption, as both EPA and DHA are positively associated with iron stores (SF). Therefore, future studies should consider a direct effect of EPA and vitamin E status on RBC fragility and turnover as a possible explanation of low Hb among Inuit.

6.5 Dietary intake and nutritional anemia

Also of interest, food insecurity was not a determinant of hemoglobin or UA as it was for SF and iron depletion, suggesting that diet quality may not be as important a determinant for Hb as it is for iron status. Intake of nutrients that contribute to nutritional anemias (including vitamin C, folate, and vitamin A) was low, with median intakes <EAR for both men and women >50 y of age (Appendix E). Vitamin A intake requires many days of dietary recalls for a valid population assessment, however, so adequacy is difficult to assess for this nutrient. Limited vitamin C intakes (median: 31 and 43 mg/d for men and women, respectively) could limit non-heme iron absorption in those >50 y but this does not impact heme iron absorption. Indeed, median heme iron intake was twice as high in adults >50 y compared to 19-50 y olds. Dietary folate intake was low but not likely low enough to cause macrocytic anemia considering the frequent consumption of bannock and other fortified flour products among >50 y olds. Intakes of other nutrients of interest for nutritional anemia, vitamin B_{12} and riboflavin, appeared adequate (median intake>RDA). However, given that Inuit TF species are consumed at irregular intervals and species availability varies considerably by region and territory (Sheikh et al., 2011), usual intake patterns of TF are not easily assessed in this population. It is possible that sporadic but high intakes of pre-formed retinol in organ meats are sufficient to maintain vitamin A status while chronic low intakes of water-soluble vitamins may be inadequate over time.

6.6 Anemia and aging

The significance of anemia in older adults and the elderly is a current topic of interest in the literature (Roy, 2011; Thomson et al., 2011). AOI (also known as anemia of chronic inflammation and, formerly, anemia of chronic

disease (Ganz, 2006)), the most common type of anemia among hospitalized patients, is characteristic of disease processes involving acute or chronic immune activation (Weiss and Goodnough, 2005a; Weiss, 2008). This condition can be self-limiting if the source of inflammation is resolved but anemia severity is positively associated with advanced disease stage. The unexplained anemia of aging shares some features of ACI although the pathophysiology is poorly understood. The WHO criteria for anemia are based on data collected from otherwise healthy individuals with no underlying disease process. However, there is some evidence that optimal Hb levels for the elderly (>80 y) may be higher than current WHO cut-offs (130 g/L, men; 120 g/L, women) (Milman et al., 2004; Guralnik et al., 2004) and the functional status of older women (70-80 y) was reportedly worse for those with Hb=120-129 g/L compared to Hb≥130 g/L, further questioning the appropriateness of the current cut-offs (Chaves et al., 2006). However, there are less data available on late middle age (50-65 years of age). Analysis of NHANES III survey data in the U.S. reported that anemia increased with age, ranging from 4.4-8.5 % for men and women from 50-74 years of age (Guralnik et al., 2004). Causes of anemia were attributed to nutrient deficiency (1/3), ACI and/or chronic renal disease (1/3) and unexplained anemia (1/3). Anemia rates, it should be noted, in the present study are 4-5 fold higher than the NHANES data (Guralnik et al., 2004) and more common in men than women after 50 years of age. Although mild anemia is often dismissed as clinically unimportant, a growing body of literature (Penninx et al., 2004; Cesari et al., 2004) is demonstrating functional impairments in muscle strength, mobility, and physical performance at low to normal Hb levels (above the WHO cut-offs) (Roy, 2011). Therefore, it is important from a public health perspective to further investigate the etiology and clinical significance of mild anemia among Inuit in late middle age to elderly age.

6.7 Lead exposure as a contributor to low hemoglobin

As discussed in manuscript four, blood lead levels were independently associated with lower Hb concentrations in men and were also much higher in Nunavut participants than participants from other regions. Studies have not been able to establish clear links between consumption of game (hunted with lead shot) and blood lead levels (Dietz et al., 1996; Bjerregaard et al., 2004). However, the exposure to lead through the act of hunting through inhalation and hand to mouth routes is now being considered another possible route of exposure. Although blood lead levels are not high enough to cause overt anemia, there may be inhibition of several hematopoietic enzymes at lower but elevated levels. Furthermore, blood mercury concentrations (also found in TF sources) were not associated with Hb concentrations in exploratory analyses. Therefore, the link between blood lead and Hb cannot necessarily be explained by higher consumption of TF species (which may contain more lead).

6.8 Relationship of alcohol use and smoking to iron status and anemia

Smoking was associated with a lower SF before and after adjustment for confounders, including adiposity and age in Inuit men, but not women. This may be related to diet quality as men who smoked also had lower adjusted iron intake than non-smokers (3 mg/d), consistent with the literature (Dallongeville, et al., 1998) and despite similar caloric intakes. Although non-smokers were older, they also had better *n*3-PUFA status and were more likely to have consumed TF on the previous day. The high prevalence of smoking in the sample (70 %), however, likely limited the ability to detect a difference in nutritional status.

The effect of smoking on hemoglobin was adjusted for according to WHO protocols and therefore smoking was not used as an independent variable in multivariate modeling for anemia.

Excessive alcohol use is associated with iron overload and hepatic damage (Ford et al., 1995; Bell et al., 1994) but mild to moderate alcohol intake (up to 2 drinks/day) has also been linked with reduced risk of iron deficiency (Ioannou et al., 2004). The mechanism for increased iron stores with chronic alcohol use is unknown but may include the additional iron content of alcoholic beverages, increased iron absorption through the paracellular intestinal route, hepatic inflammation, and altered iron regulation at the gastrointestinal level (Ioannou et al., 2004).

Alcohol intake, as assessed, was not associated with SF or Hb in exploratory analyses. Alcohol use was categorized as self-reported use in the past year or no use in the past year. Heavy consumers were further identified by a positive response to ever drinking alcohol to the point of losing consciousness in the past year. There was likely non-response bias in the self-reporting of alcohol use as there were 171 more missing values for this question than for other standard questions on the health questionnaire. This bias also prevented the use of alcohol as a variable in regression modeling. Similarly, the 1993-94 Greenland Inuit health survey reported no difference in SF between low and high alcohol consuming Inuit men (Milman et al., 2001a). Biomarkers for alcohol use would likely be more useful in investigating the relationship between iron status and alcohol in population surveys because of reporting bias. Identification of alcoholism and the severity of alcoholism would be helpful in considering the effects on nutritional status. Finally, red wine has higher iron content than spirits and beer and the type of alcohol consumed may impact the response in iron stores according to several reports (MacDonald and Blaumsberg, 1964; Perman, 1967; Olalla et al., 2000).

6.9 Strengths and limitations

There are several important limitations to this work. Primarily, the cross-sectional nature of this study does not allow for conclusions on causality. Inflammation status was defined by hs-CRP alone and therefore may have underestimated the prevalence of chronic inflammation in the population. More comprehensive inquiry into the medical history of infectious or inflammatory diseases of participants and inclusion of a chronic marker of inflammation such as alpha$_1$-acid glycoprotein may have captured more of the impact of inflammation on the biomarkers. Measurement of serum sTfR on the entire sample would have provided more confidence in our assessment of functional ID but was limited by the expense of the assay (*approximately $10.00 per sample*).

Concern is raised over the lack of repeat dietary recalls on the sample, as logistical restraints of the survey limited dietary assessment to a single 24 recall hour and semi-quantitative FFQ. In order to estimate usual intake of iron, within person variability from a 1998-99 dietary survey of Canadian Inuit (n=1600) using seven day food records were relied upon. The coefficient of variation for iron intake may have changed over time, although this is not known.

Field-based measurements necessitated the use of a portable hemoglobinometer, rather than an automated complete blood count, which would have aided in anemia classification using RBC size and number counts. In addition, fasting venous samples were collected in some participant homes, on land, in order to prevent prolonged fasting into the afternoon for participants assessed later in the day. This necessitated that a significant number of Hb determinations be made on capillary samples. For this reason, only venous determinations were used in estimates of anemia prevalence. Notably, including blood sampling technique as a covariate did not change the interpretation of any independent variables in the multivariate modeling.

Despite these limitations, this study has considerable strengths and was able to build significantly on the knowledge in this area. This is the first representative sample of Inuit men and women to investigate anemia and iron status. Notably, it is the first study to report the influence of inflammation on iron status markers for an Inuit population, as previous surveys were biased by relying upon SF, an acute-phase protein, without any assessment of inflammation. It was also the first study with enough power to go beyond correlation analysis and examine independent determinants of iron status and anemia. For too long high rates of anemia among Inuit have been dismissed as a differences in physiology. We provide the first evidence that biology cannot explain the patterns of anemia observed in men and women, but that one or more environmental factors associated with a more traditional lifestyle appear to be involved.

6.10 Future directions

This work has opened the door to several interesting areas of future exploration. Studies designed to investigate the etiology of anemia in Inuit adults by sex and lifestage are needed using complete blood counts, mulit-parameter iron indices, biochemical markers of folate, vitamin E and retinol status. The establishment of standard methodology and reference values for sTfR assessment are also needed, and this standardization should reduce the cost of this important measure which is not influenced by inflammatory conditions. Absorption studies utilizing stable or radioisotopes to investigate iron absorption and excretion in Inuit would be informative towards understanding the disparity between iron intake and iron stores. These studies should also consider the presence of chronic *H. pylori* infection and other inflammatory conditions in iron absorption rates, as well as polymorphisms in the iron transporters ABCG2 and DMT1. Furthermore, measurement of hepcidin, the hormonal regulator of iron

absorption and an acute phase protein, could confirm or refute the down-regulation of gastrointestinal iron absorption.

Current work on the impact of Inuit body composition on chronic disease risk factors should be complemented with more investigation on the relationship between body composition and iron status. The double burden of malnutrition, including the obesity-IDA phenotype, is an important global public health issue, and may not be applicable to the Inuit population. Additionally, follow-up investigations on RBC fragility and turnover rates according to % RBC EPA and vitamin E status are needed. Vitamin E status assessment is of course limited by the lack of a biomarker to reflect body stores and the great deal of missing nutrient composition database values. However, a functional indicator such as the erythrocyte hemolysis test could be an useful alternative. In addition to physiological studies, comparisons of hemoglobin concentrations and n3-PUFA status between surveys of Alaska Natives, Greenlandic and Nunavik Inuit could lend additional support to this hypothesis. Markers of a traditional lifestyle, including high n3-PUFA status and increased risk of infectious disease, are common characteristics in these populations that may explain anemia prevalence.

Finally, studies to assess the functional and clinical significance of this mild anemia among older Inuit adults are needed in order to gage the public health significance of this issue. A study designed to test the appropriatenesss of the WHO cut-offs for anemia in Inuit of different ages would help to clarify this issue. Demonstration of functional impairments at low but mild hemoglobin levels will be important in stimulating action on this public health issue.

6.11 Public health implications

Health assessment in remote regions such as the Canadian Arctic are challenging on many fronts including equipment and sample collection methods. Although hemoglobin is a relatively easily measured indicator, its use as a marker of iron status for Inuit adults is clearly inappropriate, as ≤1 % of men >50 years of age had IDA or functional ID, while 30 % had anemia (Hb<130 g/L). Therefore, hemoglobin testing of adults in remote Inuit health centres should be interpreted with caution. The development of portable equipment to rapidly test specific iron indicators would be valuable for remote populations.

The use of body iron stores (sTfR:SF) was comparable to the conventional criteria of low Hb (<WHO cut-off) + SF<15 µg/L for men, producing estimates of 1. 6 % and 2.2 %, respectively, well within the 95 % CI around each estimate (Appendix G). For women, the conventional model produced an estimate 1.5-fold higher than the body iron store model and therefore should be used more cautiously in population surveys, especially among pre-menopausal women.

In addition to high rates of IDA (up to 15 %) the prevalence of depleted iron stores among pre-menopausal women was very high (up to 40 %). This level of prevalence should be considered a public health issue requiring immediate action. ID during pregnancy has serious implications for both maternal health and healthy fetal development.

6.12 Conclusion

Inuit are in a period of dietary and health transition with emerging and re-emerging issues of both overnutrition and undernutrition. Comparison of data from the International Polar Year Inuit Health Survey 2007-2008 and the 2004 Inuit Health Survey for Nunavik, reveals that the prevalence of both ID and anemia have risen considerably since the 1970-72 Nutrition Canada Survey of Inuit (Canada Bureau of Nutritional Sciences, 1975), yet these conditions are not often associated with Inuit because of the central role of animal foods in the traditional diet. Iron deficiency and iron deficiency anemia are public health concerns among young Inuit women and should be addressed in order to ensure healthy pregnancies and child development. Anemia, unrelated to iron status, remains a public health concern among older Inuit adults and is associated with one or more environmental factors including high n3-PUFA status, lead exposure, and low socioeconomic status, all of which tend to concentrate in more traditional Inuit regions and communities. Unexplained anemia for Inuit should not be dismissed as a difference in physiology, without evidence for revising the current WHO cut-offs for Inuit. Iron deficiency and anemia remain important health issues for Canadian Inuit adults and are related to food insecurity, TF access and consumption, and diet quality.

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APPENDICES

Appendix A. *Study setting and methodology*

Appendix B. *Home-based Questionnaires*

Appendix C. *Ship-based Questionnaires*

Appendix D. *Study population descriptive statistics*

Appendix E. *Dietary intake assessment*

Appendix F. *Sources of dietary iron*

Appendix G. S*ubset: iron deficiency models*

Appendix H. *Distributions of iron status biomarkers and dietary intakes*

Appendix A. Study setting & methodology

Study setting

A cross-sectional Inuit health survey of adults residing in randomly selected households was conducted between August to September, 2007, and August to October, 2008, in 36 Arctic communities across ISR, Nunavut, and Nunatsiavut. All non-pregnant household members 18 years and older from selected houses were eligible for participation. Of the 2796 Inuit households approached, 1901 households (68 %) agreed to participate, with a total of 2595 adult participants, of whom 38.5 % were male. Fasting venous blood samples, anthropometric measurements, a 24-hour dietary recall, a semi-quantitative food frequency questionnaire, and 5 general questionnaires were administered either on-land or upon the Canadian Coast Guard Ship (CCGS) Amundsen.

Methodology

Survey Sample size calculation - *Qanuippitali* – International Polar Year Inuit Health Survey, 2007-08.

The study design was a cross-sectional survey of private Inuit households in Nunavut, Inuvialuit Settlement Region (ISR), and Nunatsiuvut. Stratified random sampling procedures were used to survey 12% of the Inuit population in these regions, with a sample size of approximately 2500 adults. Stratification was done by municipality and randomization by household. Thirty-six communities across all 3 regions were included in the survey. Based on current population estimates of the target Inuit regions (17 726) from the 2005 Statistics Canada Census (accounting for a 2 % population growth rate) an estimated sample size of 2000 adults was required in order to confidently estimate population prevalence of selected health indicators within accepted errors of margin and to analyze determinants of health by multivariate modeling. A total of 2595 participated in the IPY Adult Inuit Health Survey.

Study Sample size calculation[1] – Prevalence of Anemia and Iron deficiency

To estimate a predicted 30 % rate of anemia among 17726 adults, within a 5 % acceptable margin of error, would require a sample size of 317 with a 95 % confidence level.

To estimate a predicted 20 % rate of iron deficiency among 17726 adults, within a 5 % acceptable margin of error, would require a sample size of 242 with a 95 % confidence level.

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[1] EpiInfo™ Version 3.5.3. Center for Disease Control. Atlanta, GA, USA

Height Protocol

Purpose

Height is a simple, convenient and reliable anthropometric index to be used along with body weight to determine body mass index (kg/m2) which represents a measure of obesity.

Equipment Required

Portable height rod (stadiometer)

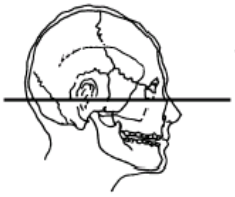
Installed dimensions: 36cm x 42cm x 168cm

Time Required

Approximately 5 minutes

Measurement Procedure

1. Ask the participant to remove their shoes. The subject should stand as tall and as straight as possible with the head level (see illustration), the shoulders and upper arms relaxed.



2. Measure the vertical distance between the standing surface and the top of the head with the height rod (see illustration). Measure at the maximum point of quiet (normal) respiration.

3. The measurement should be taken to the nearest millimeter (0.1 cm). If the measurements fall between two millimeters, record to the nearest even millimeter. Repeat height measurement until results are similar (no more than 1 cm difference).

Waist Circumference Protocol

Purpose

Waist circumference is a simple, convenient and reliable anthropometric index for determining the extent of abdominal obesity and the risk for related metabolic complications.

Equipment Required

Flexible tape measure
Clothes pins

Measurement Procedure

1. Politely ask the participant to undo a few buttons or lower the zipper of their pants only to clear the abdominal area. Use clothes pins to hold up the shirt.

2. The participant should stand straight with the abdomen relaxed, the arms on the sides and the feet together.

3. Standing in front of the participant, use your hands to gently feel for the tops of both hips and for the last loose rib. Locate the midpoint between the top of the hip and the last loose rib. Try to remember as best you can where this point is.

4. Place the measuring tape around the participant, positioning it by eye so that it lies on the midpoint between the top of the hip and the last loose rib. Use your hand to find it again if you need to.

5. Measure the waist circumference making sure that the measuring tape is not twisted, too tight or too loose. Measure from a standing position between the front and the side. The measurement should be taken at the end of a normal expiration.

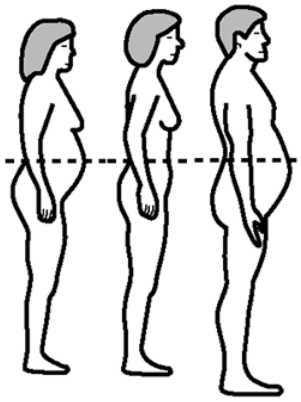
6. The measurement should be taken to the nearest millimeter (0.1 cm). If the measurement falls between 2 millimeters, record to the nearest even millimeter.

7. If the tape is too short, fasten two measuring tapes and measure the participant with two people.

Interpretation

Normal waist circumference: men <102 cm amd women <88 cm,

according to Health Canada criteria.



Bioelectrical Impedance Analysis Protocol

Important: NOT to be used on anyone with a pacemaker!

Purpose

Bioelectrical impedance analysis (BIA) combines the impedance value with anthropomorphic data to provide body compartment measures (i.e. body fat %) but does not specify the location of body fat.

Equipment Required

Tanita scale
Electrical power/Extension cord
Rubbing alcohol
Paper towels
Stapler (to attach printout to clinical measurement sheet)

Information Required

Gender and body type (standard or athletic – use standard for all participants)
Weight of clothing (use 0.4 kg for all participants).
Height (not self-reported height, use measured height)
Age

Time Required
Approximately 5 minutes

Measurement Procedure

1. Ask if participant has a pacemaker. If "YES", DO NOT use Tanita. Take body weight with the SECA scale as described on the next page.

2. Ask the participant to remove his/her shoes and socks.

3. Enter clothing weight of 0.4 kg (0.9 lbs), the participants' gender and whether standard or athletic (standard for all), age, and height.

4, When the Tanita screen indicates to STEP ON, the participant should stand on the platform, making sure that his/her feet are within the metal foot pads.

5. The participant must stay on the platform until the test is complete. Observe the machine.

6. Remove the printout when measurement is complete, record and staple to the clinical measurement sheet.

7. Clean the machine for the next participant with rubbing alcohol spray and a paper towel.

For Those with a Pacemaker:

Equipment Required

Seca scale
Rubbing alcohol
Paper towels

Measurement Procedure

1. Ask the participant to remove his/her shoes.

2. Touch the scale briefly (0.5 sec) to activate it. When the display reads 0.0, have the participant stand on the scale.

3. Record the weight on the clinical measurement sheet. Add 0.4 kg (0.9 lbs) to account for clothing weight.

4. Clean the machine for the next participant with rubbing alcohol spray and a paper towel.

Interpretation

Percent body fat was classified according to the manufacturer's age-appropriate healthy body fat ranges. For men, at-risk body fat percentage was defined as >20 %, >22 %, and >25 % for ages 18-39, 40-59, and ≥60 years, respectively. For women, an at-risk body fat percentage was defined as >33 %, >34 %, and >36 % for ages 18-39, 40-59, and 60-99, respectively. Normal weight (BMI 18.5-24.9), overweight (BMI 25-29.9) and obesity (BMI≥30) were defined by the WHO classification system for BMI.

Blood Collection Protocol

Purpose
To obtain appropriate blood specimen, without harm to participant or technician.

Equipment Required

Venipuncture Tray

- Tourniquets
- Vacutainer needles (20, 21 22G)
- Vacutainer butterfly sets (21, 23G)
- Vacutainer holder(s)
- Vacutainer tubes for each participant
  - **Grey top tubes**
    1 fasting sample (labeled with green tape) and 1 post-OGTT sample
    Contains sodium fluoride (anticoagulant) + potassium oxalate
  - **Lavender top tubes:**
    Contains K_2 EDTA (anticoagulant)
  - **Red and grey (tiger) top tubes**
    Contains a clot activator and gel for serum separation
- Spare vacutainer tubes (grey, lavender, tiger top) & marking pen
- Red and green round stickers (red for women ≥ 40; green for women < 40)
- Alcohol swabs
- X 2 gauze squares
- Bandaids
- Bottle of BacDown Gel
- Gloves
- Spray bottle with Isopropyl alcohol
- Paper Towels
- Pillow
- Blue underpads

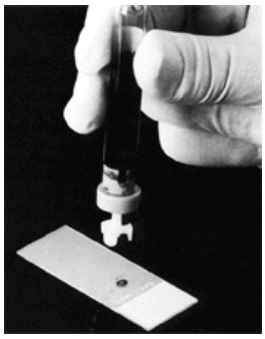
Venipuncture Procedure

1. Check the venipuncture tray, to ensure all supplies are ready.
2. Ask the participants if they consent to have their blood taken, and whether they understand the reason for the blood tests.
3. Ask participant when they last ate or drank anything (other than water).
4. Ask participant when they last smoked.
5. Have the participant to sit down.
6. Check that labels on vacutainers correspond to the participant's study number.
If participant is a female **<** 40, put a **GREEN** sticker on sample bag.
If participant is a female **≥** 40, put a **RED** sticker on sample bag.
7. Wear gloves.
8. Select a venipuncture site (usually antecubital).
9. Feel vein and make sure it's suitable. Slap vein very lightly, or use a warm cloth to make it more prominent.
10. Apply tourniquet above site. (Before using latex tourniquet, check that the participant does not have a latex allergy).
11. Swab area with alcohol pad.
12. Select the appropriate needle and assemble the vacutainer.
13. Perforate the vein with the needle bevel up.
14. Insert tube into vacutainer holder in the following order:
    - 1 grey (marked with green tape) – **label tube as G1** for fasting blood
    - tiger top
    - lavender top
    - **Make sure vacutainers are completely filled** – if necessary take an additional tube and label it with the spare labels. 3 <u>full</u> tiger tops and 2 <u>full</u> lavender tops are needed for all the tests to be done.
15. Loosen tourniquet when enough blood has been taken and before needle is withdrawn.
16. Withdraw needle and apply pressure with a 2 X 2 gauze square for at least one minute. (For bleeding disorders or easy bruising, longer times may be needed).
17. Apply bandaid.
18. DO NOT recap the needle. Press auto-release button on sides of vacutainer holder to release needle directly into the sharps container.
19. Place all tubes into plastic bag.
(Please make sure tube for toenail samples is not packaged with the tubes going into the lab).
20.Store samples on ice until they go to the lab

Laboratory: Samples were processed into serum, plasma or red blood cell fractions, aliquoted, and frozen at -80^{o} within 4-8 hours.

Diff-Safe Blood Dispenser Protocol

Purpose



Allows blood to be removed from a vacutainer without removing the stopper, and eliminates the need to use a lancet for a finger prick when testing for hemoglobin.

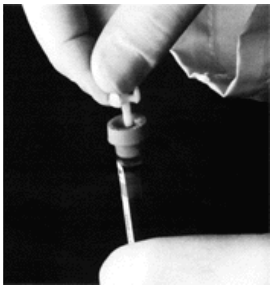
Equipment required

Diff-Safe dispenser

Vacutainer tube

Parafilm

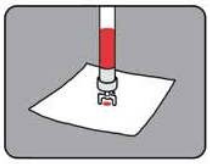
Procedure



1. With the vacutainer tube held upright, insert the cannula through the rubber stopper.

2. Turn the tube upside down and press against a piece parafilm to dispense a drop of blood.

3. Leave the Diff-Safe in the stopper. It will be removed in the lab.



Hemocue Hb 201+ Protocol

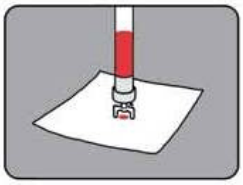
Purpose

The HemoCue Hb 201+ provides determination of hemoglobin.

Equipment required

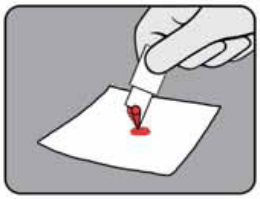
Hemocue Hb 201+
Hemocue Hb 201 Microcuvettes
Diff-Safe blood dispenser
Parafilm
Lavender Top vacutainer containing freshly collected blood sample
KimWipes

Measurement Procedure



Use Diff-Safe blood dispenser to place a drop of blood on a square of parafilm.

1. Fill the microcuvette in one continuous process.
   Do not refill!
   Wipe off excess blood from the outside of the cuvette with a clean Kimwipe. Be careful not to touch the open end of the cuvette.



2. Check for air bubbles. If present, discard the cuvette and fill a new one. Small bubbles around the edge can be ignored.
Place microcuvette in the cuvette holder.

3. Push cuvette holder gently to the measuring position.
"Slamming" the cuvette holder shut can splatter blood drops inside and cause erroneous high reading.

4. After 15-60 seconds, the hemoglobin value is displayed.

5. Record the result. Result will remain on the display as long as the cuvette holder is in the measuring position.

Interpretation

Anemic = <120 g/L (women) and <130 g/L (men) according to WHO criteria. Sampling was done on venous blood drops (morning participants) or blood drops from a finger prick (afternoon participants) onboard CCGS Amundsen.

Daily Maintenance

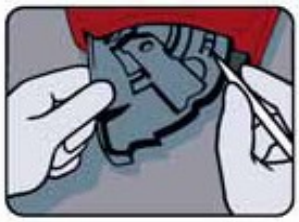
1. Clean cuvette holder at the end of each day of use.

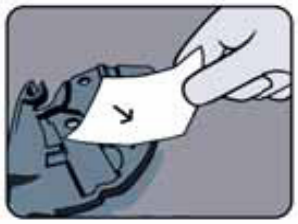
2. Turn the unit off.

3. Pull cuvette out to its loading position

4. While pressing the catch, carefully rotate the cuvette holder towards the left as far as possible.

5. Carefully pull the cuvette holder away from the analyzer





6. Clean the cuvette holder with alcohol or mild detergent. Cuvette holder must be completely dry before being replaced.

7. Clean the inside of the unit with a Q-tip moistened with alcohol or water. Move Q-tip side-to-side 5-10 times. If swab is stained, repeat with a new swab.

8. Wait 15 minutes before replacing the cuvette holder

For problems and additional information, consult Troubleshooting Guide in the HemoCue™ manual.

Hemocue Hb 201+ Quality Control Protocol

Purpose

The HemoCue Hb 201+ is a precision instrument. Accuracy of each instrument must be verified daily for accurate results.

Equipment required

Hemocue Hb 201+
Hemocue Hb 201 Microcuvettes
Pipettor and pipette tips
Parafilm
Hemotrol control standards - Low, Normal, High
KimWipes

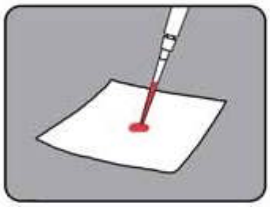
Time required

15 minutes for control standards to stand at room temperature
Approximately 10 minutes to analyze the three standards

Procedure

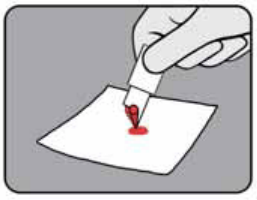
1. The standards are stored tightly capped at 4oC.

2. At the start of each clinic day, allow the standards to sit at room temperature for 15 minutes before analyzing them.



3. Gently mix each vial 8-10 times before sampling.

4. With a pipettor dispense a drop of standard onto a piece of parafilm. (Use a clean pipette tip and Parafilm for each standard.)



5. Fill the microcuvette in one continuous process.

6. Do not refill.

7. Wipe off excess material from the outside of the microcuvette with a clean KimWipe. Be careful not to touch open end of microcuvette.

8. Wait 1 minute.

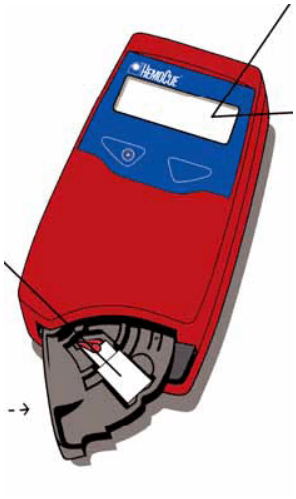
9. Place microcuvette in the cuvette holder.

10. Push cuvette holder gently to the measuring position.

11. After 15-60 seconds, the hemoglobin value is displayed.

12. Record the values in the Quality Control Notebook.

13. Wipe any excess material from the outside of standard vial and cap with a clean KimWipe. Recap the vial tightly and store at 4oC.



Laboratory analyses

Serum ferritin

Equipment:

Automated chemiluminescence assay (Liaison Ferritin; Diasorin, Italy) at McGill University, Montréal, Québéc.

Detection limit: 0.5 µg/L.

Accuracy: Level 1 Lyphocheck control (Biorad, Hercules, CA, USA) was assessed daily to ensure accuracy (<2 % difference from expected concentration).

Mean inter-assay precision: 1.4 % (range=0.2-4.1 %CV) for quality controls measured in duplicate.

Interpretation: <15 µg/L, depleted iron stores (iron deficiency); <32 µg/L, low iron stores, >200 µg/L elevated iron stores.

Serum soluble transferrin receptor (sTfR)

Equipment: sTfR ELISA (R&D Systems, Minneapolis, USA) measured on a Synergy HT microplate reader (BioTek; Winooski, CT, USA) at McGill University, Montréal, Québéc.

Detection limit: 0.225 mg/L.

Accuracy: 1 % difference from expected concentration of two manufacturer quality controls.

Mean inter-assay precision: 3.3 % (range=0.4-6.0 %CV) for quality controls measured in triplicate.

Notes: sTfR measured on a subsample (n=1039) of participants from all 3 regions collected in 2008. sTfR is stable for 1 year at -80^{o} C.

Interpretation: >2.76 mg/L, iron deficient erythropoieisis (according to manufacturer's instructions.

High-sensitivity C-reactive protein (hs-CRP)

Equipment: Auto-analyzer (Beckman Coulter, Brea, CA, USA) at Montreal General Hospital, Montréal, Québéc.

Detection limit: 0.2 mg/L.

Accuracy: <3 % difference from expected concentration of Liquicheck Levels 1-3 (Biorad, Hercules, CA, USA).

Mean inter-assay precision: <10 %.

Interpretation:

Blood lead

Equipment: Inductively coupled plasma mass spectrometry (ICP-MS; DRC II (M-572)) at the Institut national de santé publique Laboratoire de Toxicologie (Québec City, Québec, Canada).

Detection limit: 0.95 µg/L.

Accuracy: verified through inter-laboratory quality control program.

Mean inter-assay precision: precision was ≤3.4 %.

Interpretation: Blood lead concentrations >100 µg/L were considered elevated and >600 µg/L overt toxicity (Centre for Disease Control criteria).

Serology

Equipment: Immunoenzymatic methods (ELISA) were used to detect IgG antibodies against *H. pylori* (Calbiotech; Spring Valley,CA, USA), *Toxocara, Trichinella, Echinococcus* (IVD Research Inc; Carlsbad, CA, USA), and *Toxoplasma* (Abbott Axsym; Abbot Park, IL, USA) in serum at Montreal General Hospital, Montréal, Québéc.

Interpretation: Results were interpreted as positive, negative or equivocal according to the assay protocol. All equivocal results were re-tested once and excluded from analyses if the test remained equivocal.

Fatty acid composition red blood cell membranes

Equipment: Fatty acid methyl esters were prepared by standard techniques and analyzed on a Varian 2400 gas-liquid chromatograph (Palo Alto, CA, USA) with a 60-metre DB-23 capillary column (0.32 diameter) at Lipid Analytical Laboratories Inc., University of Guelph Research Park, Guelph, ON). Lipid extraction was based on the methodology of Folch *et al.*[2]

Interpretation: EPA, DHA, and other fatty acids of interest were expressed as % of total fatty acids.

Serum 25-hydroxy vitamin D ([25-(OH)D]

Equipment: Automated chemiluminescence assay (Liaison Ferritin; Diasorin Inc, Stillwater, MN, USA) at McGill University, Montréal, Québéc. The laboratory obtained a certificate of proficiency for 2009-10 from the Vitamin D External Quality Assessment Scheme program.

Inter-assay CV%: 4.5 % with low control (38.2 nmol/L) and 6.2 % for high control (127.2 nmol/L)

Intra-assay CV%: 11.0 % with low control (38.2 nmol/L) and 5.3 % for high control (127.2 nmol/L)

[2] FOLCH, J., LEES, M. & SLOANE-STANLEY, G. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, 226, 497-509.

Multivariate Modeling Procedures

Multivariate mixed linear models

1. $\log_{10}$ serum ferritin
2. hemoglobin

Random effects: household, community, and region were included if they significantly contributed to model (P<0.05 in a likelihood ratio test).

Diagnostics:

- Multicollinearity testing (variance inflation factors<3 and tolerance>0.4)
- Histogram of standardized residuals to confirm normality
- Q-Q plot of standardized residuals to confirm normality
- Scatter plot of residuals and predicted values to verify the assumption of constant error variance
- Linearity of continuous predictors was verified with component residual plots
- AV plots for each predictor were produced to identify potential outliers, high leverage points, and influential points
- Cook's distance was calculated for each participant in the model
- Identified potential outliers, high leverage points and influential points with a high Cook's distance and visual confirmation on the AV plot were individually removed from the model and changes in significance or interpretation of predictors was compared to the original model. No data points had to be removed from either model because of high influence or leverage.

Multivariate mixed logistic models

1. low or depleted iron stores in men (SF< 32 μg/L)
2. iron deficiency in women (SF<15 μg/L)
3. unexplained anemia

Random effects: household, community, and region were included if they significantly contributed to model (*P*<0.05 in a likelihood ratio test).

Diagnostics:

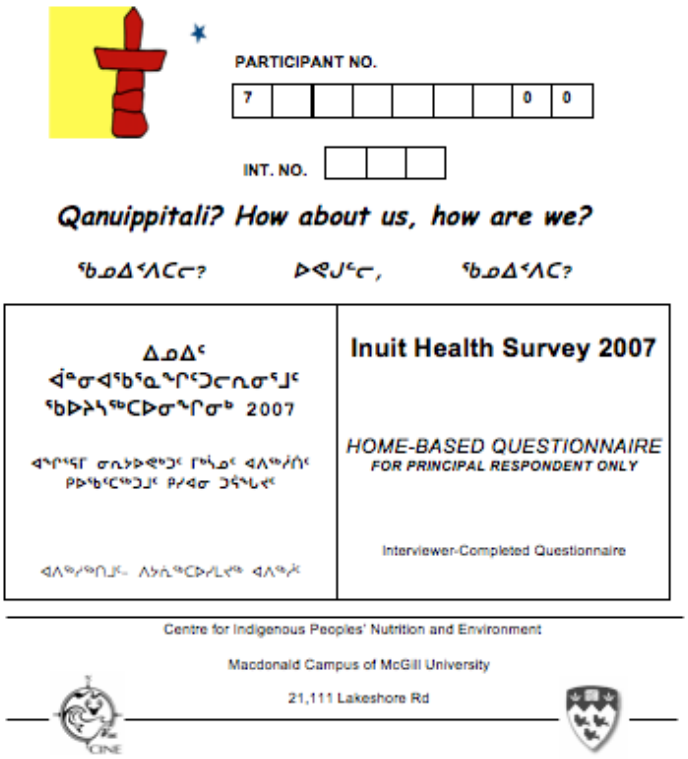
- Multicollinearity testing (variance inflation factors<3 and tolerance>0.4)
- Model verification: STATA link test was used to investigate specification errors and confirm that no important predictors were excluded from the model.
- STATA goodness-of-fit test and the pseudo R^2 statistic were used to assess model fit
- Standardized pearson residuals and deviance residuals were plotted against predicted values to verify the assumption of the maximum likelihood principle (minimize the sum of deviance residuals) and identify potentially influential points
- Visually identified potential outliers, high leverage points and influential points were individually removed from the model and changes in significance or interpretation of predictors was compared to the original model. No data points had to be removed from either model because of high influence or leverage.

Missing values in linear and logistic models:

There was a considerable amount of missing values due to the inclusion of partial participants who did not complete all stages of assessment during the survey. The largest number of missing values was due to lack of a venous blood sample (14 %) and lack of dietary data (19 %). Data was tested for

monotonicity but was shown to be non-monotonic and therefore standard multiple imputation methods could not be used to impute missing values. Participants with missing values did not vary in terms of basic demographic information from the household questionnaire and therefore it was assumed that values were missing at random.

Appendix B. Home-based Questionnaires



Home-based Questionnaire and Demographic ID Chart

One Home-based Questionnaire and the Demographic ID Chart were completed by the head of the household. The ID Chart was used to collect name, age, gender and birth date information of each person living in the participant's household. The relationship of each person to the principal respondent was also recorded.

The Home-based Questionnaire contains questions about languages spoken in the home, household crowding, smoking in the home, hunting practices, household expense and income and food security. Questions about food security were based on the USDA 18-item Household Food Security Survey Module[3]. Indian and Northern Affairs Canada modified the standard USDA module based on cognitive testing with Inuit interviewers to improve

[3] BICKEL, G., NORD, M., PRICE, C., HAMILTON, W. & COOK, J. 2000. Guide to measuring household food security. *In:* UNITED STATES DEPARTMENT OF AGRICULTURE. Alexandria, VA.

acceptability among Inuit. Two questions were added to the USDA module on reasons why the household was not able to buy enough food and coping strategies.

Key variable selected:

HS_Q12: Is there an active hunter in your household?

Response: 1-Yes, 2-No, 98-Do not know, 99-No response

Medicine and Supplement Use Questionnaire

A brief questionnaire about the participant's current medicine and supplement use was also administered. Participants were asked to give information on any medicines or supplements that they were taking, including frequency of use and reason for use.

Appendix C. Ship-administered questionnaires

BAFFIN INUKTITUT VERSION

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Qanuippitali? How about us, how are we?

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Inuit Health Survey 2007	ᐃᓄᐃᑦ ᐋᓐᓂᐊᖃᕐᓇᙱᑦᑐᓕᕆᓂᕐᒧᑦ
24-HOUR RECALL	ᖃᐅᔨᓴᖅᑕᐅᓂᖏᓐ 2007
Interviewer-Completed Questionnaire	ᖃᐅᔨᓴᖅᑕᐅᓂ ᐃᑲᕐᕋᐃᑦ 24 ᐊᓂᒍᖅᓯᒪᓕᖅᑐᓂ ᓂᕿᔾᔪᑎᒋᔭᐅᖅᑐᓂᒃ
	ᐊᐱᕐᓯᖅᑐᖅ- ᐱᔨᑦᑎᕐᒪᑦ ᐊᐱᕐᖁᑦ

Centre for Indigenous Peoples' Nutrition and Environment

Macdonald Campus of McGill University
21,111 Lakeshore Rd
Ste-Anne-de-Bellevue, (QC) H9X 3V9
Tel. (514) 398-7544

Completion Date: ___/___/2007
m/ d / y

Starting Time: ____/____
h / m

Time 0:00 24:00	Food description *Include junk food and drinks (even water and alcohol)*	Number of Servings	Serving description: Serving model	Serving description: Thickness	Office use only: Weight
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
11.					
12.					
13.					
14.					
15.					
16.					

24-hour dietary recall

One 24-hour dietary recall was conducted for each participant. First, participants were asked to list everything they ate and drank in the past 24 hours (from midnight to midnight). Once a list of food and drink was generated, participants were asked to give more detailed information about the foods and drinks (ex: brand name, flavour or method of cooking) and were further probed to try to remember foods and drinks that they may have forgotten such as water, juice and snacks. Three-dimensional food model kits (Santé Québec) were used to help estimate portion sizes. Some participants knew the volumes of liquid of food consumed and this information was recorded. Recipes were noted if needed. Listed food and beverages were reviewed and probes were used for any missing items.

Analysis

Dietary analysis was done using CANDAT software (Godin London Inc,. London, Ontario, Canada) and the following nutrient databases:

- 2007b Canadian Nutrient File (CNF);
- a database containing foods not available on the CNF, recipes, and information from food labels;
- data from an Indigenous food nutrient file developed by the Centre for Indigenous Peoples' Nutrition and Environment (CINE);
- a database of imputed values for nutrients missing in the CNF housed at the School of Dietetics and Human Nutrition (McGill University);

Twenty-five recalls were excluded due to incompleteness, resulting in 2097 recalls. T here were no missing values for the foods and nutrients included in the analysis.

Assessment of iron inadequacy:

Dietary iron inadequacy was assessed by the Estimated Average Requirement (EAR) probability method (pre-menopausal women) or the cut-point method (post-menopausal women and men) using the SIDE method and SIDE software. Within-subject variation estimates for iron intake were obtained from a 1998-99 CINE dietary surveys with Canadian Inuit populations. Nutrient coefficients of variation were available for Inuit <40 and ≥40 years of age. Supplement content was not included in nutrient analysis of the 24 h recalls as the majority of supplement users could not recall the brand or amount of supplement taken.

Median intakes of vitamins A, C, B_{12}, riboflavin, folate, and calcium:

Median intakes of nutrients important for erythropoiesis or relevant to iron absorption were calculated and compared to the age and sex appropriate DRI.

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Inuit Health Survey 2007	ᐃᓄᐃᑦ ᐋᓐᓂᐊᖃᕐᓇᖏᑦᑐᓕᕆᓂᕐᒧᑦ
FOOD FREQUENCY QUESTIONNAIRE	ᖃᐅᔨᓴᖅᑕᐅᓂᖏᓐᓂ 2007
PART 1: COUNTRY FOOD	ᓂᕆᔭᕆᐊᖃᕐᑐᑦ ᒥᒃᓵᓄᑦ ᐊᐱᖅᑯᑎᑦ
PART 2: MARKET FOOD	ᐃᓚᖓ 1: ᐃᓄᓕᖅᑕᐃᑦ ᓂᕿᖏᑦ
Interviewer-Completed Questionnaire	ᐃᓚᖓ 2: ᓂᕿᐅᕙᐅᔭᐃᑦ ᓂᕿᖏᑦ
	ᐊᐱᕐᓱᖅᑐᑦ-ᐱᔭᕇᖅᓯᒪᔪᑦ ᐊᐱᖅᑯᑎᑦ

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ᐃᓚᖓ 1: ᐃᓄᐃᑦ ᓂᕿᖏᑦ ᓂᕆᔭᑦ

Part 1: Country Food

[illegible] Legend: # = number, D=day, W=week, M=month, S=season	Was it eaten in the past year?			Frequency		Usual serving			Office use only
Country Food	Yes or No (circle)	Season	✓	#	D / W / M / S	# of servings	serving model	Thickness	Weight
Sea mammals									
1. Beluga meat (fresh, cooked or frozen)	Y N	IN season							
		OFF season							

Food Frequency Questionnaire

Each participant was asked to complete a semi-quantitative Food Frequency Questionnaire (FFQ). The FFQ was designed to capture consumption information about a comprehensive list of common country foods (37 items) that are available in the regions of ISR, Nunavut and Nunatsiavut based on older CINE FFQs which were updated as needed through feedback from steering committee members and hunters and trappers organizations. The FFQ was adapted to reflect the species available in each region. The participant was asked about how often a particular traditional food was eaten in the past year (in and off season). Harvest calendars from each community helped identify the time periods for the in and off season by community. An abbreviated list of market foods (5 items) with a focus on sugar drinks, fruit juices, milk and chips was also included in the FFQ. The participant was asked how often a particular market food was eaten in the past month. For all food items, the participant was asked to quantify his usual serving using the food models and provided pictures if needed.

Frequency and usual quantity of intake was assessed over the previous year during the in-season and off-season for each species. Seasons were determined according to regional wildlife harvest calendars and intakes adjusted to frequency per month (assuming 30.4 days per month).

List of traditional foods on FFQ:

Beluga meat (fresh, cooked or frozen)

Beluga, dried

Beluga muktuk with blubber (raw or boiled)

Beluga muktuk without blubber (raw or boiled)

Beluga blubber (raw or cooked)

Beluga oil or misirak

Narwhal meat (fresh, cooked or frozen)

Narwhal blubber (raw or cooked)

Narwhal muktuk with blubber (raw or boiled)

Narwhal muktuk without blubber (raw or boiled)

Ringed seal, blubber (raw or boiled)

Ringed seal, liver (raw or cooked)

Ringed seal, meat (raw, cooked or frozen)

Walrus blubber (raw, cooked, aged)

Walrus meat (raw, boiled, aged)

Caribou meat (raw, baked, cooked, boiled, aged)

Caribou meat (dried)

Caribou liver (raw, baked, cooked)

Caribou heart (raw, boiled)

Caribou tongue (raw, cooked)

Caribou stomach (walls, contents)

Caribou kidney (raw, boiled)

Polar bear, meat (raw, boiled)

Rabbit meat (raw, cooked)

Arctic char, meat (raw, boiled, frozen, dried)

Halibut

Turbot

Mussels

Clams (with shells or without)

Shrimp

Ptarmigan

Canada goose

Eider duck

Eggs of goose or eiderduck

PLANTS:

Blueberries, crowberries, cranberries, other picked berries

Sour leaves

Welk (seaweed)

Categories:

Analyses were conducted on frequency of all animal-source TF items as well as categories of marine mammals, game, fish, birds, birdeggs, seafood, and liver (*all species*).

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Qanuippitali? How about us, how are we?

ᖃᓄᐃᑉᐱᑕᓕ? ᐅᕙᒍᑦᑕ,

ᖃᓄᐃᑉᐱᑕ?

Inuit Health Survey 2007	ᐃᓄᐃᑦ ᐋᓐᓂᐊᖅᑲᓐᓂᕐᑐᓕᕆᓂᕐᒧᑦ ᖃᐅᔨᓴᖅᑕᐅᓂᖓᓂᒃ 2007
INDIVIDUAL QUESTIONNAIRE	ᐃᓄᒃᑯᑦ ᐊᑕᐅᓯᕐᑯᑦ ᐊᐱᖅᑯᑏᑦ
Interviewer-Completed Questionnaire	ᐊᐱᖅᓱᖅᑐᖅ-ᐱᔾᔨᓇᖅᓯᒪᔪᑦ ᐊᐱᖅᑯᑏᑦ

Centre for Indigenous Peoples' Nutrition and Environment

Macdonald Campus of McGill University

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Individual Questionnaire

The Individual Questionnaire focused on dental health history, medical health history which included diabetes, high blood pressure and cholesterol, reproductive health history (women only), sun exposure, smoking, alcohol consumption, and socio-demographic information which included education, income and current employment status.

Key variables selected:

IND_Q43: At the present time, do you smoke cigarettes?

Response: 1-Yes, 2-No, 98-Do not know, 99-No response

IND_Q44: On average, how many cigarettes do you smoke each day

Response: _____(#/day) ➔ Go to Q46

IND_Q45: Have you ever smoked?

Response: 1-Yes, 2-No, 99-No response

IND_Q49: What is your marital status?

Response:

1-Single,
2-Married/common law partner
3-Separated, still legally married
4-Divorced
5-Widowed
98-Do not know
99-No response

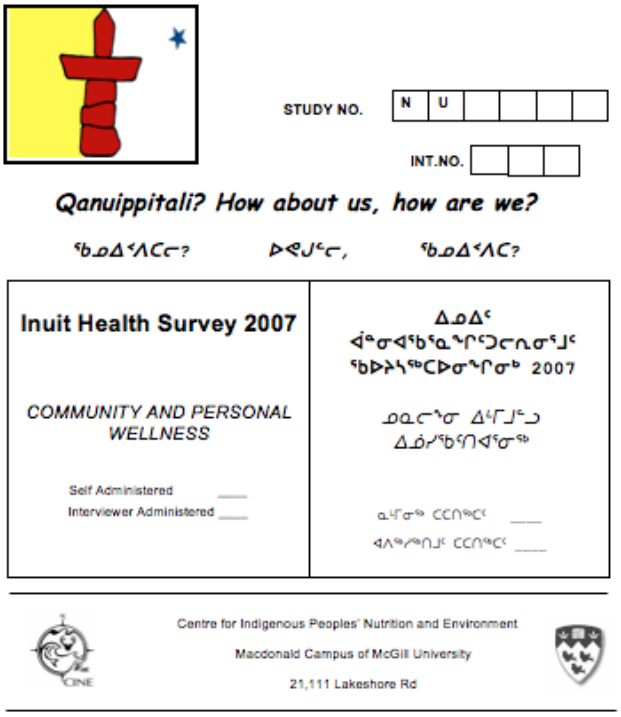
IND_Q50: What is the highest level of schooling you have completed (even if you are still in school)?

Response:
1-No formal schooling
2-Some years of elementary school
3-Elementary school completed
4-Some years of secondary school
5-Secondary school completed
6-Partial training in community college, a trade school or a private commercial college, a Nunavut Sivuniksavut program, a technical institute, a nursing school, or a normal school (teaching school)
7-Diploma or certificate from a community college, a trade school or a private commercial college, a technical institute, a Nunavut Sivuniksavut program, a nursing school, or a normal school (teaching school)
8-Some university (not completed)
9-University degrees (completed), Certificate, Bachelor, Masters, PhD
98-Do not know
99-No response

IND_Q53: What is your best estimate of your total personal income from all taxed and untaxed sources, in the past 12 months (before taxes and other deductions)?

Response:

1-Less than $20,000
2-$20,000 to less than $40,000
3-$40,000 to less than $60,000
4-$60,000 or more
98-Do not know
99-No response



Community and Wellness Questionnaire

Because of sensitive and very personal aspects of the Community and Wellness Questionnaire, participants were offered to complete this questionnaire by themselves if they felt more comfortable than with an interviewer. In that case, the participant completed the questionnaire alone, folded it and placed it in a sealed envelope identified with the participant ID. The envelope was then added to the participant's file with the other questionnaires. Questions asked were about land activities, life in the community (ex: organized activities, violence), quality of sleep, anxiety and other feelings, suicide, gambling, drug use, violence and sexual abuse in the participant's own life.

Key variables selected:

CF_Q52. In the last 12 months, did you drink alcohol?

1-Yes →Continue
2-No →Go to Q60
3-Have never drank →Go to Q60
98-Do not know →Go to Q60
98-No response →Go to Q60

When we use the word 'drink' it means:
-1 bottle or can of beer, OR
-1 glass of wine or a wine cooler, OR
-1 shooter, OR
-1 drink or cocktail with 1 ½ ounces of liquor.

CF_Q57: In the last 12 months, how many times have you blacked out or passed out from drinking?

1-Number of times, specify _____
2-Not in the last 12 months
3-Never
98-Do not know
99-No response

Appendix D. Study population descriptive statistics

	Men (n=994)		Women (n=1550)	
LIFESTYLE & DEMOGRAPHIC	**Median**	**IQR**	**Median**	**IQR**
Continuous variables				
Age (years)	42	31-53	41	30-51
BMI (kg/m2)	26.1	23.0-30.1	28.4	23.7-33.7
Waist circumference (cm)	89	80-103	93	80-106
% Adiposity	22.1	16.0-29.1	36.8	27.6-42.8
C-reactive protein (mg/L)	1.2	0.5-2.9	1.3	0.4-3.4
Blood lead (μg/L)	26	7-49	15	4-35
Categorical variables	%		%	
No hunter in home	19.9		34.4	
Smoking	67.8		70.9	
Single marital status	31.4		39.2	
Household crowding (>1 person/room)	33.5		30.0	
Personal Income <$20 000/year	49.2		56.4	
≤Elementary school education	21.4		22.6	
Food insecure (moderate/severe)	66.2		63.2	
H pylori seropositive	75.5		71.9	
Toxocara seropositive	1.6		1.0	
Trichinella seropositive	14.4		11.9	
Toxoplasma seropositive	26.5		21.2	
Echinococcus seropositive	4.6		4.7	
Daily ASA use	4.9		5.0	

Self-reported hypertension	22.8		28.2	
Self-reported diabetes	6.4		6.7	
Self-reported high cholesterol	10.0		12.4	
DIETARY				
Continuous variables				
Hemoglobin (g/L)	142	131-152	128	119-136
Serum ferritin (µg/L)	55.9	30.4-95.6	24.5	14-51
Soluble serum transferrin receptor (mg/L)	1.27	1.11-1.46	1.33	1.12-1.63
sTfR:log(ferritin)	0.31	0.25-0.38	0.41	0.31-0.56
EPA in RBC (% of total fatty acids)	1.15	0.66-2.10	1.04	0.57-2.06
DHA in RBC (% of total fatty acids)	2.40	1.36-3.61	2.44	1.21-3.69
Plasma 25(OH)D (nmol/L)	55.2	34.7-79.4	51.7	30.8-78.6
Categorical variables				
Fe-containing supplement	1.8 %		4.4 %	

Appendix E

Population mean and median nutrient intakes from the 24 hour recall in comparison to the Dietary Reference Intakes and adequacy of intakes.

Men	**EAR mg iron/d**	**% below EAR, adjusted intake from SIDE (n/N)***
19-30 years	6	2.9 % (5/175)
31-50 years	6	2.6 % (10/388)
51-70 years	6	1.1 % (2/189)
>70 years	6	2.8 % (1/36)
		2.3 % (18/788)

*Analyzed by the EAR cut-point method

All men (n=788)

Nutrient	EAR	RDA	mean	SD	median
Iron (mg/d)	6	8	24.0	29.7	16.7
Adjusted iron (mg/d)	6	8	25.6	20.0	20.3
Heme iron (mg/d)	-	-	15.0	28.4	6.3
Vitamin C (mg/d)	75	90	128	175	61.6
Vitamin A (µg RAE/d)	625	900	768	1132	416
Folate (µg DFE/d)	320	400	381	298	318
Vitamin B_{12} (µg/d)	2.0	2.4	10.7	14.5	4.9
Riboflavin (mg/d)	1.1	1.3	3.1	2.3	2.5

Men 19-50 y (n=563)

Nutrient	EAR	RDA	mean	SD	median
Iron (mg/d)	6	8	23.1	25.7	16.6
Adjusted iron (mg/d)	6	8	24.6	18.4	20.3
Heme iron (mg/d)	-	-	13.2	24.1	4.8
Vitamin C (mg/d)	75	90	147	190	77.1
Vitamin A (µg RAE/d)	625	900	818	1194	456
Folate (µg DFE/d)	320	400	404	321	333
Vitamin B_{12} (µg/d)	2.0	2.4	10.5	14.4	4.4
Riboflavin (mg/d)	1.1	1.3	3.2	2.4	2.6
Calcium (mg/d)	800	1000	609	480	468

Men >50 y (n=225)

Nutrient	EAR	RDA	mean	SD	median
Iron (mg/d)	6	8	26.2	37.8	16.8
Adjusted iron (mg/d)	6	8	27.9	23.6	20.9
Heme iron (mg/d)	-	-	19.4	36.7	9.0
Vitamin C (mg/d)	75	90	80.3	115	31.1
Vitamin A (µg RAE/d)	625	900	642	951	359
Folate (µg DFE/d)	320	400	322	219	286
Vitamin B_{12} (µg/d)	2.0	2.4	11.4	14.7	6.2
Riboflavin (mg/d)	1.1	1.3	2.88	2.10	2.25
Calcium (mg/d)	1000	1200	430	328	361

Women	EAR mg iron/d	Probability below EAR (adjusted intake from SIDE)*
19-30 years	8.1	**15.7 %**
31-50 years	8.1	**17.3 %**

*Analyzed by the probability method

Women	EAR mg iron/d	% below EAR, adjusted intake from SIDE (n/N)*
51-70 years	5	**0.4** % (1/280)
>70 years	5	**2.2** % (1/46)
		0.6 % (2/326)

*Analyzed by the EAR cut-point method

All women (n=1221)

Nutrient	EAR	RDA	mean	SD	median
Heme iron	-	-	9.8	19.5	4.0
Vitamin C (mg/d)	60	75	122	171	58.3
Vitamin A (µg RAE/d)	500	700	619	889	355
Folate (µg DFE/d)	320	400	324	217	275
Vitamin B_{12} (µg/d)	2.0	2.4	6.8	10.4	3.6
Riboflavin (mg/d)	0.9	1.1	2.3	1.5	1.9

Women 19-50 y (n=895)

Nutrient	EAR	RDA	mean	SD	median
Iron (mg/d)	8.1	18	16.9	17.2	12.4
Adjusted iron (mg/d)	8.1	18	18.0	12.2	15.0
Heme iron (mg/d)	-	-	8.5	16.5	3.1
Vitamin C (mg/d)	60	75	134	186	64.0
Vitamin A (µg RAE/d)	500	700	617	893	340
Folate (µg DFE/d)	320	400	333	223	279
Vitamin B_{12} (µg/d)	2.0	2.4	6.5	10.9	3.2
Riboflavin (mg/d)	0.9	1.1	2.3	1.5	1.9
Calcium (mg/d)	800	1000	494	357	409

Women >50 y (n=326)

Nutrient	EAR	RDA	mean	SD	median
Iron (mg/d)	5.0	8	20.3	26.8	14.0
Adjusted iron (mg/d)	5.0	8	21.3	15.9	17.2
Heme iron (mg/d)	-	-	13.7	25.9	6.9
Vitamin C (mg/d)	60	75	88.8	113	43.1
Vitamin A (µg RAE/d)	500	700	623	880	383
Folate (µg DFE/d)	320	400	300	196	266
Vitamin B_{12} (µg/d)	2.0	2.4	7.7	9.0	4.6
Riboflavin (mg/d)	0.9	1.1	2.3	1.5	1.9
Calcium (mg/d)	1000	1200	447	360	351

Appendix F. Sources of dietary iron

Sources of dietary iron on the day prior to the survey, according to age and sex.

Men 19-50 y		**Men ≥51 y**	
	% iron		**% iron**
Traditional meats	28.4	Traditional meats	48.0
Market food meats	12.4	Market food meats	11.2
Other	12.7	Breads	9.6
Mixed meats	9.3	Bannock	5.9
Breads	8.9	Cereals	5.4
Cereals	4.0	Other	3.9
Bannock	3.1	Vegetables	3.4
Vegetables	4.4	Meat alternatives	2.8
Pasta	4.4	Mixed meat dishes	2.8
Meat Alternatives	2.8	Baked products	2.2
Baked products	3.4	Pasta	1.4
Mixed grain dishes	2.6	Fruit	1.4
Fruit	2.1	Milk products	1.4
Milk products	2.0	Traditional fats	0.9
Soup (non-dairy)	1.6	Mixed grain dishes	0.7
Traditional fats	1.2	Soup (non-dairy)	0.6
Traditional plants	0.6	Traditional plants	0.1

Women 19-50 y		**Women ≥51 y**	
	% iron		**% iron**
Traditional meats	22.5	Traditional meats	43.6
Baked products	13.1	Baked products	12.4
Other	14.1	Bannock	8.4
Market food meats	13.0	Breads	7.5
Mixed meat dishes	9.3	Market food meats	6.1
Breads	9.5	Other	5.2
Cereals	5.0	Cereals	5.1
Vegetables	5.0	Vegetables	2.6
Pasta	3.5	Meat alternatives	2.1
Meat Alternatives	3.4	Mixed meat dishes	2.1
Fruit	2.8	Fruit	1.5
Bannock	2.4	Milk products	1.2
Mixed grain dishes	2.7	Pasta	1.2
Milk products	1.6	Mixed grain dishes	1.0
Soup (non-dairy)	1.4	Soup (non-dairy)	0.9
Traditional fats	0.4	Traditional fats	0.8
Traditional plants	0.1	Traditional plants	0.3

Appendix G. Subset: iron deficiency models

Comparison of weighted prevalence of functional iron deficiency by two different models in the population subset (n=941) with measurements for serum ferritin and serum soluble transferrin receptor.

MEN

Age, *y*	Anemia[1]		Depleted iron stores[2]		**MODEL 1** Iron deficiency anemia[2]		**MODEL 2** R:F body iron[3]	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
18-50	241	8.5 (5.2-13.7)	249	7.3 (4.3-12.2)	241	2.8 (1.3-6.2)	249	2.1 (0.9-5.2)
≥51	110	29.6 (20.4-40.8)	117	4.1 (1.2-12.7)	110	1.1 (0.3-4.6)	117	0.6 (<0.1-4.4)
Total	**351**	**15.7 (11.7-20.9)**	**366**	**6.2 (3.8-9.9)**	**351**	**2.2 (1.1-4.5)**	**366**	**1.6 (0.7-3.6)**

[1] Where anemia = Hb<130 g/L.

[2] Where depleted iron stores = serum ferritin <15 µg/L or ferritin 15-50 µg/L + CRP>10 mg/L; iron deficiency anemia = depleted iron stores + anemia.

[3]R:F body iron = <0 mg/kg (calculated as: -[log(sTfR/SF ratio) – 2.8229]/0.1207).

WOMEN

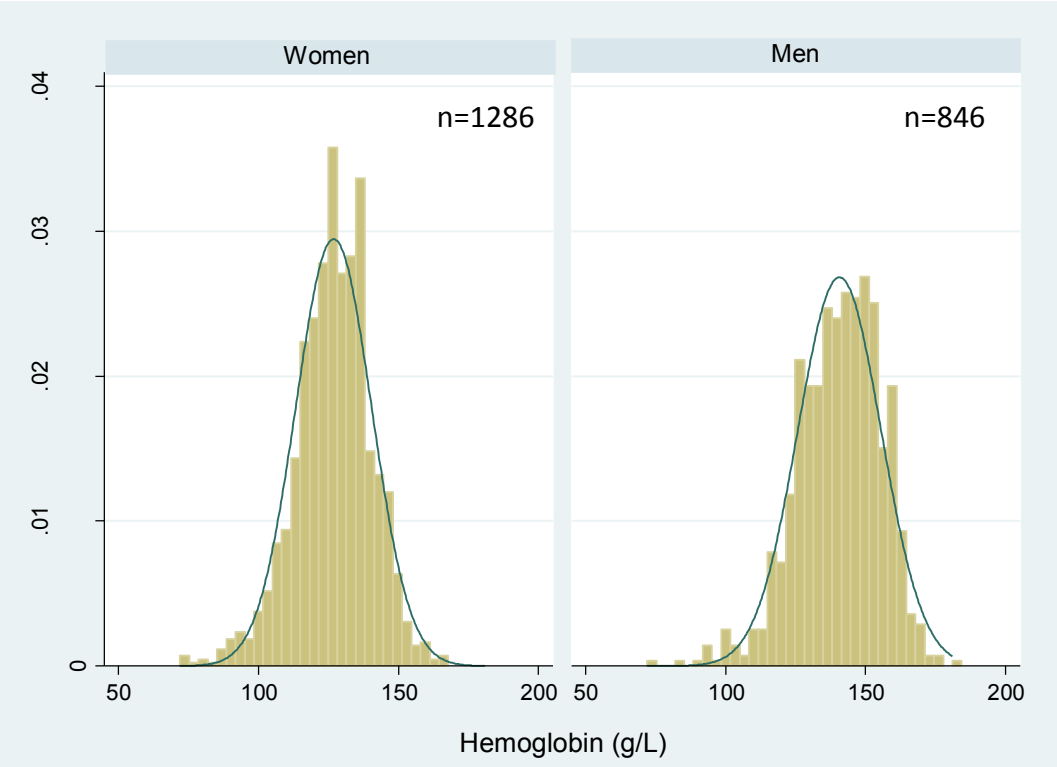
Age, *y*	Anemia[1]		Depleted iron stores[2]		**MODEL 1** Iron deficiency anemia[2]		**MODEL 2** R:F body iron[3]	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
18-50	392	20.9 (16.9-25.5)	413	37.4 (32.4-42.7)	392	12.4 (9.4-16.2)	413	8.6 (6.0-12.3)
≥51	151	20.3 (14.1-28.4)	162	6.7 (3.5-12.3)	151	2.9 (1.1-7.6)	162	0
Total	**543**	**20.7 (17.3-24.6)**	**575**	**28.3 (24.3-32.7)**	**543**	**9.7 (7.4-12.6)**	**575**	**6.1 (4.2-8.7)**

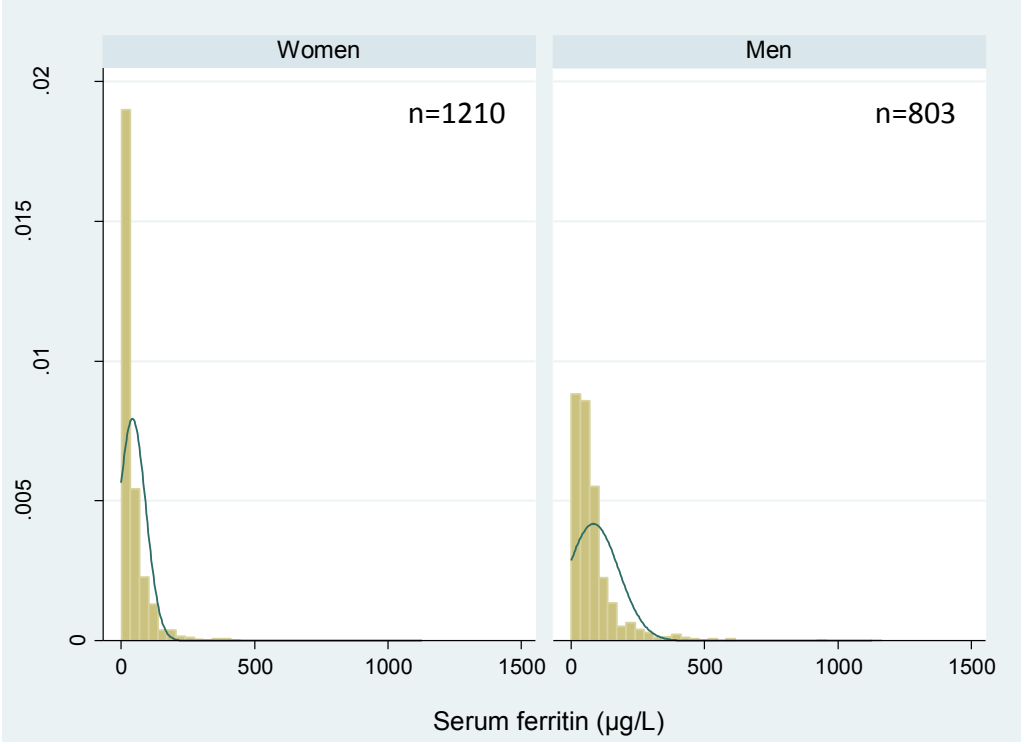
[1] Where anemia = Hb<120 g/L.

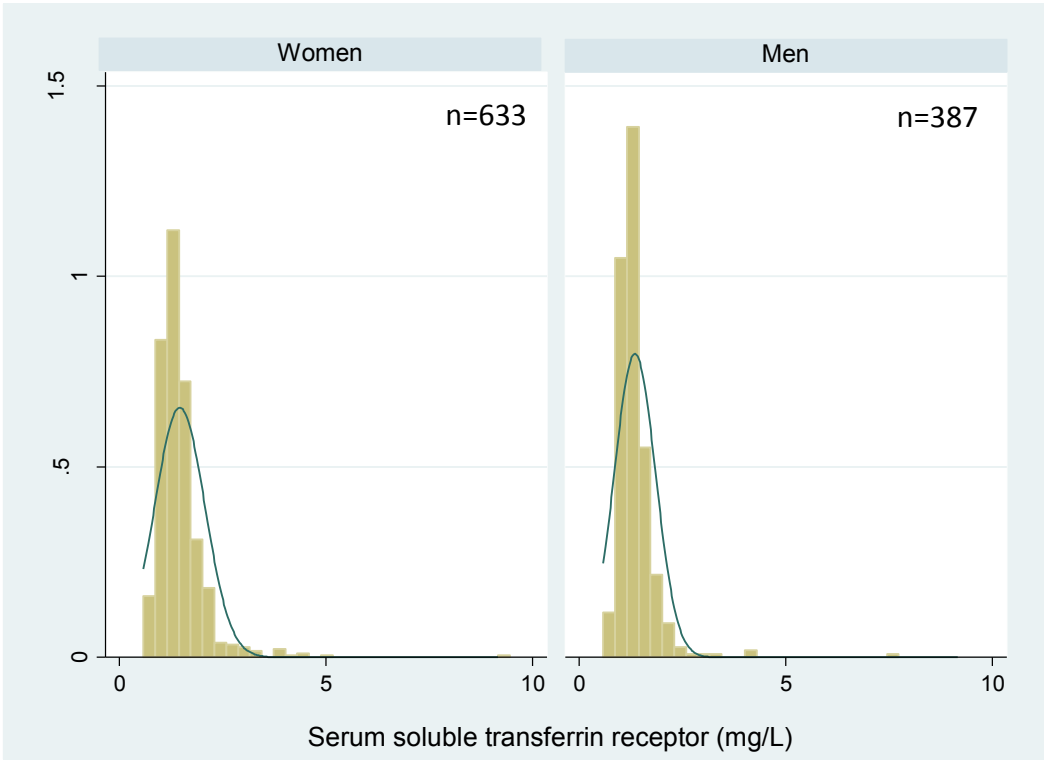
[2] Where depleted iron stores = serum ferritin <15 µg/L or ferritin 15-50 µg/L + CRP>10 mg/L; iron deficiency anemia = depleted iron stores + anemia.

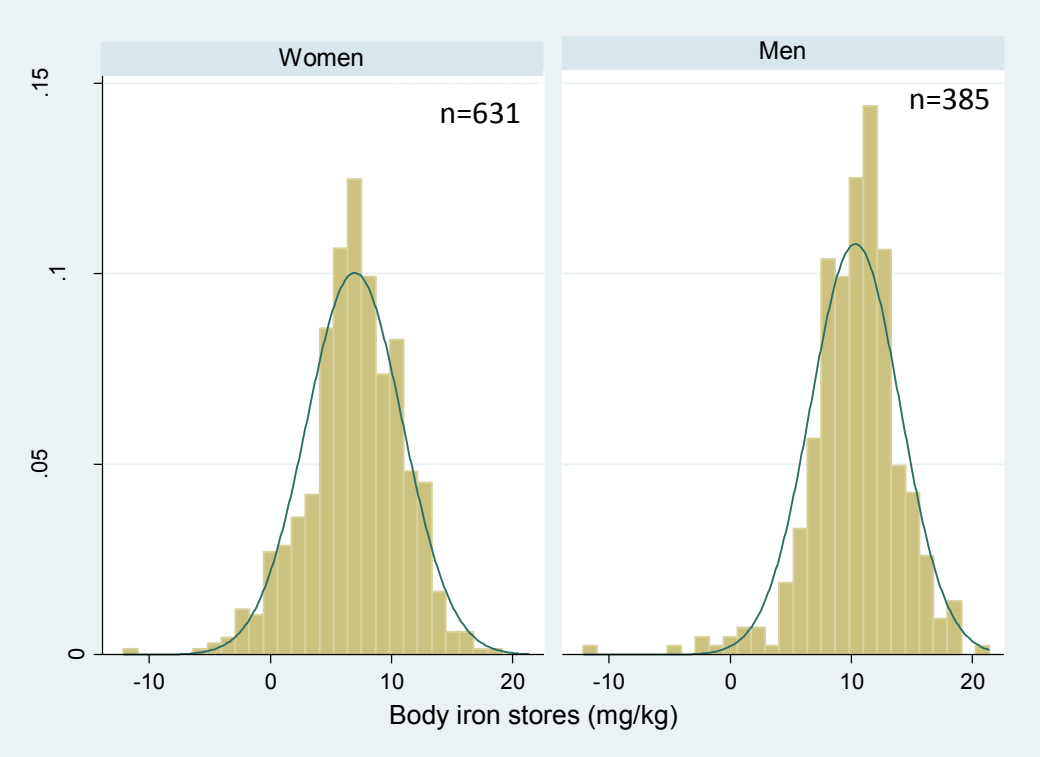
[3]R:F body iron = <0 mg/kg (calculated as: -[log(sTfR/SF ratio) – 2.8229]/0.1207).

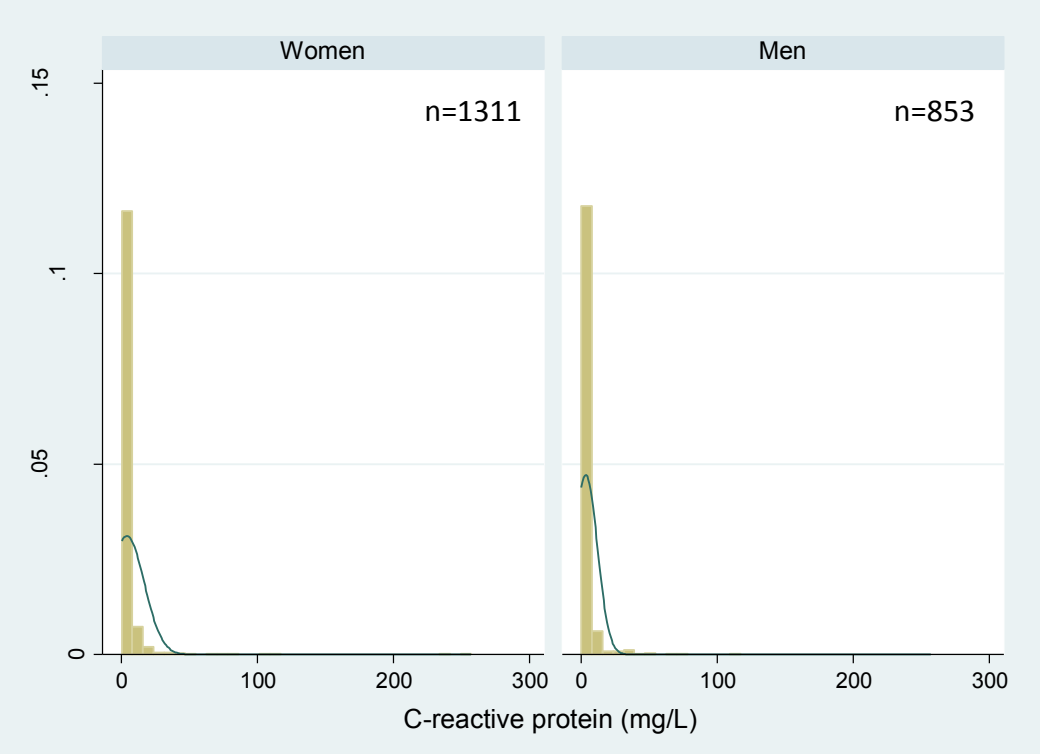
Appendix H. Distributions of iron status biomarkers and dietary intakes

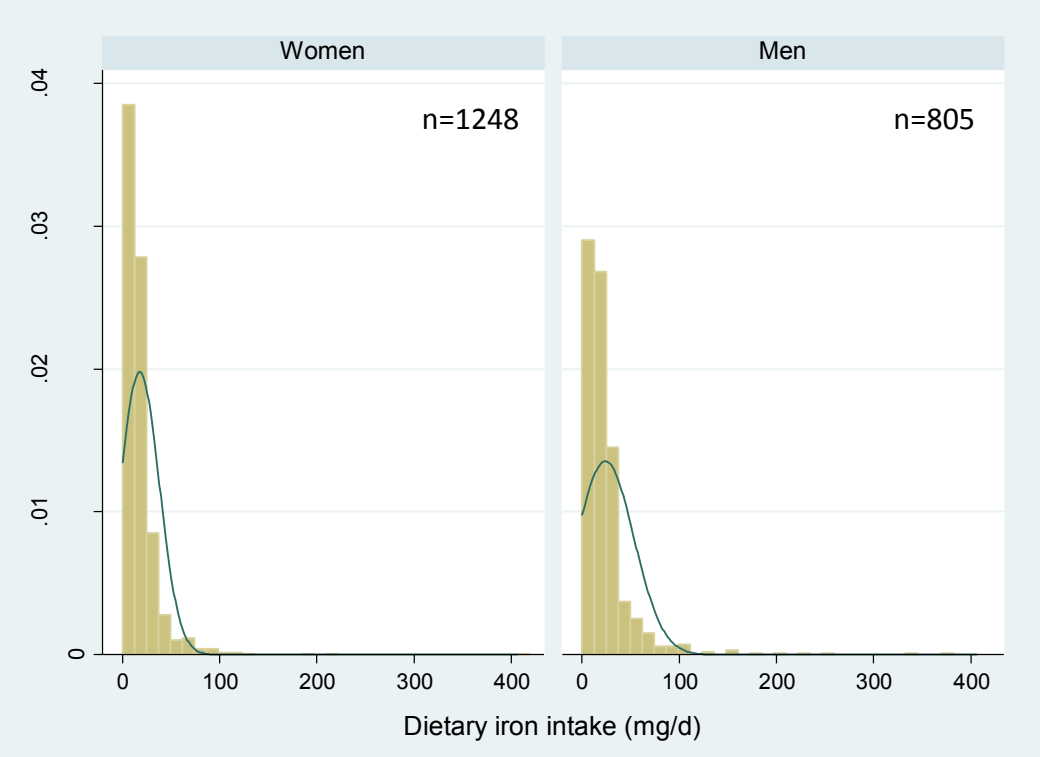


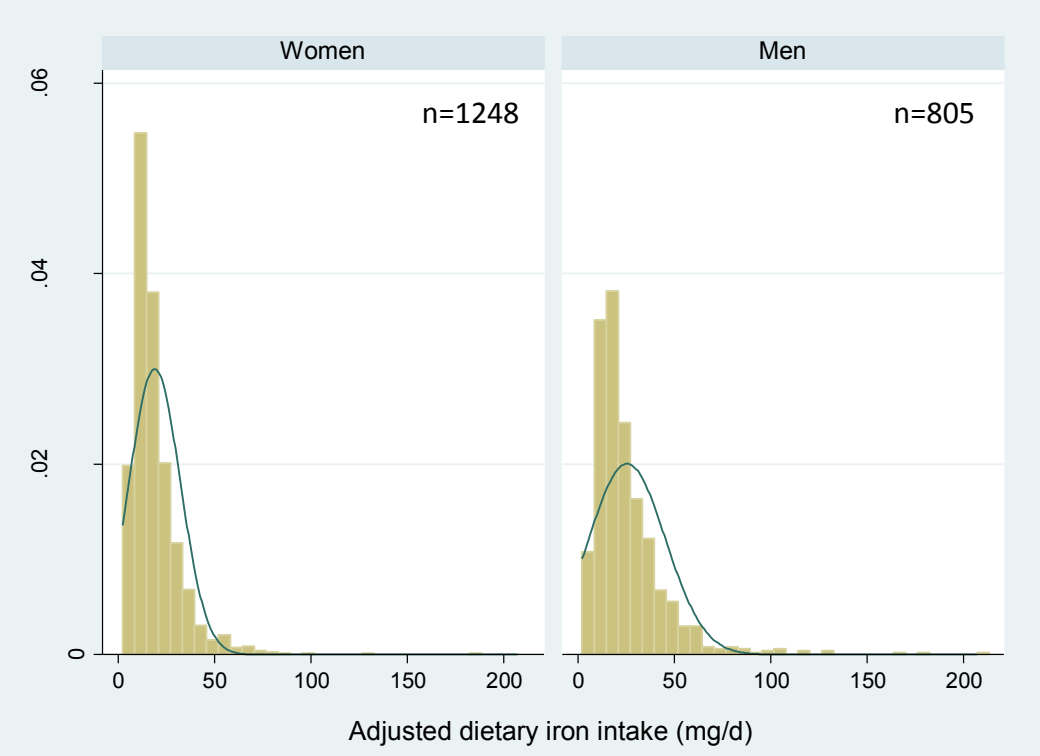

Women
Men
n=1210
n=803
.02
.015
.01
.005
0
0
500
1000
1500
Serum ferritin (µg/L)


Women
Men
n=633
n=387
1.5
1
.5
0
0
5
10
Serum soluble transferrin receptor (mg/L)


Women
Men
n=631
n=385
.15
.1
.05
0
-10
0
10
20
Body iron stores (mg/kg)


Women
Men
n=1311
n=853
.15
.1
.05
0
0
100
200
300
C-reactive protein (mg/L)


Women
Men
n=1248
n=805
0
.01
.02
.03
.04
0
100
200
300
400
Dietary iron intake (mg/d)


Women
Men
n=1248
n=805
0
.02
.04
.06
0
50
100
150
200
Adjusted dietary iron intake (mg/d)