Iron deficiency and iron deficiency anemia among preschool aged Inuit children living in Nunavut

Angela Pacey

School of Dietetics and Human Nutrition, McGill University, Montréal

October 2009

A thesis submitted to McGill University in partial fulfilment of the requirements of the degree of Master of Science

© Angela Pacey 2009
ABSTRACT

Limited information is available about iron deficiency and iron deficiency anemia (IDA) among preschool-aged Inuit children. A cross-sectional survey was conducted with 388 Inuit children, aged 3 to 5 years, from 16 Nunavut communities. Interviews were conducted on dietary and household characteristics. Height, weight and biomarkers of iron status and *Helicobacter pylori* (*H. pylori*) exposure were measured. The prevalence of iron deficiency and IDA was calculated and risk factors were examined. The prevalence of iron deficiency was 19.2%, of IDA was 4.5% and of anemia was 20.3%. Only 0.3% of children had usual iron intakes below the Estimated Average Requirement. *H. pylori* exposure, food insecurity and household crowding were not associated with iron deficiency or IDA. Three to four year olds were more likely to be iron deficient than 5 year olds. Boys were more likely to be iron deficient than girls.
RÉSUMÉ

Peu d’informations sont disponibles sur la carence en fer et l’anémie due à une carence en fer (ACF) chez les Inuits d’âge pré-scolaire. Un sondage transversales a été conduit avec 388 enfants Inuit âgés de 3 à 5 ans, dans 16 communautés du Nunavut. Des interviewers ont conduit des entrevues alimentaires et des questionnaires à propos des caractéristiques des ménages. La taille, le poids, ainsi que des marqueurs biologiques du niveau de fer et de l’exposition à *Helicobacter pylori* ont été mesurés. La prévalence de la carence en fer et de l’ACF a été calculée et les facteurs de risque ont été examinées. La prévalence de la carence en fer a été 19.2%, de l’ACF a été 4.5% et de l’anémie a été 20.3%. Seulement 0.3% des enfants avaient des apports habituels en fer sous le besoin moyen estimatif. L’exposition à *H. pylori*, l’insécurité alimentaire et le nombre d’habitants par ménage n’étaient pas associés à une carence en fer ou à de l’ACF. La carence en fer était plus élevée chez les enfants âgés de 3 à 4 ans que chez ceux de 5 ans. La carence en fer était aussi plus élevée chez les garçons que chez les filles.
STATEMENT OF SUPPORT

Funding for this study was provided through Government of Canada International Polar Year, Government of Nunavut Department of Health and Social Services, Canadian Institutes for Health Research. Ms. Pacey was financially supported by a stipend provided by Dr. Grace Egeland and through a grant from the Nasivvik grant.
ACKNOWLEDGEMENTS

I am very grateful to my supervisor, Dr. Grace Egeland, for exposing me to so many fascinating experiences, for her on-going input, support and patience. I feel lucky to have had such insightful committee members, Dr. Hope Weiler and Dr. Katherine Gray-Donald, who offered encouragement and their expertise throughout. Special thanks to Dr. Nelofar Sheikh for her dedication to data management and to Louise Johnson-Down who coordinated the dietary data entry and performed the nutrient intake analyses. Special thanks also to Donna Leggee, Sherry Agellon and Jennifer Jamieson for their assistance and teachings in laboratory analyses of iron status. My Master’s of Nutrition training was truly a collective effort by all of the above-mentioned mentors.

We would like to acknowledge the work of the 2007 and 2008 research teams including Nancy Faraj, Christine Ekidlak, Laureen Pameolik, Kathy Morgan, Lauren Goodman and Jessy El Hayak. We whole-heartedly appreciate the assistance provided to us by the communities, hamlet offices, the schools, the health centre staff and our steering committee. Finally, thank you to the participating children and their families.
CONTRIBUTION OF AUTHORS

Ms. Pacey assisted considerably in data collection, research team training, data entry, laboratory analyses and thesis and manuscript writing. Dr. Grace Egeland planned and guided the research methods and statistical analyses and reviewed and gave feedback on this thesis and manuscript.
TABLE OF CONTENTS

ABSTRACT.......................................................................................................................... II

RÉSUMÉ ............................................................................................................................... III

STATEMENT OF SUPPORT ............................................................................................... IV

ACKNOWLEDGEMENTS ........................................................................................................ V

CONTRIBUTION OF AUTHORS ....................................................................................... VI

LIST OF TABLES .................................................................................................................. IX

LIST OF FIGURES ................................................................................................................ X

LIST OF APPENDICES ........................................................................................................ XI

LIST OF ABBREVIATIONS .................................................................................................. XII

1 STUDY BACKGROUND .................................................................................................. 1
  1.1. ABORIGINAL PEOPLES AND INUIT IN CANADA .................................................... 1
  1.2. HEALTH CARE DELIVERY IN NUNAVUT .......................................................... 2
  1.3. INUIT CHILD HEALTH SURVEY .......................................................................... 2

2 LITERATURE REVIEW ................................................................................................ 3
  2.1. IRON METABOLISM, REQUIREMENTS AND DEFICIENCY .................................. 3
      2.1.1. Iron metabolism .................................................................................................. 3
      2.1.2. Iron requirements and measuring dietary intake .............................................. 5
      2.1.3. Health outcomes of iron deficiency .................................................................... 9
  2.2. POPULATION-BASED RESEARCH IN IRON DEFICIENCY ................................... 11
      2.2.1. Measuring iron status ....................................................................................... 11
      2.2.2. Iron deficiency among Inuit children: review of prevalence estimates .......... 14
  2.3. ETIOLOGY OF IRON DEFICIENCY AND ANEMIA AMONG CHILDREN .............. 17
      2.3.1. Overview of causes of iron deficiency and IDA in children ......................... 17
      2.3.2. Dietary factors related to iron deficiency ......................................................... 17
      2.3.3. Helicobacter pylori .......................................................................................... 20
      2.3.4. Underlying risk factors for iron deficiency ..................................................... 30

3 RATIONALE .................................................................................................................. 38
  3.1. OBJECTIVES ........................................................................................................... 39
  3.2. HYPOTHESES .......................................................................................................... 39

4 METHODS .................................................................................................................... 40
  4.1. PARTICIPATORY RESEARCH PROCESS ............................................................ 40
  4.2. SAMPLE SIZE CALCULATION ............................................................................. 40
  4.3. STAFFING AND TIMEFRAME FOR DATA COLLECTION .................................. 41
  4.4. RECRUITMENT ........................................................................................................ 42
  4.5. ETHICS APPROVAL ............................................................................................... 43
  4.6. INTERVIEWS .............................................................................................................. 43
      4.6.1. Interview training ............................................................................................. 43
      4.6.2. Inuktitut translations ....................................................................................... 44
      4.6.3. Written informed consent .............................................................................. 45
      4.6.4. Study numbers and confidentiality .................................................................. 45
      4.6.5. Participant compensation ................................................................................ 45
      4.6.6. Demographic information and household characteristics ......................... 46
      4.6.7. 24-hour dietary recall ...................................................................................... 46
      4.6.8. Food frequency questionnaire ....................................................................... 46
      4.6.9. Quality control for interview component ...................................................... 47

vii
4.7. **CLINICAL DATA COLLECTION** .............................................................................................................. 47
  4.7.1. Anthropometry ................................................................................................................................. 48
  4.7.2. Blood sample collection .................................................................................................................... 48
  4.7.3. HemoCue™ .................................................................................................................................. 49
4.8. **PLASMA SAMPLE PREPARATION** .................................................................................................. 50
4.9. **LABORATORY ANALYSES** .............................................................................................................. 51
  4.9.1. Measurement of C-reactive protein .................................................................................................. 51
  4.9.2. Measurement of *Helicobacter pylori* exposure status .................................................................. 52
  4.9.3. Measurement of ferritin ................................................................................................................. 53
4.10. **DATA MANAGEMENT** .................................................................................................................. 54
4.11. **STATISTICAL ANALYSES** ........................................................................................................... 56

5 **MANUSCRIPT** .................................................................................................................................... 61

6 **DISCUSSION** ...................................................................................................................................... 83

7 **REFERENCES** ..................................................................................................................................... 89

8 **APPENDICES** ..................................................................................................................................... 103
LIST OF TABLES

Table 2-1. Institute of Medicine (2001) reported absolute requirements and Dietary Reference Intakes (DRIs) for iron in male and female infants, children and adults. ....32

Table 2-2. Summary of prevalence studies in anemia and iron deficiency for Inuit and northern First Nations children, and comparison groups. ..............................33

Table 2-3. Estimated iron content of some traditional Inuit foods and market foods. ....34

Table 2-4. Summary of reported prevalence rates of Helicobacter pylori infection in northern or Arctic regions, and in comparison groups. ...............................................35

Table 4-1. Nunavut communities, location and population sizes. ...................................58

Table 4-2. Inuit Child Health Survey 2007-2008 data collection schedule. ..................59

Table 4-3. Descriptions of measured outcome and exposure variables. .......................60

Table 5-1. Population and household characteristics. ..........................................................76

Table 5-2. Summary of serum ferritin and hemoglobin concentrations for Nunavut and by region. .................................................................................................................77

Table 5-3. Prevalence of iron deficiency, anemia, iron deficiency anemia and Helicobacter pylori infection among participating children. ....................................78

Table 5-4. Mean, median and percentage of individuals with intakes below the EAR, not including supplements, for energy, vitamin C and iron in Inuit children, ages 3 to 5 years (n = 374). .................................................................79

Table 5-5. Frequency of consumption of traditional and market food sources of iron among Inuit children, ages 3 to 5 years. .................................................................81

Table 5-6. Bivariate analyses of explanatory factors for iron deficiency and iron deficiency anemia using two different ferritin cut-off values to define iron deficiency. .....................................................................................82
LIST OF FIGURES

Figure 2-1. Map of Inuit regions and communities in Canada. .................................36

Figure 2-2. Age-sex pyramid of the predominantly Inuit population in Nunavut and the total population of Canada, 2006 [6]. ........................................................................................................37

Figure 5-1. Adjusted iron intake distribution for Inuit children, aged 3 to 5 years, in Nunavut. The Estimated Average Requirement (EAR) for children aged 3 years is 3.0 mg and for children 4 to 5 years is 4.1 mg..........................................................80
LIST OF APPENDICES

Appendix A. Quality control material for dietary questionnaires............................... 104

Appendix B: Clinical protocols and quality control procedures for clinical equipment. 107
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGP</td>
<td>alpha-acid glycoprotein</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate Intake</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DMT1</td>
<td>Divalent metal transporter 1</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Reference Intakes</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbant assay</td>
</tr>
<tr>
<td>FPN1</td>
<td>Ferroportin 1</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food frequency questionnaire</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High sensitivity C-reactive protein</td>
</tr>
<tr>
<td>ICHS</td>
<td>Inuit Child Health Survey</td>
</tr>
<tr>
<td>IDA</td>
<td>Iron deficiency anemia</td>
</tr>
<tr>
<td>IDE</td>
<td>Iron deficiency erythropoiesis</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>IREG1</td>
<td>Iron regulated transporter 1</td>
</tr>
<tr>
<td>KHAS</td>
<td>Keewatin Health Assessment Survey</td>
</tr>
<tr>
<td>NCNS</td>
<td>Nutrition Canada National Survey</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PR</td>
<td>Prevalence ratio</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Dietary Allowance</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SF</td>
<td>Serum ferritin</td>
</tr>
<tr>
<td>sTfR</td>
<td>Serum transferrin receptor</td>
</tr>
<tr>
<td>TIBC</td>
<td>Total iron binding capacity</td>
</tr>
<tr>
<td>TS</td>
<td>Transferrin saturation</td>
</tr>
<tr>
<td>UBT</td>
<td>Urea breath test</td>
</tr>
<tr>
<td>UL</td>
<td>Tolerable Upper Intake Limit</td>
</tr>
<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
1 STUDY BACKGROUND

1.1. ABORIGINAL PEOPLES AND INUIT IN CANADA

There is no internationally recognized definition of Indigenous Peoples but it is generally agreed that they are a group of people who are native to the land [1]. It is generally accepted that around 15,000 years before present, the Indigenous group known today as the Inuit, migrated across the Bering Strait, settling in the circumpolar regions of the Russian Chukotka peninsula, central and south-western Alaska, northern Canada and Greenland [2]. Inuit, with First Nations and Dene/Métis, make up three distinct Indigenous, or Aboriginal groups in Canada [3, 4]. In Canada, Inuit predominantly live in four distinct regions that make up Canada’s northern-most lands (Figure 2-1). These are Nunavik in northern Quebec, Inuvialuit Settlement Region in the Northwest Territories, Nunatsiavut in Labrador, and finally Nunavut, the area where the following study took place.

Political borders once defined Nunavut as part of the Northwest Territories but this changed with the 1993 Nunavut Act when it became Canada’s third distinct territory. Situated in north-central Canada, Nunavut’s borders range from 56° N to 76° N in latitude and from 64° W to 115° W in longitude. Within Nunavut there are three regions, Kivalliq, Baffin and Kitikmeot and 25 settled communities. All of these communities are Hamlets except for Iqaluit, which is the only city in the territory, the capital of Nunavut and the most populated area (Figure 2-1).

Eighty-four percent of Nunavut’s total population are Inuit. The remaining population is of other Aboriginal identity (1%) or non-Aboriginal (15%) [5]. The total population in Nunavut is 51% male [6]. Among all children under the age 5 living in the territory, 92% are Inuit [5]. Approximately 11% of the total Nunavut population is under the age of 5, compared to approximately 5% overall in Canada [6], (Figure 2-2).
1.2. **HEALTH CARE DELIVERY IN NUNAVUT**

Within each Nunavut hamlet there is a health centre staffed with at least two full-time nurses and with on-call after hours service. The only hospitals in Nunavut are found in the capital city, Iqaluit. Most hamlet health centres are equipped to provide neo-natal care, minor surgery, x-ray, in-patient care and commonly needed prescription medicines and vaccines. Tele-health technology is available and allows for audio-visual long-distance conferencing between health centres in Nunavut and in major Canadian cities. Physicians and dentists are available full-time in some communities while others are visited for a few weeks at a time. If a community member requires medical attention that cannot be provided by the local health centre they are flown to Iqaluit or a major Canadian city for care. Regarding child health specifically, health centres routinely provide vaccinations and preschool screenings. Preschool screenings include vision and hemoglobin testing and growth monitoring. Child and infant vitamin and mineral supplements such as iron, vitamin D, fluoride and multivitamin supplements are available through health centres. The costs of these and most prescription medications are covered by government-provided public health insurance.

1.3. **INUIT CHILD HEALTH SURVEY**

The project “Iron deficiency and iron deficiency anemia among preschool aged Inuit children living in Nunavut” is one component of a broader child health survey. Known as the Inuit Child Health Survey (ICHS), this comprehensive cross-sectional health survey of preschool Inuit children, ages 3 to 5 years, living in Nunavut looked at various health indicators in addition to iron status. Further, the survey is a component of a broader survey called “Qanuippitali? How about us, how are we?”, which includes adults as well as children. Data collection for Qanuippitali? took place in 2007 and 2008. The adult health survey was larger in scope than the child health survey. It included data collection in 39 communities in Inuvialuit Settlement Region, Nunavut and Nunatsiavut and employed the use of a Coast Guard ice-breaker vessel to travel to communities and conduct research activities. The ICHS was land-based, taking place in 16 Nunavut communities, independent of the ship-based adult survey.
2 LITERATURE REVIEW

Iron is a nutrient that is essential for growth and proper function of many organ systems. When iron requirements are not met, it is generally referred to as iron deficiency. Iron deficiency can develop in men and women, boys and girls and at any age. Within each of these groups, there may be differing etiologies and health outcomes. This chapter begins with a general review of iron metabolism and requirements. The Inuit context is then emphasized with a review of previous research in childhood iron deficiency in circumpolar populations. It will be shown, however, that there is a research gap for Inuit preschoolers in Canada. The final sections of this chapter describe potential risk factors for iron deficiency, again, as they relate to Inuit children.

2.1. IRON METABOLISM, REQUIREMENTS AND DEFICIENCY

2.1.1. Iron metabolism

Iron is an essential nutrient that must be obtained from the diet and absorbed in the upper gastrointestinal tract (GI). Iron requirements change depending on sex and life stage and in order to understand these requirements, it is necessary to first understand the mechanisms by which iron is absorbed, circulated and utilized by different cells.

There are two forms of dietary iron: non-heme and heme iron. Non-heme iron takes the more simple form of free iron atoms, such as ferric (Fe3+) or ferrous (Fe2+) iron. Non-heme iron is ubiquitous in many foods such as grains, pulses, legumes, fruits and vegetables. In most populations throughout the world, non-heme iron is the main form of dietary iron [7, 8]. Heme iron is a more complex form of iron and is available only from animal meats and organs.

When either form of iron is consumed, it is absorbed from the upper regions of the small intestine. In the acidic environment of the stomach, non-heme ferric iron (Fe3+) is reduced to ferrous iron (Fe2+) [7, 9-12]. If this reduction reaction has not occurred by the time ferric iron reaches the small intestine, the DcytB brush border enzyme converts iron to its ferrous state [7, 9-12]. The divalent metal transporter 1 (DMT1) transports ferrous
iron across the luminal membrane of enterocytes lining the duodenum or upper jejunum [7, 9-12]. Once inside the enterocyte, ferrous iron may be stored in the main iron storage protein, ferritin, or directly exported out of the cell and into the portal vein [7, 9-12]. Ferritin stores iron in many cells and is a 24-subunit protein with a central cavity that can hold up to 4500 iron atoms [13]. If iron is not immediately needed, it remains stored in the enterocytes and is excreted when they are sloughed off [7, 9-12]. When iron is needed, the protein transferrin transports ferrous iron to the basolateral side of the cell [7, 9-12]. Iron is then transported across the cell membrane via ferroportin 1 (FPN1) also known as iron-regulated transporter 1 (IREG1) [7, 9-12]. Hephaestin, a ferroxidase enzyme found on the basolateral cell membrane, oxidizes iron to its ferric (3+) state to allow for transport through the circulation [7, 9-12].

The above describes non-heme iron absorption and heme iron absorption differs. As mentioned, heme iron is derived from animal meats and organs, but more specifically is derived from hemoglobin, an oxygen transport protein characteristic of red blood cells, and from myoglobin, an oxygen storage and transport protein in muscle [14]. Hemoglobin is a tetrameric protein made up of four subunits [14]. Each subunit consists of a globin protein bound to a heme molecule made up iron-protoporphyrin ring complex [14]. Myoglobin is similar in structure but consists of a single heme-globin subunit [12].

When meats or organs are consumed, heme molecules are split from globin in the small intestine [10]. The iron-protoporphyrin complex is transported into the enterocyte via a heme transport protein [10]. Once inside the enterocyte, a hemoxygenase enzyme releases ferrous (2+) iron from the porphyrin ring at which point, the free iron has a similar fate as free non-heme iron entering the enterocyte [10].

Once released into the blood stream, iron is handled and used in different ways. Iron is transported bound to transferrin to various cells. Inside cells, iron may be stored or used as functional iron to create iron-containing proteins [7, 15]. The majority of functional iron is contained in hemoglobin which is further incorporated into red blood cells in the bone marrow [15]. As described above, each hemoglobin protein contains four iron-protoporphyrin rings. The presence of these rings in hemoglobin allows for erythrocytes
to reversibly bind oxygen for transport and delivery to tissues throughout the body [12].

In addition, when carbon dioxide concentrations are high, hemoglobin preferentially
binds carbon dioxide and delivers it to the lungs for excretion [12].

Functional iron is also found in myoglobin which stores and transports oxygen in
muscle tissue [12]. Iron containing proteins called cytochromes are involved in electron
transfer reactions that occur in mitochondria as part of cellular energy production [12]. In
addition, iron is a component of enzymes involved in non-water soluble drug excretion,
DNA synthesis, synthesis of certain neurotransmitters and synthesis of myelin which
surrounds certain neurons and aids in signal transduction [12, 15].

When iron is not needed for the various functions described above, it is stored. Storage
iron is found in all cells but mostly in the liver, bone marrow and spleen [7, 15]. Zero to
50% of the body’s total iron may be in storage, either bound to ferritin or hemosiderin
within cells [15]. In the liver, hepatocytes take up iron mostly from circulating iron bound
to transferrin and release it back into the circulation when needed by tissues [16].

In healthy human beings, iron loss is minimal except through menstrual blood loss in
women and girls. Iron is recycled from proteins by macrophages and liver Kupffer cells
[15, 16]. For example, these cells perform phagocytosis of senescent, or deteriorating,
erthrocytes [17]. Macrophages and Kupffer cells remove iron from bound protein and
recycle it back into circulation to be picked up by transferrin and re-used [17]. This
process of iron recycling in addition to the low solubility of iron in water results in
minimal excretion of iron [15]. Some daily iron loss does occur through enterocyte
shedding, bile excretion, urine and the skin [12].

2.1.2. Iron requirements and measuring dietary intake

Iron is required from the diet in general to replace daily losses. In infants, children and
adolescents, iron is also required to meet the needs of growth and development. There
exists a set of age and sex specific iron requirements that describe absolute daily required
iron, that is, the amount that needs to be absorbed from the diet [18], (Table 2-1). Only a
portion of consumed iron will be absorbed and the remainder will be lost through
excretion from the GI tract. The absorbed portion is said to be bioavailable. As such, the Daily Recommended Intakes (DRIs), which are used by health researchers and professionals to assess dietary iron intake, take into account both the body’s absolute need for iron at the various life stages, as well as limited iron bioavailability.

Iron has low bioavailability because it is not readily absorbed and the presence of other foods in the diet may inhibit absorption [7]. For example, 25% of heme iron is absorbed from a meal [7]. Dietary calcium, high cooking temperatures and lengthy cook times are reported to impair heme iron absorption in the gut [7]. About 5 to 15% of non-heme iron is thought to be bioavailable depending on the presence of iron absorption enhancers and inhibitors in the diet [7, 8]. Inhibitors of iron absorption include phytates found in cereals, grains, nuts, seeds, vegetables, roots and fruit, phenolic compounds found in tea and coffee, calcium and soy protein [8, 19]. Enhancers of iron absorption include ascorbic acid and the presence of meat, fish or poultry in the diet [8].

The DRIs include the recommended dietary allowance (RDA), the estimated average requirement (EAR), adequate intake (AI) and the tolerable upper intake level (UL). The RDA is the amount of a given nutrient that when consumed, is anticipated to meet the needs of 97.5% of the population [7]. The estimated average requirement (EAR) is the amount of a given nutrient that will meet the needs of 50% of the population [7]. The EAR and RDA for iron are set at levels that are thought to maintain iron needs without creating too much storage iron [7], (Table 2-1). The EAR and the RDA were determined based on a mixed North American diet, in which dietary iron is approximately 90% non-heme iron and 10% heme iron, resulting in an overall 18% bioavailability of iron [7]. As can be seen in Table 2-1, when accounting for low bioavailability, the DRIs for iron are much higher than the absolute requirement.

When there is insufficient evidence to establish an EAR for a nutrient, the adequate intake (AI) is given instead. The AI is the observed or experimentally determined amount of a nutrient that is consumed in a healthy population [7]. For iron, an AI is given for infants aged 0 to 6 months and reflects the average amount of iron in breast milk (Table 2-1). Healthy term infants are born with iron stores and these in addition to breast milk
meet the infant’s iron needs until 4 to 6 months of age [11]. Beyond this iron from other
dietary sources is needed [11]. The tolerable upper intake level (UL) is the highest limit
of intake that is likely to pose no risk of adverse health outcomes for most people [7]. For
iron, the UL was determined based on evidence of gastrointestinal effects such as nausea,
vomiting and diarrhea at intakes more than 70 mg/day [7]. This was lowered to 40 to 45
mg/day to allow for a certain level of uncertainty with respect to the UL for iron [7].

The IOM recommends that only the EAR be used to assess the nutrient intake of
individuals and populations [18]. Further, using the EAR in dietary assessment involves
taking intake account the day-to-day variability in nutrient intake (i.e. one cannot simply
compare observed intake to the EAR) [18]. Overall, the EAR is used to estimate
probability of adequate intake in individuals and populations [18]. The EAR is just a
guideline to aid in determining the risk of inadequate intake. It is not a cut-off value that
can classify people with intakes below the EAR as having inadequate intakes. Recall that
in a healthy population, 50% of people will have iron needs below the EAR. The RDA is
published as a target usual intake for individuals, but should not be used to assess the
intake of individuals or populations [18].

One of the challenges of applying the DRIs is accurately measuring dietary intake.
Different tools that can be used for this purpose include observation, food records, food
frequency questionnaire and 24-hour dietary recalls [20, 21]. Each of these can provide
an estimate of dietary intake, however, the information differs within each and each has
its strengths and weaknesses, especially with respect to its estimation of usual diet, which
varies from day to day.

Dietary records require a participant to keep a quantitative record of all foods eaten
within a specified period of time [21]. The dietary food record is typically done from 3 to
7 days and is considered a gold standard in dietary assessment [21]. Food records are
advantageous because diet is recorded at present time and does have to be recalled,
leaving less room for missing or forgotten information [21]. When many days of diet are
recorded, dietary records can provide an estimate of usual diet for the individual [21].
However, food records impose participant burden and perhaps measurement bias if
participants change their usual diet because they are keeping a record of it. In addition, food records are inappropriate for young children. As such, food records are usually inappropriate for large epidemiological studies especially where time constraints are an issue.

Food frequency questionnaires (FFQs) ask a participant to recall their usual diet over a longer period of time and are advantageous because they can be completed rapidly relative to food records while still providing an estimate of usual intake [20, 22]. FFQs are limited in that they only contain a list of foods and foods not listed are likely missed. As such, FFQs need to be developed carefully and often validated to ensure the appropriate foods are included. In addition, in order to estimate usual nutrient intake from an FFQ, one needs quantitative information about each food listed, that is, the typical portion size and frequency of consumption. This requires participants to recall this information and is likely less accurate than quantitative information from a food record [21]. In addition, when they are long, FFQs can impose burden on the participant. While FFQs are thought to provide better estimates of usual intake for an individual than 24-hour dietary recalls, which are discussed below, they can also be logistically difficult [21].

The 24-hour dietary recall method is commonly used and has been shown to be valid for short-term intake for preschoolers [23]. Validation studies typically involve comparing 24-hour recall data to observed dietary intake [23]. The 24-hour recall method involves an interview to record detailed, quantitative information about the foods and drinks consumed by the participant for a 24-hour period. A five-step multiple pass interviewing method is typically used and has been previously validated for many nutrients [24].

Twenty-four hour recalls are advantageous because they can obtain quantified dietary information rapidly [22, 25, 26]. However, one recall can only provide short-term dietary information, and unlikely information about an individual’s usual diet [22, 25, 26]. With respect to iron specifically, this is reflected in low correlations between 24-hour recall
intake data and iron status biomarkers [7, 25, 26]. As such, multiple recalls on varying days are required to assess usual individual intake [22].

2.1.3. Health outcomes of iron deficiency

Iron deficiency varies in severity and is generally classified into three stages. The mildest stage of iron deficiency is iron depletion where there is a decrease in iron stores, such as those in the liver [20]. Iron deficiency erythropoiesis (IDE) is slightly more severe. It is characterized by depleted iron stores, decreased iron supply to the tissues but no observed effect on circulating erythrocytes [20]. Finally, iron deficiency anemia (IDA) is the most severe stage of iron deficiency. It occurs when there is a lack of iron to synthesize hemoglobin and hence, healthy erythrocytes [20]. In IDA, erythrocytes appear small and pale, otherwise known as hypochromic, microcytic anemia [14, 20].

Anemia is not always hypochromic and microcytic or always a result of iron deficiency. Anemia occurs when there is a severe enough reduction in erythrocyte mass or in blood hemoglobin concentration [14]. In addition to iron deficiency, there are numerous other causes of anemia in children including disorders involving the bone marrow, impaired erythropoietin production, disorders impairing erythroid maturation and hemolytic anemias [14]. These are beyond the scope of this review, but are important to recognize since it is estimated by the WHO that world-wide that only 50% of observed anemia is related to iron deficiency and the remainder is related to other causes [27].

Childhood is a vulnerable age for iron deficiency because children have rapid growth and a subsequent high requirement for iron relative to body mass [8]. Iron deficiency is of particular concern in children because they are undergoing growth and development and these processes can be impaired in the presence of iron deficiency. For example, IDA can lead to impaired cognitive development and neurological malfunction, fatigue and growth delay [7, 12, 15, 20, 28]. Further, treatment with iron therapy is not necessarily able to reverse the effect of cognitive impairment later in life, resulting in behavioural and developmental problems [29, 30]. IDA can also result in impaired resistance to infection specifically decreased leukocyte killing and decreased cells available for cell mediated
immunity [15, 28]. Severe IDA with hemoglobin less than 50 g/L can lead to respiratory distress, congestive heart failure, and, rarely, cardiac arrest [30].

The above health outcomes were observed only in children with IDA, and it is unclear if milder stages of iron deficiency result in developmental deficits [28]. Some studies in infants have reported that cognitive development and motor deficits are seen even with iron deficiency without anemia [15, 30]. In the United States, school-aged children with iron deficiency but not anemia had lower math scores than children with healthy iron status [31]. Other studies have reported reduced aerobic or work capacity in adults with iron deficiency but not anemia [32, 33].

There are numerous causes of iron deficiency and IDA in children. They are briefly reviewed here, but those more relevant to Inuit children will be reviewed in more detail later. Celiac disease or inflammatory bowel disease can result in inflammation and damage to the intestinal epithelium, which can impair iron absorption [14]. The presence of phytates, polyphenols, calcium or soy protein may also limit iron absorption in addition to lack of heme-iron or ascorbic acid in the diet [8, 19]. Gastrointestinal blood loss can also result in iron deficiency. This may be a result of anatomical defects in the gastrointestinal tract and bleeding peptic ulcers [14]. Microscopic blood loss from the gastrointestinal tract may result from inflammation, cow’s milk consumption before the first year of life, and parasitic infection with hookworm or whipworm [14]. In young children and infants in particular, in the absence of parasitic infection or chronic gastrointestinal illness, iron deficiency is usually related to low dietary iron intake, particularly prolonged breast-feeding and high cow’s milk consumption, both of which can replace iron-rich foods [34]. Evidence has also emerged that infection with the human pathogen Helicobacter pylori (H. pylori) can cause iron deficiency in young children as well as adults. H. pylori as well as dietary risk factors in relation to Inuit children will be discussed in more detail later in this review.

Causes of general anemia, as opposed to IDA, is not the focus of this study but it should be mentioned that there are various processes that can result in low hemoglobin or erythrocytes in children. Vitamin A, riboflavin, folic acid, vitamin B-12 and vitamin B-6
are required for normal erythrocyte production [35, 36]. As such, deficiency in any of these may result in anemia, even if iron stores are normal. Although less significant, it has been suggested that the anti-oxidant properties of vitamins C and E are important in maintaining hemoglobin levels since they protect against free radical destruction of erythrocytes [35]. Acute infection and inflammation can also lead to anemia in children. The inflammatory response leads to an increase in certain cytokines that block the movement of iron from cell to cell [17]. For example, macrophages that recycle iron from senescent erythrocytes can no longer move iron back to the tissues in the presence of inflammation [14, 35-37]. In addition, oxidative stress causes lyses of senescent erythrocytes thus reducing the concentration of hemoglobin in the blood [17]. The end result may be mild anemia with normal iron stores.

Overall, the health consequences of IDA are severe in children and potentially irreversible. As such researchers and health organizations have conducted studies to measure the prevalence of iron deficiency and IDA in children for decades. Inuit have been the subject of some of this research for almost 40 years now. After reviewing the methods for measuring iron status, the next section will provide a review of population-based research in iron deficiency. The focus will be Inuit children, and as will be shown, it is reasonable to suspect that rates are high compared to American children. Also, there is a need for current information on this important issue in preschool aged Inuit children.

2.2. POPULATION-BASED RESEARCH IN IRON DEFICIENCY

2.2.1. Measuring iron status

The currently accepted method for assessing prevalence of iron deficiency is to measure concentrations of biomarkers in blood samples. Various biomarkers can be used and each can provide information about the severity of iron deficiency.

Hemoglobin or hematocrit concentrations in the blood are used to assess anemia irrespective of iron status [20, 28]. Hemoglobin concentrations less than 110 to 115 g/L are indicative of anemia in children [20, 28]. Hemoglobin can be measured with an automated Coulter counter system in a clinical or laboratory setting, or using portable
haemoglobinometer called Hemocue™ [20]. Blood samples are inserted into the Hemocue™ in specially manufactured cuvettes that haemolyse free hemoglobin and convert it to a measurable form called azidemethaemoglobin, which is measured via light absorbance [38]. Hemocue™ is reported by the manufacturer to be accurate within ± 3 g/L of hemoglobin and studies have confirmed its accuracy for clinical and research use using venous and capillary blood samples [39-41]. However, one study reported larger variations in repeat test results with capillary blood samples than when using venous blood [42]. Another study reported that capillary samples showed higher results than venous samples but the difference was not significant [43]. They also showed low coefficients of variation (CVs) with repeat capillary samples. It seems overall that there is ample evidence supporting the accuracy and precision of the HemoCue™ method of measuring hemoglobin concentration, and this tool is recommended by the World Health Organization (WHO).

Anemia can also be assessed by measuring hematocrit, which is the volume fraction of red blood cells in a blood sample [20]. When hemoglobin synthesis is reduced, hematocrit also becomes reduced [20]. It can be measured by calculating the ratio of packed red cell height to total sample height or using an automated coulter counter [20]. Hematocrit fraction less than 0.33 or 0.34 is indicative of anemia in children [20]. Measuring hematocrit is limited because it is prone to measurement error and can be affected by high white blood cell counts [20]. Overall, hemoglobin is a more sensitive measure of anemia since hemoglobin concentration tends to drop before reduced hematocrit can be detected [20].

Low hemoglobin or hematocrit measurements indicate anemia, but other biomarkers are required to assess iron deficiency specifically. The status of iron stores can be measured via plasma or serum ferritin [20, 28]. Ferritin within cells stores iron but ferritin is also present in the circulation, where it serves as an acute phase protein produced by liver [20, 28]. It correlates well with total body iron stores and low serum ferritin levels indicate low or depleted iron stores [20, 28]. It has been proposed that serum ferritin less than 12 µg/L indicates storage iron deficiency in young children [20, 28]. The National Health and Nutrition Examination Survey (NHANES) III, a large longitudinal study in
the United States, used a lower cut-off of 10 µg/L for children [44]. This cut-off value has also been used in some Canadian studies with infants [45, 46]. Another study suggests that ferritin less than 5 µg/L be used to identify iron deficiency in 9 month old infants [47]. Serum ferritin can be measured in serum or plasma samples using an immunoradiometric assay or an enzyme linked immunosorbance assay [20, 28]. A method of measuring ferritin in dried blood spots has also been recently developed [48].

Plasma or serum ferritin values are of limited use during infection because they may become falsely elevated with the acute phase response. As such, ferritin results should be interpreted with a biomarker of acute infection status such as C-reactive protein (CRP) or alpha₁-acid glycoprotein (AGP). CRP is an acute phase protein made up of five identical subunits [49]. It plays a crucial role in pathogen killing, removal of damaged apoptotic cells and complement activation [49]. When infection occurs, CRP concentrations quickly rise, but also quickly decline 24 to 48 hours after stimulation [50]. AGP concentrations remain elevated for 5 to 6 days and ferritin remains elevated for up to 10 days [50]. As such, in the presence of elevated CRP or AGP, ferritin values should be interpreted with caution. As is the case with ferritin, the cut-off values for CRP are unclear. Some researchers use higher cut-off values for CRP, such as 8 ng/mL and 10 ng/mL to indicate infection [51, 52]. Others use lower values, such as 3 ng/ml or 2 ng/ml [50, 53].

Low ferritin only indicates early stages of iron deficiency. When coupled with a measure of hemoglobin, IDA can be assessed. However, more moderate stages such as IDE cannot be determined from these measures. Transferrin saturation can be used to assess whether iron deficiency as defined by low serum ferritin has progressed to IDE. Transferrin saturation is a measure of the ratio of serum iron to total iron binding capacity (TIBC) [20, 28]. In IDE, serum iron decreases and TIBC increases [20, 28]. As such, very low transferrin saturation indicates nutritional iron deficiency that affects tissue iron supply [20, 28]. Transferrin saturation is useful because when it is at the low end of normal range, it indicates infection since serum iron is decreased, but TIBC does not increase as it does in nutritional iron deficiency [20, 28]. In children, transferrin saturations below 10 to 14% have been proposed as cut-off values, but are unclear since
serum iron changes with age [20, 28]. Serum iron is measured using a clinical chemistry autoanalyzer and TIBC is determined by measuring the amount of iron required to saturate transferrin [20, 28].

Erythrocyte protoporphyrin also measures IDE and specifically indicates decreased iron supply for erythrocyte synthesis. It becomes elevated when iron levels are insufficient to produce heme for erythrocyte protoporphyrin [20, 28]. This method is limited because it is falsely elevated during infection, lead poisoning and haemolytic anemia [20, 28]. Erythrocyte protoporphyrin can be measured in a research setting using fluorescence or haematofluorometer [20, 28].

Serum transferrin receptor (sTfR) is another biomarker that can be used to assess IDE. If iron deficiency is severe enough and supply to tissues is limited, sTfR levels in the blood increase, reflecting the up-regulation of cellular transferrin receptors to capture more iron in the tissues [20, 28]. Elevated levels of sTfR indicate tissue iron deficiency, but cut-off values are unclear, even for adults [20, 28]. Serum TfIR is useful because it is not influenced by infection and recent studies have shown that the ratio of sTfR to serum ferritin can distinguish between low circulating iron due to nutritional iron deficiency and that due to infection and inflammation [54, 55]. Serum transferrin receptor can also be measured in small volumes of serum [20, 28].

There are numerous biomarkers of iron deficiency and anemia, each with their strengths and limitations. Overall, biomarkers exist to measure every stage of iron deficiency. The WHO suggests that in a population-based research setting, the ideal combination of biomarkers for measuring iron status is hemoglobin, plasma or serum ferritin coupled with a marker of infection and serum transferrin receptor [28]. This allows one to assess all stages of iron deficiency using biomarkers that can reasonably measured for large groups of people.

2.2.2. Iron deficiency among Inuit children: review of prevalence estimates

Some information is available on the iron status of Inuit children, although more recent studies focus more on infants or Alaska Native children. However, these and results from
the latest nation-wide nutritional survey in Canada will be reviewed below to show that the prevalence of iron deficiency is typically higher among Inuit compared to non-Aboriginal groups. In addition, information for Nunavut preschoolers currently is not available.

One problem with many of the studies that investigate childhood iron deficiency among Inuit is that rates for preschoolers are not reported separately than those for infants. This makes it difficult to truly know about the iron status of Inuit preschoolers since infants tend to have higher rates of iron deficiency. This difference between age groups was seen in the 1995 Keewatin Health Assessment Survey (KHAS) where anemia was found in 11.5% of Inuit aged 9 months to 17 years but then was much higher at 27% in only those aged 9 months to 2 years [56]. Similarly, among Northwestern Ontario First Nations children, those aged 3 to 30 months had rates of anemia of 38% to 79%, but preschoolers aged 30 to 60 months had lower rates of 12 to 28% [57]. Among Alaska Natives a similar trend is seen where among children aged 0 to 5 years iron deficiency was found in 70% and anemia in 17% [58]. Then, among older children of 7 to 11 years, iron deficiency was found in only 38% and anemia in 15% [59].

As such, data for age groupings including both infants and preschoolers need to be interpreted carefully. For example, the most recent data on the iron status of Canadian preschoolers comes from the 1970-1972 Nutrition Canada National Survey (NCNS), but these children are grouped with infants [60]. Anemia from all causes was around 4 to 5% for both Inuit and non-Aboriginal children aged 0 to 4 years [60]. IDE was detected in 5% of non-Aboriginal children and in 12% of Inuit children [60]. From these results, one cannot whether Inuit infants are more at risk for IDE, or preschoolers, or perhaps both.

Although the NCNS only included 29 Inuit children from the Kivalliq region and did not separate children from infants, it suggests that Canadian Inuit are at higher risk than the general population. A later study assessed iron status of Inuit in the high Arctic [61]. Here, IDE was 3 to 7%, but again, the authors did not report age specific rates so it is difficult to derive conclusions for preschoolers [61]. In addition, because the NCNS only measured transferrin saturation and hemoglobin, a sub-sample of serum was analyzed for
ferritin, but not for Inuit [62]. Among this sub-sample, iron deficiency was found in 30% and IDA in 2%. Given that the rate of IDE was higher among Inuit, it is possible that rates of iron deficiency among Inuit children 0 to 4 years were higher than 30% and those of IDA were higher than 2%. However, this is merely speculation and overall, the latest information on the iron status of Canadian Inuit is outdated with a small sample size and inappropriate age groupings.

More recent studies are available for Canadian infants and seem to show a continued trend that Inuit are at higher risk for iron deficiency. Among Inuit infants from Nunavut and Nunavik, iron deficiency was found in 37 to 60% compared to about 33% in non-Aboriginal Canadian infants [45, 46, 51]. IDA was found in about 26% compared to about 5% in non-Aboriginal Canadian infants and 24% in low-income Montréal infants [45, 46, 51, 63]. Anemia from all causes was found in 37% to 48% compared to 8% in non-Aboriginal Canadian infants [45, 46, 51].

A similar trend was seen among Alaska Native children aged 0 to 5 years where the prevalence of iron deficiency was 70% and among children 7 to 11 years, the prevalence of iron deficiency was 38% and IDA was 7.8% [58, 59]. In the United States, iron deficiency rates are much lower than this. Recent data from NHANES (1999-2000) showed that among children 3 to 5 years, 0.5% had IDA and among children 6 to 11 years, 0.1% had IDA [64].

From reviewing studies in iron deficiency among Inuit, it is revealed that current information has not been reported for Inuit preschoolers in Canada. While some estimates exist from the 1972 NCNS, they are for both infants and preschoolers together so age-specific rates were not available. It was explained above that infants typically have higher rates of iron deficiency, IDA and anemia than children in older age groups. As such, it may be hypothesized that Inuit preschoolers experience rates of iron deficiency less than 36 to 60% and IDA less than 26%, which are current estimates for Inuit infants. However, while preschoolers may have lower rates than infants, data from Inuit populations compared to non-Aboriginal population suggests that Inuit have higher rates
than the national population. The uncertainties around this issue in addition to the detrimental health outcomes of IDA precedent the need to fill this information gap.

2.3. Etiology of Iron Deficiency and Anemia Among Children

2.3.1. Overview of causes of iron deficiency and IDA in children

Various causes of iron deficiency in children were mentioned above and the final section of this review will discuss some of these in detail as they relate to Inuit children. As mentioned previously, bleeding from the GI tract due to certain parasitic infections may result in iron deficiency in children. There is no evidence that these particular parasites exist in the Arctic. Iron absorption impairment and microscopic bleeding resulting from inflammatory bowel or celiac disease is possible in Inuit children, but is unlikely to explain large prevalence rates of iron deficiency and IDA should they exist in this population. In addition, genetic factors leading to IDA or hemoglobinopathies are possible, but again, unlikely to explain any large prevalence rates of this condition. The main causes of iron deficiency in Inuit children may be related to the diet and infection with the common human pathogen *H. pylori*.

2.3.2. Dietary factors related to iron deficiency

Young children rely on iron from the diet to meet their growing needs. When they consistently have iron intakes below their needs, it can result in iron deficiency or IDA. The issue of iron deficiency among Inuit has been described as a paradox because the traditional Inuit diet consists of numerous sources of land and sea animal meats and their organs (Table 2-3). Assuming that children are eating a traditional diet, one would suspect that the risk of inadequate iron is low. However, as explained above, previous studies report that iron deficiency exists in Canadian and Alaskan Inuit, and that these rates are higher than national averages. This seeming paradoxical trend may be explained by a nutrition transition that has likely been occurring in Arctic communities since the early 20th century [2].
In the late 1970s in a remote Inuit community it was reported that 75% of Inuit households were using commercially available market food “half of the time” or “most of the time” while still hunting caribou and seal [65]. Other studies report similar trends where market food continues to make up some proportion of the Inuit diet [66-68]. In addition, although there are many sources of iron in market foods such as meats and cereal grains, as shown in Table 2-3, some of these foods with limited shelf life may be expensive or less available in remote communities [68, 69]. In addition, studies with Canadian Inuit and Dene/Metis show that diets high in market foods are also higher in simple carbohydrates and fat [70, 71]. This transition from traditional foods towards market foods, particularly those that are less micro-nutrient rich, and high in simple sugars and fat, is known as the nutrition transition and evidence shows that it is occurring in among Canadian Inuit [56].

Another characteristic of the nutrition transition seems to be that younger Inuit consume less traditional food than older Inuit [66, 71, 72]. Overall, it has also been shown that iron intake is lower on days where Inuit traditional food is not consumed or when traditional food intake is lower [70, 71, 73]. However, despite this, iron intake has consistently reported to be high among Alaska Native and Canadian Inuit even among younger age groups [70, 74]. With respect to iron specifically, the 1972 Nutrition Canada Eskimo Survey found a high median dietary iron intake in four Inuit communities [56]. More recently, in a Kivalliq Inuit community, most were eating above two-thirds of the RDA and seal and caribou meat were the most common sources of iron among infants [51].

Studies on Inuit diet consistently suggest that iron intake is likely adequate in the Canadian Inuit population. However, given that the nutrition transition seems to occur to a greater extent in younger generations, it is unclear what the situation is like for Inuit preschoolers today. The most recent study on Inuit iron intake was for infants from only one community in 2003 and remaining evidence of iron intake comes from studies in the early 1990s [51, 56].
While inadequate dietary iron intake is one potential cause of iron deficiency and IDA, low dietary iron bioavailability may be another issue. Enhancers and inhibitors of iron deficiency were discussed previously. The known inhibitors of iron absorption are phytates, phosphates, calcium, polyphenols and certain dietary fibers. Enhancers include vitamin C and heme iron consumption. It is unknown to what extent each of these factors can limit iron absorption since controlled iron intake studies do not reflect habitual iron intakes [75].

The RDA and EAR for iron assume a dietary iron bioavailability of 18% in a mixed diet of 90% non-heme iron and 10% heme iron [7]. When these proportions are different, perhaps the RDA and EAR are not as appropriate. The current recommendation is to increase the RDA and EAR by 1.8 times in diets verging on 5% bioavailability [7]. The Food and Agriculture Organization proposes similar adjustments for diets with 5%, 10% and 15% bioavailability [8]. Other studies have proposed algorithms for determining iron absorption but in none of these does iron absorption explain differences in iron status [19, 76-78]. Overall, this aspect of the diet is difficult to study and in theory, if the typical Inuit diet has a dietary iron bioavailability of 18%, the EAR and RDA should be appropriate reference standards for assessing iron adequacy. If dietary iron bioavailability is thought to be lower, adjustments to the EAR and RDA could be made.

One factor that might require particular consideration is milk consumption. In young children, cow’s milk consumption is a risk factor for iron deficiency, especially when introduced at an early age or instead of fortified infant formulas [79]. While it is a rich source of other essential nutrients, cow’s milk lacks iron and when consumed too much it tends to replace other food sources of iron [12]. In addition, calcium is an inhibitor of iron absorption although it is unclear if over the long-term, calcium intake can result in iron deficiency [80]. Aside from one study in infants that found that cow’s milk consumption was the only risk factor independently associated with iron deficiency after controlling for other factors there have been no data reported on milk intake in Inuit children [51].
2.3.3. *Helicobacter pylori*

*H. pylori* is a gram-negative spiral bacterium that commonly infects the human stomach [81]. Barry Marshall and J. Robin Warren won the 2005 Nobel Prize for its discovery in 1983 [82]. It is not known exactly how infection with this pathogen occurs but person-to-person transmission is the most likely candidate, as will be discussed. The worldwide prevalence of *H. pylori* infection is thought to be around 50% and is typically higher in low income settings and low overall in higher income such as Canada and the United States. *H. pylori* is commonly associated with gastric cancer or peptic ulcers. Since it’s discovery, evidence has emerged that this common pathogen is also associated with iron deficiency and IDA in adults and children.

**Transmission of and risk factors for Helicobacter pylori infection**

There is limited evidence supporting zoonotic or water-borne transmission and *H. pylori* is most likely transmitted from person to person. Human *H. pylori* has never been isolated from pigs, cats or sheep, ruling these out as reservoirs for infection [83]. Monkeys can carry certain Helicobacter species but do not come in contact with enough people to explain the high world-wide prevalence [83]. In controlled experiments houseflies were exposed to human *H. pylori* and have transmitted the pathogen to Petri dishes but transmission has not been shown to occur when flies are exposed to fresh human faeces [83].

Regarding water-borne transmission, *H. pylori* has been found in water supplies such as lakes, rivers and water delivery trucks, including those in northern regions [84, 85]. However, the bacteria were identified using polymerase chain reaction, which only show that *H. pylori* genetic material was present in the water and not viable bacteria.

While the possibility of animal and water reservoirs have not been completely ruled out, it is generally believed that *H. pylori* is transmitted form person-to-person through a fecal-oral or oral-oral route [83]. Viable *H. pylori* has been cultured from diarrheal samples, vomitus and also 30 centimeters in the air from vomitus and recent history of vomiting in siblings was found to be a risk factor for infection [86]. Recent progress in
identifying *H. pylori* specific genetic markers has shown that people living in the same home tend to carry the same strain, suggesting that person to person transmission occurred [87].

Even though there remains some uncertainty about the transmission route, certain risk factors for *H. pylori* infection have been established. These include lack of hot water access, household crowding, household age distribution characteristics, socioeconomic status and race/ethnicity. Retrospectively, 227 adults were asked about their household living situation when they were 8 years old [88]. Lack of hot water access (OR = 4.34, 95% CI: 1.34-10.0) and more than 1.3 people per room relative to less than 0.70 people per room (OR = 6.15, 95% CI: 1.84-18.6) were associated with *H. pylori* infection in adulthood [88]. Among 245 healthy children, aged 3 to 5 years, the prevalence of infection was higher among those living in low-income homes [89]. Risk factors surrounding socioeconomic status and race have been reported in other studies [64, 90]. Age has also emerged as a risk factor for infection. In the Colombian Andes where infection rates are high, the strongest significant predictor of infection in children aged 2 to 9 years was the age gap to the next oldest sibling, where those closer in age were more at risk [91].

**Measuring Helicobacter pylori**

There are various diagnostics tests for *H. pylori* infection and each have their limitations. The gold standard method is endoscopy, which allows physicians to visually examine the gastric wall for signs and extent of infection [92, 93]. Children with *H. pylori* infection may have healthy looking stomachs and biopsy and culturing for the pathogen can be used to affirm infection [92]. Endoscopy is performed in a clinical setting, is invasive and while it has been used on in the research setting, it is not practical in population level studies. Less invasive and more practical diagnostic tests for *H. pylori* are antibody testing, urea breath test or stool antigen testing. Serum or plasma samples can be tested for IgG or IgA antibodies against *H. pylori* using commercial ELISAs. This method, also known as serodiagnosis, is inexpensive and requires only a small volume of blood sample. However it is limited in children because it cannot distinguish between current
and past infection [93]. More importantly, some studies have reported that serodiagnosis will underestimate the prevalence of infection in children. It is thought that this occurs because antibody concentrations may take months to increase to detectable levels after infection occurs [93-95]. One study reported that IgG has a specificity of 54% in children under 10 years compared to stool antigen testing [95]. In a pilot study in Alaska, 86% were positive for infection using a urea breath test (UBT) but only 41% were positive using IgG diagnosis [96]. Where venipuncture is not possible, saliva samples can also be used for measuring antibodies using commercial ELISAs, but are subject to the same limitations as serum or plasma antibodies.

Other less invasive tests for infection included the UBT and stool antigen. The UBT takes advantage of the unique property of *H. pylori*; the possession of a urease enzyme that breaks down urea [92]. Patients swallow a pill containing carbon labeled urea. If the patient has a current infection, labeled carbon dioxide will be detectable in a breath sample obtained after a fasting period. While there are many reports about the high accuracy of the UBT, it is has been suggested that there are few studies on UBT in children so its accuracy in this age group is still unclear [93]. They also suggest that testing is more costly than other tests and cumbersome in field research setting [93]. *H. pylori* stool antigen testing indicates current infection, is lower cost than the UBT, and its good accuracy has been reported in both adults and children [93]. Where collection of fecal samples is feasible, *H. pylori* stool antigen test is reasonable diagnostic test for use in the research setting.

The gold standard for diagnosing infection remains endoscopy, but this is not feasible in large studies. Among the less invasive tests in children, UBT and stool antigen testing seem to be the most accurate however their practicality is limited because obtaining samples may be difficult [92]. Serodiagnosis is often more practical but is less accurate in young children and may underestimate the prevalence of infection [93-95].

*Prevalence of Helicobacter pylori infection in children*

In general, *H. pylori* infection occurs in childhood and infection prevalence increases with age [97-99]. Further, in low income countries where risk factors for infection are
more common, infection is thought to occur at a younger age than in higher income countries and as such, infection prevalence is higher in children [97, 99]. For example, in 1991, it was reported that 60% of children in India aged 0 to 9 were positive for infection while around this same time, only 4 to 5% of children in this age group were positive in Australia, France and England respectively [99]. Currently in the United States, prevalence follows the predicted age-related pattern where 5.5% ± 1.4% of young children aged 3 to 5 years are infected, and peaks at about 30 to 45% in adulthood [64]. In Canada, the prevalence estimate for children aged 5 to 18 years is 7.1%, but they were selected from children referred for gastrointestinal symptoms and are not representative of the entire population [100].

Consistent with previous findings for children in low-income settings, *H. pylori* prevalence rates are high in Canadian northern First Nations and Inuit communities (Table 2-4). In Wasagamack, a Cree community in northeastern Manitoba, 56.4% of children age 6 weeks to 12 years were positive for *H. pylori* infection in 2002 [101]. Earlier, 95.1% of adults were infected in this community [84]. Among Inuit in two Kivalliq communities in 1999, the seroprevalence was 50.8% in adults [85]. In 2003, it was reported that 39% of infants age 4 to 18 months in one Kivalliq community were seropositive for *H. pylori* [51]. Using the UBT test, it was found that 86% of Alaskan Native children, aged 7 to 11 years, were infected by UBT diagnosis [59]. In a younger age group, the seroprevalence was 32% in Alaskan Native children aged 0 to 4 years [102]. In addition, the overall prevalence of *H. pylori* infection among Alaska Natives was 74.8% and increased with age as occurs in other populations [102]. Overall, it seems that among Canadian and Alaskan northern populations, the prevalence of *H. pylori* infection in young children is 39 to 56%.

In contrast, the prevalence of infection among Inuit children in Greenland was low. In West Greenland between 1996 and 1998, the seroprevalence was 41% for all age groups [103]. However, the *H. pylori* prevalence rate was only 6.1% (95% CI: 0-15.8%) among children aged 0 to 4 years [103]. In this study, the authors suggest that the lower seroprevalence compared to other Arctic regions may be due to better housing. They propose that a birth cohort effect may have occurred in Greenland where the prevalence
of \textit{H. pylori} infection is declining as reflected through low prevalence rates in younger populations. Similar trends have been seen in other populations in Finland, the Netherlands and in Germany where infection rates have declined significantly likely due to improved socioeconomic conditions, decreased household crowding or increased antibiotic use [104-106].

Evidence suggests that prevalence of \textit{H. pylori} infection is high in Canadian Inuit communities [51, 85]. It further suggests that infection occurs in early childhood and continues throughout adolescence and adulthood until the overall prevalence in Inuit is higher than the overall world-wide prevalence [84, 101, 102]. The epidemiological pattern of \textit{H. pylori} infection is consistent with that of a low-income setting and as such, young children may be at risk for \textit{H. pylori}-related illness such as iron deficiency as will be discussed next in greater detail.

\textbf{Pathophysiology of \textit{H Helicobacter pylori}}

An \textit{H. pylori} bacterium has 4 to 6 flagella that allow it to penetrate the gastric mucous layer and subsequently adhere to the gastric epithelium [92]. It is not known to penetrate the gastric epithelium [92]. Bacterial damage to epithelial cells lining the stomach elicits an immune response, which can lead to chronic inflammation and histological changes in the gastric mucosa [92]. This is known as gastritis [92]. Gastritis occurs in both adults and children and its severity varies [92]. Gastritis in children is typically characterized by immune cell infiltration to the site of infection as well as the presence of lymphoid follicles [92]. It is typically superficial because the glandular tissue in the gastric epithelium is undamaged, or intact [92]. More severe forms of gastritis involve atrophy of the glandular epithelium [92]. In children, metaplasia, which signifies early onset of gastric carcinoma, and severe gastritis are rare [92]. While the risk ratio of gastric cancer is 5.9 in infected individuals compared with non-infected individuals, carcinoma usually only develops in a minority of individuals in the 4\textsuperscript{th} to 5\textsuperscript{th} decade of life [92, 107]. Peptic ulcer in the stomach or duodenum caused by \textit{H. pylori} is also rare in children [108]. In children one symptom of infection may be dyspepsia or pain in the stomach. However not all \textit{H. pylori} infection causes dyspepsia and not all dyspepsia is related to \textit{H. pylori}
and can be related to other gastrointestinal conditions such as celiac disease [92]. As such, there are no classic signs of *H. pylori* infection in children and usually infection is asymptomatic. Because of this children are rarely tested for infection despite the high worldwide prevalence. The seemingly benign nature of *H. pylori* infection in childhood has been questioned over the past decade as health researchers established the association between *H. pylori* infection and iron deficiency.

**Helicobacter pylori and iron deficiency**

*H. pylori* infection is a known cause of iron deficiency and IDA in children and adults. The biological mechanism for this is still unclear but evidence from case reports has supported the cause-effect relationship between the two. Cross-sectional studies also tend to support the association but at the population level, the association is typically significant but weak. Below is an extensive review of studies examining the relationship between *H. pylori* infection and iron deficiency and IDA.

There are several theories about the mechanism by which *H. pylori* causes iron deficiency. These include bacterial mechanisms that sequester iron for growth, impair iron absorption and/or gastrointestinal bleeding [109, 110].

There is good evidence that *H. pylori* requires iron for its growth but there is limited evidence to show that it can compete for iron in the human stomach. *H. pylori* bacteria possess a lactoferrin binding protein which supports their growth *in vitro* [111, 112]. Lactoferrin, which is not normally present in the healthy adult stomach, was found in the stomachs of patients and biopsy specimens with both mild and severe *H. pylori* associated gastritis [111, 113-115]. Another study reported that less injected, labeled iron than expected was incorporated into red blood cells and was not diverted to the reticuloendothelial system as occurs in inflammation [116]. They theorized that iron was diverted to the patients stomachs [116]. Despite the above findings, there has been no evidence showing in vivo that *H. pylori* can sequester iron from within the body or competes for it in the stomach, and as such, these mechanisms remain theories.
Another theory is that *H. pylori* alters the pH environment of the stomach which can impair iron absorption [109]. *H. pylori* infection typically occurs in the corpus or fundus of the stomach [92]. These regions of the stomach contain most of glandular tissue responsible for hydrochloric acid (HCl) secretion which lowers stomach pH [117]. When *H. pylori* infection occurs in these region, damage to glandular tissue and inflammation results in decreased HCl output, increasing stomach pH [109]. The reduction of non-heme iron requires a low acidity and thus *H. pylori* infection may reduce the bioavailability of non-heme iron.

There is good evidence for the above theory. There has been some evidence of glandular atrophy occurring in young children, suggesting that it is possible that acid disturbances can occur, even in children who have not been infected as long as adults [118]. Studies have shown that in adults and children, subjects with *H. pylori* infection coupled with IDA, have increased stomach pH and reduced ascorbic acid concentrations compared to subjects with infection but no IDA or those with just IDA and no infection [109, 119, 120]. In addition, certain strains of *H. pylori* are known to be more aggressive than others and these are associated with more severe mucosal damage in adults [110]. One recent study reported that among 52 school age children, those infected with more severe strains had lower ascorbic acid concentrations in gastric juice than those with less aggressive strains [121].

Finally, it has been suggested that gastrointestinal blood losses associated with *H. pylori* induced gastritis could lead to iron deficiency [122]. It is known that bleeding peptic ulcers can cause iron deficiency however, it usually takes years to develop this condition and is unlikely to explain *H. pylori* associated iron deficiency in children [107]. However, inflammation of the gastric mucosa, which can occur in children, could result in microscopic blood loss but currently little is known about the histological changes that occur in the gastric mucosa in children with *H. pylori* infection [109].

There are various theories about how exactly *H. pylori* could induce iron deficiency. The above studies provide support for these but are not definitive. The mechanism thus
remains unclear and as will be reviewed next, the strongest evidence for the relationship between \textit{H. pylori} and iron deficiency comes from case report studies.

Five articles, published between 1993 and 2003, reported 15 cases of children who had IDA that could not be managed by conventional treatment, also known as refractory IDA [116, 123-126]. In all cases, \textit{H. pylori} was diagnosed and IDA resolved after eradication of the infection and where follow-up was done months later, there was no indication of IDA. Another study reported a case series of 28 adult premenopausal female patients who presented with a long history of IDA and \textit{H. pylori} infection [127]. Follow-up for infection status and iron status occurred at 3, 6 and 12 months. At 6 month, 75% of the women had recovered from IDA and 12 months 91.7% had recovered [127]. While serum ferritin increased significantly from pre-treatment levels (6.2 ± 0.8 \(\mu\)g/L to 23.9 ± 6.7 \(\mu\)g/L), it remained below the adult cut-off values at 12 months [127]. From this report, it is possible that iron status improved only enough to prevent anemia but storage iron deficiency was still present at 12 months.

The above case reports reveal a cause-effect relationship between the bacteria and iron deficiency. Evidence from cross-sectional studies has been mixed. In 2000, \textit{H. pylori} antibodies and IDA were measured in 375 Korean boys and girls aged 10 to 15 years [128]. There were significantly more \textit{H. pylori} positive subjects in the IDA group. Similar results were found in another study with 660 Korean adolescents suggesting that \textit{H. pylori} infection is a risk factor for IDA [129]. However, neither study group assessed the independent effect of infection over diet or potential sociodemographic confounders so it is unclear as to whether \textit{H. pylori} was independently associated with IDA.

In the United States from data for participants older than 2.99 years from NHANES (1999-2000), \textit{H. pylori} significantly explained differences in IDA status with an odds ratio of 2.7 (95% CI: 1.5-4.8) when adjusted for age, sex, poverty, pregnancy and gestational history [64]. The association remained significant when further adjusting for race/ethnicity, vitamin C intake, some chronic illnesses and country of birth (OR = 2.6, 95% CI: 1.5-4.6) [64].
Among Inuit, the evidence is mixed. Among Alaskan native children, *H. pylori* infection is independently associated with iron deficiency, but the association was modest [59]. Prevalence ratios (PR) for iron deficiency and *H. pylori* were significant (PR = 1.6, 95% CI: 1.1–2.4) [59]. After controlling for sex, village of residence and household size, *H. pylori* explained a significant amount of the variation in iron deficiency status, but only for children 9 or older (9 years: OR = 5.1, 95% CI: 1.1-23.0; 10-11 years: OR = 5.3, 95% CI: 1.5-19.0) [59]. While they did not control for household size, a similar study in Alaska found that infection was significantly associated with iron deficiency but only in those under 20 years of age [102]. In both of these studies, only iron deficiency, and not IDA, was associated with *H. pylori* infection.

Overall in Alaska Natives there is a significant but modest association between *H. pylori* and iron deficiency, but perhaps in people aged 9 to 20 years and not with more severe forms of iron deficiency [59, 102]. Canadian Inuit are similar in social and cultural practices to Alaska Natives and perhaps there exists a similar relationship between *H. pylori* and iron deficiency in Nunavut.

In a northern Manitoba First Nations community, 57% of children were anemic but there was no association between hemoglobin level and *H. pylori* status [101]. However, the participants ranged in age from 6 weeks to 12 years and they did not examine age-specific relationships as was done in Alaska. They also did not assess iron deficiency. Among infants in two northern Cree communities and in one Kivalliq Inuit community, cow’s milk consumption and not *H. pylori* infection was independently associated with iron deficiency [51]. However, this age group was younger than in the Alaskan studies.

At the population level, age may be an important factor when examining this relationship where younger age groups may not be affected [51, 59, 102]. Further, although there is evidence of a significant association, it is typically weak and likely other factors are also important in determining iron status [59, 64]. However, when *H. pylori* emerged as a cause of iron deficiency, various eradication studies were conducted. Overall, they provide mixed evidence on this issue.

\[1 \% \text{ with ID and } H. \text{ pylori} / \% \text{ with ID without } H. \text{ pylori}\]
Initial small eradication trials seemed to support the use *H. pylori* eradication therapy in the management of IDA and iron deficiency. Although there is some debate about the treatment of *H. pylori* in general, standard treatment in children seems to be 2 weeks of triple antibiotic therapy [92, 107]. In Korea, a small double-blinded randomized control trial was conducted with 25 boys and girls aged 10 to 17 years, all with *H. pylori* and IDA [130]. Groups that received *H. pylori* treatment had higher hemoglobin than the group that received only iron treatment. The group that received both iron and *H. pylori* therapy showed the most prominent increase in hemoglobin. There were no significant differences in changes in serum ferritin levels, serum iron and TIBC in any of the groups. The same author conducted two other eradication trials, one among 11 girls aged 15 to 17 years and the other among 22 girls aged 15 to 17 [129, 131]. The results were similar to the first and they also found significant increases in serum ferritin levels after eradication of infection. While these three trials are promising, they were small and not generalizable enough to make health care recommendations.

In another study, 160 children aged 6 to 16 with *H. pylori* infection were treated and changes in various iron indices were observed [132]. They found that treating the *H. pylori* infection significantly improved hemoglobin, MCV and serum ferritin in the children who had IDA. In children with iron deficiency but not anemia, ferritin concentration improved after eradication treatment. However, the lack of a control group limits these findings.

To date, only two large population-level trials have been conducted [133, 134]. These were among children in Alaska and in Bangladesh, both regions with high rates of *H. pylori* infection as well as evidence of moderate iron deficiency and of *H. pylori*-associated iron deficiency [59, 120, 135]. In Alaska, the *H. pylori* treatment trial involved 219 native children aged 7 to 11 in western Alaska. In Bangladesh the study involved 4 periurban communities with 200 children aged 2 to 5 years. In both studies, *H. pylori* treatment provided no benefit over iron treatment alone.

Both of these studies are complicated by the fact that eradication therapy is not always successful and re-infection in children is more common than in adults [136, 137]. For
example, in Alaska, the children in the *H. pylori* treatment group who were infection-free throughout the entire study period had 24% less iron deficiency than compared to the group receiving iron therapy alone, even though the relative risk was not significant. The Alaskan study group suggests that longer follow-up is needed since perhaps epithelial damage caused by the bacteria takes longer to heal and hence, it takes longer to resolve iron deficiency [133]. But overall in these two studies, the effect of iron therapy alone was similar to that of *H. pylori* plus iron therapy, suggesting that nutritional iron deficiency may also be a causal factor in some children.

The evidence supporting the cause-effect relationship between *H. pylori* infection and iron deficiency comes mostly from case-studies and some large cross-sectional studies, including one from Alaska. In addition, given the likely high prevalence of *H. pylori* infection among Canadian Inuit, this risk factor is important to address in studies of iron deficiency.

### 2.3.4. Underlying risk factors for iron deficiency

The primary objectives of this study are to estimate the prevalence of iron deficiency and IDA among Inuit preschoolers in Nunavut. Should prevalence rates be high, it is important to investigate risk factors that could direct public health interventions. As described above, dietary iron intake and *H. pylori* infection are two risk factors that could reasonably affect the iron status of young Inuit children. In addition, certain underlying socioeconomic risk factors for iron deficiency, such as food insecurity and household crowding, are thought to be prevalent among Canadian Inuit.

Food security as defined by the United States Department of Agriculture (USDA) means “access by all members at all times to enough food for an active health life” This includes the “ready availability of nutritionally adequate and safe foods” and “assured ability to acquire acceptable foods in socially acceptable ways” [138, 139]. Food insecurity exists when there is “limited or uncertain availability of nutritionally adequate and safe foods or limited or uncertain ability to acquire foods in socially acceptable ways.” [138, 139] Food insecurity has been shown to increase risk of iron deficiency in young children [140]. It is also an emerging concern in Nunavut and thought to be very
prevailing throughout the territory [68]. While territory-wide information is lacking, in one Nunavut community, five out of six Inuit household were food insecure [69].

Household crowding has been shown to be common among Canadian Inuit. According to the 2006 Aboriginal Peoples Census, 43% of Inuit children under the age 6 live in a crowded home, compared to 7% of non-Aboriginal Canadian children [141]. In addition, Alaskan native children were 1.4 times more likely to be iron deficient, but not iron deficient anemic, when they lived in crowded homes [59].
Table 2-1. Institute of Medicine (2001) reported absolute requirements and Dietary Reference Intakes (DRIs) for iron in male and female infants, children and adults.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Absolute Requirement, 97.5th percentile (mg/day)</th>
<th>EAR&lt;sup&gt;a&lt;/sup&gt; (mg/day)</th>
<th>RDA&lt;sup&gt;a&lt;/sup&gt; (mg/day)</th>
<th>UL&lt;sup&gt;a&lt;/sup&gt; (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 6 mos.</td>
<td>-</td>
<td>AI&lt;sup&gt;a&lt;/sup&gt;: 0.27</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>7 to 12 mos.</td>
<td>1.07</td>
<td>6.9</td>
<td>11</td>
<td>40</td>
</tr>
<tr>
<td>1 – 3 years</td>
<td>1.23 – 1.36</td>
<td>3.0</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>4 – 8 years</td>
<td>1.45 – 2.01</td>
<td>4.1</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td><strong>Girls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 – 13 years</td>
<td>1.44</td>
<td>5.7</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>14 – 18 years</td>
<td>2.7</td>
<td>7.9</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 – 30 years</td>
<td>3.15</td>
<td>8.1</td>
<td>18</td>
<td>45</td>
</tr>
<tr>
<td>31 – 50 years</td>
<td>3.15</td>
<td>8.1</td>
<td>18</td>
<td>45</td>
</tr>
<tr>
<td>51 – 70 years</td>
<td>1.44</td>
<td>5</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>&gt; 70 years</td>
<td>1.44</td>
<td>5</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 – 13 years</td>
<td>1.44</td>
<td>5.9</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>14 – 18 years</td>
<td>1.98</td>
<td>7.7</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 – 30 years</td>
<td>1.44</td>
<td>6</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>31 – 50 years</td>
<td>1.44</td>
<td>6</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>51 – 70 years</td>
<td>1.44</td>
<td>6</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>&gt; 70 years</td>
<td>1.44</td>
<td>6</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td><strong>Pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 – 18 years</td>
<td>~1.2 - 5.6</td>
<td>23</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td>19 – 30 years</td>
<td>1.2 - 5.6</td>
<td>22</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td>31 – 50 years</td>
<td>1.2 - 5.6</td>
<td>22</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td><strong>Lactation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 – 18 years</td>
<td>1.26</td>
<td>7</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>19 – 30 years</td>
<td>1.17</td>
<td>6.5</td>
<td>9</td>
<td>45</td>
</tr>
<tr>
<td>31 – 50 years</td>
<td>1.17</td>
<td>6.5</td>
<td>9</td>
<td>45</td>
</tr>
</tbody>
</table>

<sup>a</sup>Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI), Tolerable Upper Level of Intake (UL)
Table 2-2. Summary of prevalence studies in anemia and iron deficiency for Inuit and northern First Nations children, and comparison groups.\(^a\)

<table>
<thead>
<tr>
<th>Source</th>
<th>Population</th>
<th>n</th>
<th>%</th>
<th>(a) Hemoglobin; (b) Iron deficiency when two of SF &lt; 10 (\mu)g/L, TS &lt; 12% or EP &gt; 1.24 (\mu)mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Canada, non-Aboriginal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zlotkin 1996 [46]</td>
<td>Urban areas, 8.5 – 15.5 mos</td>
<td>428</td>
<td>ID: 33.9% (SF &lt; 10 (\mu)g/L)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IDA: 5.1%</td>
<td>Anemia: 8% (Hb &lt; 110 g/L)</td>
</tr>
<tr>
<td>Gray-Donald 1990 [63]</td>
<td>Montreal, Low-income 10 – 14 mos</td>
<td>218</td>
<td>IDA: 24.3%</td>
<td></td>
</tr>
<tr>
<td>NCNS 1970-1972</td>
<td>NCNS</td>
<td>1249</td>
<td>IDE: 12% (TS &lt; 16%)</td>
<td></td>
</tr>
<tr>
<td>Valberg 1976 [62]</td>
<td>NCNS sub-sample 0 – 4 y</td>
<td>87</td>
<td>ID: 30% (SF &lt; 10 ng/mL)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anemia: 8% (Hb &lt; 110 g/L)</td>
<td>Anemia: 5% (Hb &lt; 110 g/L)</td>
</tr>
<tr>
<td><strong>American, non-Aboriginal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardenas 2006</td>
<td>NHANES (1999-2000) 3 – 5 y</td>
<td>357</td>
<td>ID: 4.6(^b)</td>
<td>Anemia: 1.5% (Hb &lt; 112 g/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IDA: 0.5%</td>
<td></td>
</tr>
<tr>
<td><strong>Inuit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCNS 1975</td>
<td>Kivalliq 0 – 4 y</td>
<td>29</td>
<td>IDE: 24% (TS &lt; 16%)</td>
<td></td>
</tr>
<tr>
<td>Verdier 1987 [61]</td>
<td>High arctic Children and adults</td>
<td>678</td>
<td>IDE: 3-7% (TS &lt; 5(^{th}) %ile)</td>
<td>Anemia: 11-26% (Hb &lt; 5(^{th}) %ile)</td>
</tr>
<tr>
<td>Young 1995 [56]</td>
<td>Kivalliq 9 mos – 17 y</td>
<td>440</td>
<td>Anemia: 12% (criteria not stated)</td>
<td></td>
</tr>
<tr>
<td>Young 1995 [56]</td>
<td>Kivalliq 9 mos – 2 y</td>
<td>NR</td>
<td>Anemia: 27% (criteria not stated)</td>
<td></td>
</tr>
<tr>
<td>Willows 2000 [45]</td>
<td>Nunavik 12 mos</td>
<td>95</td>
<td>ID: 60% (SF &lt; 2 SD below mean)</td>
<td>Anemia: 37.8% (Hb &lt; 100 g/L)</td>
</tr>
<tr>
<td>Christofides 2005 [51]</td>
<td>Kivalliq 4 – 18 mos</td>
<td>50</td>
<td>ID: 36.9% (SF &lt; 12 (\mu)g/L)</td>
<td>ID: 25.5% (sTfR &gt; 8.5 mg/L)</td>
</tr>
<tr>
<td>Bagget 2006 [59]</td>
<td>Alaska Native 7 – 11 y</td>
<td>686</td>
<td>ID: 38% (SF &lt; 10 (\mu)g/L)</td>
<td>Anemia: 7.8%</td>
</tr>
<tr>
<td>Peterson 1996 [58]</td>
<td>Alaska Native 0 – 5 y</td>
<td>51</td>
<td>ID: 70% (SF &lt; 12 (\mu)g/L)</td>
<td>Anemia: 17% (1989 CDC cut-off)</td>
</tr>
</tbody>
</table>

\(^a\) Hemoglobin; \(b\) Iron deficiency when two of SF < 10 \(\mu\)g/L, TS < 12% or EP > 1.24 \(\mu\)mol/L
Table 2-3. Estimated iron content of some traditional Inuit foods and market foods.

<table>
<thead>
<tr>
<th>Traditional foods&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Scientific name</th>
<th>Per 100 g of food:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Energy (kcal)</td>
</tr>
<tr>
<td>Bearded seal meat, boiled</td>
<td><em>Erignathus barbatus</em></td>
<td>169</td>
</tr>
<tr>
<td>Bearded seal meat, raw</td>
<td><em>Erignathus barbatus</em></td>
<td>121</td>
</tr>
<tr>
<td>Ringed seal liver, raw</td>
<td><em>Pusa hispida</em></td>
<td>127</td>
</tr>
<tr>
<td>Ringed seal meat, boiled</td>
<td><em>Pusa hispida</em></td>
<td>164</td>
</tr>
<tr>
<td>Ringed seal meat, raw</td>
<td><em>Pusa hispida</em></td>
<td>127</td>
</tr>
<tr>
<td>Beluga meat, dried</td>
<td><em>Delphinapterus leucas</em></td>
<td>356</td>
</tr>
<tr>
<td>Narwhal meat, dried</td>
<td><em>Monodon monoceros</em></td>
<td>425</td>
</tr>
<tr>
<td>Walrus meat, aged</td>
<td><em>Odebenus rosmarus</em></td>
<td>170</td>
</tr>
<tr>
<td>Walrus meat, boiled</td>
<td><em>Odebenus rosmarus</em></td>
<td>191</td>
</tr>
<tr>
<td>Walrus meat, raw</td>
<td><em>Odebenus rosmarus</em></td>
<td>117</td>
</tr>
<tr>
<td>Arctic char, dried</td>
<td><em>Salvelinus alpinus</em></td>
<td>436</td>
</tr>
<tr>
<td>Arctic char, flesh boiled</td>
<td><em>Salvelinus alpinus</em></td>
<td>158</td>
</tr>
<tr>
<td>Arctic char, flesh raw</td>
<td><em>Salvelinus alpinus</em></td>
<td>105</td>
</tr>
<tr>
<td>Clams, meat boiled</td>
<td><em>Mya spp.</em></td>
<td>65</td>
</tr>
<tr>
<td>Mussels, meat boiled</td>
<td><em>Mytilus edulis</em></td>
<td>81</td>
</tr>
<tr>
<td>Duck</td>
<td><em>Anas platyrhynchos</em></td>
<td>166</td>
</tr>
<tr>
<td>Ptarmigan meat, cooked</td>
<td><em>Lagopus spp.</em></td>
<td>174</td>
</tr>
<tr>
<td>Canada Goose, flesh</td>
<td><em>Branta canadensis</em></td>
<td>200</td>
</tr>
<tr>
<td>Caribou liver, raw</td>
<td><em>Rangifer tarandus pearyi</em></td>
<td>124</td>
</tr>
<tr>
<td>Caribou meat, boiled</td>
<td><em>Rangifer tarandus pearyi</em></td>
<td>213</td>
</tr>
<tr>
<td>Caribou meat, dried</td>
<td><em>Rangifer tarandus pearyi</em></td>
<td>317</td>
</tr>
<tr>
<td>Caribou meat, raw</td>
<td><em>Rangifer tarandus pearyi</em></td>
<td>127</td>
</tr>
<tr>
<td>Musk-ox</td>
<td><em>Ovibos moschatus</em></td>
<td>10</td>
</tr>
<tr>
<td>Polar bear meat, boiled</td>
<td><em>Ursus maritimus</em></td>
<td>208</td>
</tr>
</tbody>
</table>

| Market Foods<sup>b</sup>:     |                  |                    |
| Cereal                        | 408              | ~13                |
| Chicken breast                | 156              | 0.5                |
| Fish sticks                   | 274              | 0.7                |
| Tuna                          | 116              | 1.5                |
| Pork chops                    | 261              | 1.0                |
| Ribs                          | 317              | 1.4                |
| Bacon                         | 568              | 1.6                |
| Hotdog                        | 242              | 2.3                |
| Ground Beef                   | 237              | 2.4                |
| Stewing Beef                  | 194              | 3.8                |

<sup>a</sup>Adapted from Traditional Food Composition Nutribase, Centre for Indigenous People’s Nutrition and Environment [142].

<sup>b</sup>Adapted from Nutrient Value of Some Common Foods [143].
Table 2-4. Summary of reported prevalence rates of *Helicobacter pylori* infection in northern or Arctic regions, and in comparison groups.

<table>
<thead>
<tr>
<th>Source</th>
<th>Population</th>
<th>Test</th>
<th>Age</th>
<th>n</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Canadian Inuit and First Nations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bernstein 1999 [84]</td>
<td>Northern Ontario James Bay Cree First Nations</td>
<td>IgG</td>
<td>20 – 50 y</td>
<td>306</td>
<td>95.1%</td>
</tr>
<tr>
<td>Sinha 2002 [101]</td>
<td>Northern Manitoba First Nations</td>
<td>Stool</td>
<td>6 wk – 12 y</td>
<td>163</td>
<td>56.4%</td>
</tr>
<tr>
<td>McKeown 1999 [85]</td>
<td>Kivalliq Inuit</td>
<td>IgG</td>
<td>15 y +</td>
<td>256</td>
<td>50.8%</td>
</tr>
<tr>
<td><strong>Alaska</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baggett 2006 [59]</td>
<td>Southwestern Alaska Native</td>
<td>UBT</td>
<td>7 – 11 y</td>
<td>688</td>
<td>86%</td>
</tr>
<tr>
<td>Parkinson 2000 [102]</td>
<td>Alaska Native</td>
<td>IgG</td>
<td>0 – 4 y</td>
<td>260</td>
<td>32%</td>
</tr>
<tr>
<td><strong>Greenland</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koch 2005 [103]</td>
<td>West Greenland Inuit</td>
<td>IgG</td>
<td>0 – 4 y</td>
<td>100</td>
<td>6.1%</td>
</tr>
<tr>
<td>Koch 2005 [103]</td>
<td>West Greenland Inuit</td>
<td>IgG</td>
<td>0 – 87 y</td>
<td>685</td>
<td>41%</td>
</tr>
<tr>
<td><strong>Canada and United States</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segal 2008 [100]</td>
<td>Urban city Canadians, with GI symptoms</td>
<td>UBT</td>
<td>5 – 18 y</td>
<td>167</td>
<td>7.1%</td>
</tr>
<tr>
<td>Cardenas 2005 [64]</td>
<td>USA, NHANES (1999-2000)</td>
<td>IgG</td>
<td>3 – 5 y</td>
<td>357</td>
<td>5.5%</td>
</tr>
</tbody>
</table>
Figure 2-1. Map of Inuit regions and communities in Canada.
Figure 2-2. Age-sex pyramid of the predominantly Inuit population in Nunavut and the total population of Canada, 2006 [6].
3 RATIONALE

Current information is not available on the prevalence of iron deficiency and IDA among Inuit children aged 3 to 5 years in Nunavut. The most recent estimates, around 24% for tissue iron deficiency and 5% for anemia from all causes, are for both infants and preschoolers and are from the 1972 NCNS [60]. Recent estimates for the prevalence of iron deficiency and IDA among non-Aboriginal infants are around 33% and 5% respectively [46]. Those for Inuit infants are higher at 36 to 60% and 26% respectively [45, 51]. While rates among preschoolers are likely lower than those for infants, Inuit children seem to be at higher risk for iron deficiency than non-Aboriginal Canadian children. Given the detrimental health outcomes of IDA and possibly iron deficiency in children such as impaired growth, cognitive development and immune defense, it is important to determine prevalence rates of iron deficiency and IDA among preschool aged Inuit children [15, 33].

Should the prevalence rates estimated from this study suggest that a health intervention is needed, it will be important to provide some information around risk factors for iron deficiency. Risk factors that most likely affect Inuit are related to the diet and infection with *H. pylori*. Previous studies show that iron intake is likely adequate among Inuit children [66, 74]. However, a nutrition transition in the Arctic is occurring rapidly so current dietary information for this age group that can be matched with iron status is needed. Infection with *H. pylori* is another important risk to assess since it has been recently shown to cause iron deficiency and this pathogen is highly prevalent in most Inuit populations [51, 59, 84, 85, 101]. In addition to diet and *H. pylori*, collecting information on certain characteristics of the household may help to describe conditions that increase children’s risk of iron deficiency. An understanding of the relationship of these risk factors with iron deficiency and IDA among Inuit children, ages 3 to 5 may be used to direct future health care planning in Nunavut.
3.1. **OBJECTIVES**

The objectives of the study “Iron deficiency and iron deficiency anemia among preschool aged Inuit children living in Nunavut” are three-fold:

- To estimate the prevalence of iron deficiency, anemia and iron deficiency anemia among Inuit children aged 3 to 5 years participating in the survey
- To describe the risk of inadequate iron intake among participating children participating in the survey
- To describe the relationship of between iron status and various risk factors, including exposure to *H. pylori*, food insecurity and household characteristics among participating children

3.2. **HYPOTHESES**

It is hypothesized that Inuit children, ages 3 to 5 years, in Nunavut will have a higher prevalence of iron deficiency than North American children overall. It is hypothesized that dietary iron intake in this population will be adequate. It is also hypothesized that *H. pylori* will be an independent predictor of iron deficiency and that food insecurity and crowding in the home will be associated with increased risk of iron deficiency.
4 METHODS

4.1. PARTICIPATORY RESEARCH PROCESS

The Nunavut Inuit Child Health Survey (ICHS) was developed by a steering committee of Inuit organizations, Canadian Universities and the Government of Nunavut. The steering committee consisted of representatives from Nunavut Association of Municipalities (NAM), Nunavut Tunngavik Incorporated (NTI), Government of Nunavut Department of Health and Social Services (GN DHSS) and the University of Toronto together with the Principal Investigator, Prof. Grace Egeland of McGill University, Centre for Indigenous Peoples’ Nutrition and Environment (CINE). In particular, the Department of Health and Social Services and Nunavut Tunngavik Inc worked in developing the scope of the survey and in the revisions of the questionnaires. Nunavut Association for Municipalities coordinated the translations. The steering committee reviewed and revised the informed consent forms and played an active role throughout the development of the research project. Further, discussions with other organizations helped shape the child health survey.

Research agreements were sent to each Hamlet, requesting their approval and involvement in “Qanuippitali?” Professor Grace Egeland and “Qanuippitali?” planning staff are based out of CINE, McGill University. “Qanuippitali?” followed closely the framework presented in Indigenous Peoples and Participatory Health Research, as per CINE’s guiding principles [1].

4.2. SAMPLE SIZE CALCULATION

The sample size for ICHS was determined based upon the primary objective of determining the prevalence of iron deficiency anemia (plus or minus 5%) among the 3 to 5 years olds. 2005 population estimates for Nunavut were obtained from Statistics Canada and the Nunavut Bureau of Statistics and increased by 2% per year until 2007 to account for population growth (Table 4-1). The estimated population size for children aged 0 to 4 years was 3609. The specific age grouping of 3 to 5 years was not available. In addition this estimate included Inuit and non-Inuit children living in Nunavut. The 3 to
5 year old Inuit population size in Nunavut was estimated to be 3000 children for sample size calculations. The sample size was calculated to allow for detection of 10% iron deficiency anemia (plus or minus 5%). This prevalence estimate is consistent iron deficiency anemia studies in Alaskan Inuit children as well as some smaller studies with Canadian Inuit infants. At a 90% confidence, the required sample size for the entire territory of Nunavut was 94 children using EpiInfo Stat Calc Version 6. In order to allow for detection of less common health indicators and to enable multivariable modelling on the determinants of iron deficiency anemia, the desired sample size was tripled to 300. Also, field survey staff members were instructed to over-sample to account for a proportion of children or care givers refusing a venous blood draw.

Sixteen of the 25 communities in Nunavut were selected to participate in the ICHS. Given the high costs of travel, communities with a very small population of 3 to 5 year olds (estimates of less than 30) and/or with excessive travel costs were excluded from the child health survey: (i.e., Resolute Bay, Grise Fiord and Qikitarjuaq). Communities were selected based upon region, population size (small, medium and large size communities), latitude (from South to North), and then finally logistical feasibility due to flight routes and financial constraints. We used the estimated population sizes of 3 to 5 year olds in each community to determine what proportion of children aged 3 to 5 in each community should be recruited to allow us to reach our sampling goal. As shown it was estimated that a sample of 20% of 3 to 5 year olds in each community would allow for an overall sample size of approximately 450, which was consistent with our sampling goal of 300 children plus over-sample for refusal of venous blood draw (Table 4-1).

4.3. STAFFING AND TIMEFRAME FOR DATA COLLECTION

The 2007 research team consisted of a bilingual Inuk nurse who conducted all venipuncture and the majority of the clinical assessments, a bilingual Inuk interviewer who conducted the majority of the interviews and two research assistants from CINE, McGill University who were responsible for training, logistical arrangements, recruitment, interviewing, assisting the nurse, file management and blood sample
preparation. The 2008 research team was similar except that the nurse was a non-Inuk Northern nurse who had previous experience working in the Nunavut.

The ICHS took place in late summer and fall in 2007 and 2008 (Table 4-2). Data collection began in Sanikiluaq in early August 2007. Sanikiluaq was visited early because research assistants were already in the community to conduct the adult health survey. The remainder of the 2007 child survey took place from September 24th, 2007 – November 23rd, 2007 and 11 communities were visited. The 2008 child survey included 4 communities in the Kitikmeot region from August 20, 2008 – September 9, 2008.

4.4. RECRUITMENT

Inuit children, ages 3 to 5, were randomly selected to participate in survey. In order to reach our total sample size and to make efficient use of staff, an approximate goal was 20% of the sample of children aged 3 to 5 years in selected communities were sampled. Children were recruited through a list of homes with children ages 3 to 5 that participated in the ship-based adult health survey and through a list of names of all children ages 3 to 5 that was provided by the local health centers. A randomized list of children was created from the health centre list using a random number table. Caregivers were contacted in the order that they appeared on the randomized list.

Usually, caregivers were first contacted by telephone. If no phone number was available, attempts were made to visit the home. If no one was home, pamphlets were left at the home with the research team’s contact information. On occasion we asked the community radio to have the selected participant call the research team or we would ask the health center workers for their help in finding people. Three attempts were made to contact households. Ideally, each of these attempts employed a different method of communication. However, we had limited time in each community and often only telephone calls were possible. Refusals, no shows and reasons for refusal were recorded.

Once a caregiver was reached they were asked to participate with their child in the health survey. They were asked to come to the health centre (or other specified location) to go through the informed consent process. If they gave written informed consent, an
interview and clinic appointment was completed. As much as possible, appointments were scheduled in the morning to avoid the effects of diurnal variations in biomarkers.

4.5. **ETHICS APPROVAL**

Certification of Ethical Acceptability for Research Involving Human Subjects for the “Qanuippitali?” Inuit Health Survey was obtained from the McGill Faculty of Medicine Institutional Review Board in March 2007 (Project # A03-E08-07B). An amendment was made to include venipuncture as part of the Child Inuit Health Survey protocol. This amendment was approved in June 2007.

A Scientific Research License was obtained from the Nunavummi Qaujisaqtulirijikkut (Nunavut Research Institute) from April 01, 2007 to December 31, 2009 (Licence # 0500607N-M). The Nunavut Research License was successfully renewed after each year of data collection. A DVD was made that followed the McGill Informed Consent form word-for-word and was made available in the appropriate dialects for the 3 Nunavut regions included in surveyed.

4.6. **INTERVIEWS**

Interviews were conducted with the person who brought the children to their appointment. The recruiter asked that the person who knew the most about the child accompany the child to the appointment. We recorded information about the respondent’s relationship to the child for quality control purposes and most often, the child’s primary caregiver brought them to the appointment. After giving written informed consent, the interviewer proceeded with the questionnaires. These are described below in more detail as they relate to this study. When the interview was complete, the child would see the nurse with the caregiver to complete the clinical assessments. Typically interviews were completed prior to the clinical assessment unless time constraints required otherwise.

4.6.1. **Interview training**

All child health survey research team members were trained on interviewing skills and dietary interviewing. Interviewing consisted of reading through each of the questions to
clarify the meaning of each if it was not clear. Interviewers were instructed to read questions as worded in the questionnaire and to offer clarification only when requested. Interviewers were instructed to use an objective tone when interviewing and not to ask leading questions. No specific instructions were given as to the order in which to administer each questionnaire however, the home-based questionnaire, ID chart and food frequency questionnaire were a priority when interview time was limited. Typically, the home-based questionnaire and ID chart were administered first and dietary questionnaires were administered towards the end of the interview, with the 24-hour dietary recall being filled before the food frequency questionnaire.

Interviewers were trained to use a five stage, multiple pass technique for collecting 24-hour dietary recall information. Interviewers were trained to ask caregivers to give a list of everything that their child ate during the day, from midnight-to-midnight, before the interview. They were then asked to go back to collect more detailed information about the food and drink listed, such as brand name, flavour or method of cooking for example. They were then instructed to review the list again and collect portion size information using 3D food models. The final stage was to review the information collected and probe for any missing items such as snacks or water for example.

4.6.2. Inuktitut translations

The written consent form, the DVD consent form and the six questionnaires were translated into three Inuktitut dialects: Nattilik, Inuinnaqtun and Baffin. Translations were conducted by professional Inuit translators. Baffin Inuktitut translations were tested during a small pilot study in Iqaluit, Nunavut and minor corrections were made to the questionnaires. Nattilik and Inuinnaqtun questionnaires were translated once. Back-translations were not done. Nattilik Inuktitut translations were used in Kugaaruk. Inuinnaqtun translations were used in Cambridge Bay and Kugluktuk. Baffin Inuktitut translations were used in the remaining thirteen communities, which were part of either the Baffin or Kivalliq regions of Nunavut. Although some of these communities spoke mainly the Kivalliq dialect of Inuktitut (or the Nunavik dialect as was the case with
Sanikiluaq), an interviewer who spoke the appropriate dialect was available for any needed translations when these communities were surveyed.

4.6.3. Written informed consent

Written informed consent was obtained from the child’s caregiver prior to any participation in the study. A person was considered a child’s caregiver if they were the person primarily responsible for the child at the time of the study. Caregivers had the option of reading the consent form, having it read to them, watching a DVD information guide or all three. All options were available in English and Inuktitut. The DVD guide was a word-for-word reading of the consent form, accompanied by photographs and video-clips relevant to the study. Caregivers were asked to complete and sign two copies of the consent form. They were given one copy and the second was retained.

4.6.4. Study numbers and confidentiality

Once caregivers had watched the supplementary information DVD and/or read and completed the written informed consent form, the participating child was given two confidential study numbers, one for the individual and one linking them to their household. A list was created with the child’s first and last names, age, sex, box number, house number, community and study number. Once the list was completed and verified, identifiers were removed from the child’s file and placed in a sealed envelope. These were hand-carried with project staff. Files without identifiers were periodically shipped to McGill University through Canada Post. Upon return to Montreal, files were placed in a locked room at McGill University. The confidential list and identifiers were placed in a locked safe.

4.6.5. Participant compensation

Each child was given a Beanie Baby toy regardless of whether or not they completed the entire child survey. Caregivers were given $15 gift-cards to either the Northern Store or the Co-Op regardless of whether or not they completed the entire survey.
4.6.6. **Demographic information and household characteristics**

Interviewers asked questions about age, sex and relationships of everyone in the child’s household. They also conducted questionnaires for characteristics of the home including the USDA 18-item Household Food Security Survey Module [144]. Indian and Northern Affairs Canada (INAC) modified the standard USDA module based on cognitive testing with Inuit interviewers to improve acceptability among Inuit. For example, response options such as “always true”, “sometimes true” and “never true” were replaced with “often”, “sometimes” and “never” to avoid creating a situation where the respondent felt that the truthfulness of their responses was being questioned [69]. A brief questionnaire about the child’s current supplement use was also administered.

4.6.7. **24-hour dietary recall**

One 24-hour dietary recall was conducted for each child participant using a five stage, multiple pass technique described above. Food model kits were used to estimate portion sizes. Some caregivers knew the volumes of liquid of food consumed and this information was recorded. Often caregivers were not with their children for the entire 24-hours. If this occurred, this was recorded along with any known information on what the child ate was recorded. If possible, interviewers or the caregiver telephoned the person that the child was with to at least record what the child ate. Depending on the number of days spent in the community, all caregivers from the first or second day of appointments were asked to return for a 20-minute appointment to complete a second 24-hour dietary recall on a nonconsecutive day. The target number of repeat recalls was 20% of the total sample of participating children.

4.6.8. **Food frequency questionnaire**

Each caregiver was asked to complete a qualitative food frequency questionnaire (FFQ) for their child. The FFQ was designed to capture past month information about common country foods that are available in the three regions of Nunavut. It also included some market foods sources of iron such as beef, pork, fish, poultry and breakfast cereals. Due to the difficulty of quantifying children’s “usual” portion sizes and to time
constraints, the child FFQ was not quantified. However, caregivers were asked about how often the child ate the foods in the past month.

4.6.9. Quality control for interview component

For the 24-hour recall and the FFQ, a quality control tool was used (Appendix A). Prior to leaving each community, each 24-hour recall and FFQ was reviewed by the research team member with the most experience with dietary questionnaires. This person reviewed the 24-hour recall to ensure that appropriate level of detail was obtained and that the portion sizes and servings were being recorded correctly. If corrections were needed, they made note of them in the quality control tool and reviewed them with the interviewer in attempts to make corrections.

A similar quality control tool was used for the FFQ. The quality control tool also served as a useful training tool so interviewers could improve upon their mistakes early on in the project. These records were placed in the child participant’s file upon completion. The other questionnaires were reviewed early on in the fieldwork to ensure that the interviewers were not making any major errors.

No measures of inter-interviewer agreement were taken to assess consistency between interviewers. All data were recorded in ballpoint pen on the questionnaires. All study numbers were recorded on the front cover of the questionnaire. Questionnaires were placed in the child’s file. A quality control checklist was completed to ensure that all appropriate documents were in the file.

4.7. Clinical data collection

Clinical protocols are shown in Appendix B. If a clinical measure was not completed, coded reasons for incompletion were recorded. The caregiver was always present for the child’s clinical assessment and often along with the interviewer who would assist the nurse. It is worth noting that parents and children seemed comfortable with the research nurses who were familiar with working in Nunavut’s communities. The research team created a positive and comfortable experience for both the child and the caregiver.
4.7.1. Anthropometry

Height was measured to the nearest 0.1 cm using a portable stadiometer (Road Rod 214 Portable Stadiometer, Seca, Maryland). Weight was measured once per child to the nearest 0.1 kg using an electronic scale. Unless specified on the clinical sheet, shoes were removed for both measurements. Results were recorded on the child’s clinical sheet and the results were returned to the caregiver at the appointment. BMI-for-age, height-for-age and weight-for-age percentiles and z-scores were calculated using EpiInfo Nutrition Version 6 and the 2000 CDC reference growth curves [145].

4.7.2. Blood sample collection

Venipuncture

A certified nurse conducted venipuncture. Caregivers were asked again in the clinic if they wanted venipuncture for their child. They were informed that if they opted for the finger prick instead, that only the hemoglobin results could be given to them and not the other indices measured in the blood. The nurse performed venipuncture using 23G¾ butterfly Vacutainer® brand blood collection sets (Becton Dickinson and Company, Franklin Lakes, New Jersey). 3ml of whole blood was collected in 4.0 mL Vacutainer® blood collection tubes coated with 68 USP units of sodium heparin (Becton Dickinson and Company, Franklin Lakes, New Jersey). Venipuncture was performed in the median antecubital vein from the anterior forearm. If venipuncture from the forearm was not possible or unlikely to work, as was often the case with the 3-year old children, the nurse performed venipuncture from the dorsal hand veins. The vacutainer tube was inverted gently 10 times. One drop of whole blood was dispensed onto Parafilm (Pechiney, Chicago, Illinois) using a Diff-Safe® blood dispenser (Alpha Scientific Corporation, Southeastern, PA) for hemoglobin measurement as discussed below. The tube was placed in a fridge or on a blue medical pad placed over ice until processing within 6 hours. It was noted on the child’s clinical sheet that venipuncture was used to collect blood.

The nurse encouraged caregivers to hold the children in their lap and place their arms around their children’s arms for safety purposes. The assisting interviewer and the
caregiver tried to distract the child’s attention from the needle. When children were unaware of what was happening, they rarely cried. With small-sized butterfly needles, venipuncture feels like a small pinch and takes about 5 to 10 seconds to collect 3ml of blood if the child is well hydrated. It should be noted that it is a privilege to be allowed to collect blood for research purposes. As such, we tried as much as possible to make blood collection quick and comfortable for the children, including giving the children “fun” band-aids and allowing them to choose a toy to keep immediately after venipuncture.

If venipuncture was not possible because the nurse could not easily find a vein or the child or caregiver was unwilling, it was explained that a finger prick could be performed to test for hemoglobin only. Finger pricks were performed using OneTouch® UltraSoft™ Sterile Lancets (LifeScan, Inc., Milpitas, California). The finger prick protocol used was difficult with children. The capillary blood sample is more likely to contain extracellular fluid that can dilute the samples. Hemoglobin results using HemoCue™ may be underestimates of the child’s actual hemoglobin and this should be considered when calculating statistics of anemia. If a finger prick was used to collect capillary blood, this was recorded on the clinical sheet.

4.7.3. HemoCue™

Dispensed venous blood drops or blood drops from finger prick were analyzed for hemoglobin concentration using the cyanmethemoglobin method with HemoCue™ 201+ portable photometer (HemoCue, Inc., Lake Forest, California). Either the nurse or the interviewer assisting the nurse completed the hemoglobin measurement. Results were recorded on the clinical sheet.

The photometer itself was tested every morning of the clinic for quality control purposes. High, medium and low control samples were tested and results were compared with expected results. Results were also recorded on a log sheet and monitored to ensure consistency. The machine was also cleaned according to the manufacturers instructions every two weeks or when needed. Cleanings were noted in the log sheet.
4.8. **PLASMA SAMPLE PREPARATION**

Sample number, time of preparation and how the sample was kept cold was recorded prior to beginning plasma preparation (Appendix B). The vacutainer was inverted gently 10 times. The cap was removed and whole blood was dispensed using a wide-tipped disposable transfer pipette (UltidentBRAND) into two or three 1.5 ml centrifuge microtubes depending on the volume of blood collected. Blood was dispensed so that tubes were balanced. Tubes were labeled and spun for 20 minutes at 2000xg in a minicentrifuge (Mandel Scientific Company Inc., Guelph, Ontario). The vacutainer tubes contained heparin, which is a blood anticoagulant.

Using a fine-tipped disposable transfer pipette (UltidentBRAND), plasma was slowly removed and dispensed into a 2 ml microtube. The 2 ml microtube was labeled and placed on a blue medical pad over ice until divided into aliquots for freezing. Hemolysis or incomplete white blood cell removal were noted. The plasma was aliquoted into pre-labeled 2 mL cryovial tubes according to the protocol using 1000 µL and 200 µl pipetors (Biohit Inc., Neptune, New Jersey). A cryovial cap was tightly placed on the tube. Sometimes less than 3 ml of blood was collected and not all aliquots of plasma were obtained. As such, a record of all aliquots was made. Cryovial tubes were placed in cardboard cryovial storage boxes. Elastic bands were placed around the box and they were placed in the coldest freezer available in each community, which was usually minus 12ºC to minus 20ºC. Freezer temperature was recorded twice daily to ensure plasma samples would remain frozen.

When traveling from community to community, cryovial boxes were kept in coolers packed with icepacks. They were marked as “Keep frozen”. Because luggage is sometimes shipped later when traveling on small airplanes, airport staff were asked to give first priority to the coolers in terms of deciding which luggage would go on our airplane. When returning from Nunavut to Montreal, coolers were sent with checked baggage and marked as “Keep frozen”. Coolers were stored in freezers in any overnight stops. Cryovial boxes were placed in a locked minus 80ºC freezer upon return to CINE, McGill.
4.9. LABORATORY ANALYSES

4.9.1. Measurement of C-reactive protein

CRP was measured in the 2007 plasma samples using CRP (Human) Enzyme Linked Immunosorbance Assay (ELISA) (2007: Pheonix Pharmaceuticals, Inc., Burlingame, California). In 2008, plasma samples were sent out for CRP analyses due to time and labour constraints. They were sent to a clinical diagnostic laboratory at the Montreal General Hospital.

The 2007 plasma samples were analyzed as follows. Frozen plasma samples were thawed over ice for 1 hour. 10 µL of plasma sample was diluted in 1490 µL of 1x assay buffer concentrate to provide a 1:150 dilution of sample. A standard curve was created by creating a serial dilution of the standards provided. 100 µL of standards, samples and a control were added in duplicate to plated wells. Two wells were left blank so as to have a zero concentration value on the standard curve. The control and one of the samples were plated in triplicate to allow for calculation of the coefficient of variation (CV). The plate was sealed and incubated on the shaker at medium speed for 2 hours at room temperature (18°C - 27°C). Wells were washed with 300 µL assay buffer per well. The plate was washed four times. 100 µL of anti-human CRP-HRP Detection Antibody was added to each well except for the blank wells. The plate was sealed and incubated for 2 hours at room temperature (18°C - 27°C) on the shaker at medium speed. Wells were washed again four times with 300µL assay buffer per well. In a dark room, 100 µL of tetramethylbenzidine substrate solution was added to each well. The plate was sealed and incubated for 25 minutes at room temperature on a shaker at medium speed in the dark. 100 µL of 2N HCL stop solution was added to each well. The plate was read at 450 nm using a spectrophotometer. CRP concentrations were quantified from optical density (OD) results using GraphPad Prism 4.

The CVs for the plasma sample and control plated in triplicate ranged from 0.99% to 10.8%, except for one control CV that was high at 42%. All samples with a CV less than 10% were repeated. Outliers were repeated using a different dilution. Low outliers were
repeated using a dilution of 1:25 to 1:50. High outliers were repeated using a dilution of 1:750 to 1:1000. We were unable to determine an exact value for some samples because the concentration of CRP in the plasma was either too high or too low and the second or third attempt at diluting still produced an outlier result. These samples were noted and the low concentration samples were assumed to be below the CRP cut-off. Similarly, the high concentration samples were assumed to be above the CRP cut-off.

The 2008 plasma samples were measured for CRP using a SYNCHRON® Autoanalyzer (Coulter Beckman, USA) and a high-sensitivity CRP (hsCRP) assay at the Montréal General Hospital, Montréal, Québec. The autoanalyzer was suitable for use with human serum and plasma. According to the manufacturer, plasma samples collected with sodium heparin anticoagulant had CRP concentrations that were correlated well with serum samples (r = 0.997). The principals of the hsCRP assay are as follows. The autoanalyzer proportions one part plasma sample to twenty-six parts hsCRP reagent. The reagent contains 17.3 mL CRP antibody (particle bound goat and mouse anti-CRP antibody, 47.8 mL reagent buffer, <0.1% (w/w) sodium azide and <0.125% (w/v) bovine serum albumin). 300 µL of plasma sample was used in the analyses. CRP in the human plasma sample mixes in the autoanalyzer with particle bound goat and mouse anti-CRP antibody. The SYNCHRON® system monitors the change in absorbance at 940 nm. The absorbance is proportional to the CRP concentration in the sample. CRP concentration is then calculated from a pre-determined calibration curve.

4.9.2. Measurement of Helicobacter pylori exposure status

To measure exposure to \textit{H. pylori}, plasma samples were tested for IgG antibodies against the pathogen. The presence of anti-\textit{H. pylori} antibodies indicates exposure to the pathogen but not necessarily current infection. Current infection is assessed using gastric endoscopy, a urea breath test or a stool antigen test, all of which were not used due to logistical constraints in remote field settings.

Plasma samples were shipped in coolers from CINE to the laboratory of Dr. Brian Ward at McGill University, Centre for the Study of Host Resistance, Montreal General Hospital, where they were analyzed for \textit{H. pylori} exposure. A qualitative ELISA (Pylori
Detect IgG, Calbiotech, Spring Valley, California) was used to analyze plasma samples for the presence of anti-*H. pylori* IgG antibodies. ELISA kits were brought to room temperature (18°C to 27°C) prior to use. Plasma samples were thawed and diluted using 10 µL of plasma and 1 mL of diluent/wash solution provided by the ELISA kit manufacturer. 100 µL of each sample was pipetted into microwells coated with *H. pylori* antigen. Samples were tested in singlet. One each of a blank, calibrator, positive control and negative control well was created on each microplate. Microplates were incubated for 20 minutes at room temperature. Wells were washed three times with diluent/wash solution. 100 µL of goat anti human IgG enzyme conjugate was added to each well allowed to incubate at room temperature for 30 minutes. Wells were washed again three times using diluent/wash solution. 100 µL TMB substrate was added to each well and then after a 20 minute incubation at room temperature, 100 µL of stop solution was added to each well. Absorbance was read at 450 nm using a spectrophotometer within 15 minutes of the addition of stop solution and optical density obtained for each sample.

To interpret results, the OD of the plasma sample was divided by the OD of the calibrator. If the result, also called the ELISA value, was greater than or equal to 1, then the sample was positive for *H. pylori* exposure. ELISA values less than 0.89 was negative for *H. pylori* exposure. Samples between 0.89 and 0.99 were equivocal and were retested once. If upon retest samples were still equivocal, results were said to be indeterminate. No problems with blanks, positive controls or negative controls were reported. The reported sensitivity of the Pylori Detect IgG ELISA was 98.3% and specificity was 94.4% when compared to endoscopy. The cut-off values for exposure, non-exposure or equivocal results were determined using sera from confirmed infected and non-infected human patients.

### 4.9.3. Measurement of ferritin

Ferritin was measured from plasma samples using the Liason® autoanalyzer in the laboratory of Dr. Hope Weiler in the School of Dietetics and Human Nutrition, McGill University. Frozen plasma samples were thawed at room temperature (18°C - 27°C) and at least 200 µL was pipetted into glass test tubes. All samples were spun at 2000xg for 5
minutes at 4°C prior to loading onto the Liason®. Centrifugation of samples ensured that any clots present in the sample were spun down, lessening the chance of clogging the Liason® pipettor needles. This was especially important because the samples were plasma and not serum, and as such, contained clotting factors. The presence of clotting factors in plasma may increase the production of clots in the sample.

Racks were loaded into the Liason®. Study numbers were entered and the appropriate tests were ordered for each sample. Ferritin concentrations were measured using a Liason® Ferritin integral (REF 313551, DiaSorin, Italy). The ferritin reagent integral was gently shaken horizontally before removing seals. Magnetic particles were re-suspended by turning the thumb wheel at the bottom of the container back and forth until the suspension turned brown. The integral was placed in the Liason® reagent area and was left to stand for 30 minutes prior to using. A calibrator within the integral allows the Liason® to re-calibrate the standard curve for each use. High, low and normal ferritin controls were tested and were within expected range. The Liason® measures ferritin by adding sample to a solution containing magnetic particles coated with specified mouse monoclonal antibodies. A second monoclonal antibody conjugates to any ferritin present in the sample and is linked to an isoluminal derivative, which allows for quantitative measurement of the sample. The solution is incubated for 10 minutes and is then washed with Liason® Wash/System Liquid (REF 319100). Starter reagents from the Liason® Starter Kit (REF 319102) are added to the solution and induce a flash chemiluminescence reaction which is measurable by a photomultiplier within the autoanalyzer. Results are produced in relative light units and converted to ng/mL. The Liason® measures ferritin concentrations between 0.5 and 3000 ng/mL.

4.10. DATA MANAGEMENT

Food frequency information was entered using EpiInfo Version 6 and data was double verified. Twenty four-hour dietary recall information was entered using CANDAT. Nutrient composition of foods was determined using the Canadian Nutrient File. CANDAT was updated for various missing items using USDA nutrient composition data. All 24-hour recalls were double verified. When information was missing from 24-hour
recalls, these were considered invalid and excluded from analyses. For example, when a caregiver reported that the child ate meals at a relative’s home, but did not know what was consumed, this recall was excluded. Unreasonable intakes were adjusted.

In addition, some assumptions were made when entering the 24-hour recall data. Firstly, when caregivers reported that their child ate snacks or meals at daycare and did not know what the child had, a default meal or snack was entered. Default snacks/meals were determined using information from 24-hour recalls where this information was provided in detail. The following defaults were entered:

- **Daycare afternoon snack**, one-fifth of: Yogurt (fruit bottom 2-4%, 100 g), water (250 mL), raw apple (0.33 fruit), raw orange (0.33 fruit), raw banana (0.33 fruit), raw celery (one 10 cm strip), raw carrot (one medium strip), raw broccoli (1 stalk), raw cucumber (0.05 of one 22 cm cucumber), white bread (1 slice), cheese slice (21.0 grams), bologna (1 slice 0.3 cm thick), butter (1 pat), jam, (15 mL), Nutrigrain Bar (37.0 grams), two muffins

- **Daycare morning snack**, one-sixth of: hard boiled egg (0.75 large egg), scrambled egg (0.75 large egg), Shreddies cereal (250 mL), Cheerios cereal (250 mL), Rice Krispies cereal (250 mL), 1% milk (250 mL), 2% milk (250 mL), apple juice (2.5 glasses, 125 mL each), orange juice (2.5 glasses, 125 mL each), pancake (10 cm), raw apple (0.5 fruit)

- **Daycare lunch**, one-half of: bagel (7.6 cm), one english muffin, banana (0.25 fruit), 1% milk (250 mL)

The remaining questionnaire and clinical information was entered into a Microsoft Access Database designed for the Inuit Child Health Survey using Microsoft Access 2003. Data was entered into the database exactly as it was recorded in the questionnaire according to a standard protocol. Then, data was generated and checked for data entry errors.
4.11. **Statistical analyses**

Prevalence rates of anemia, IDA and iron deficiency were estimated as the proportion of participating children presenting with the condition. Agresti-Coull 95% CIs were calculated for each of the prevalence rates. Prevalence rates and 95% CIs were also determined for each of the binomial risk factors.

Age specific WHO 2001 cut-off values for iron deficiency and anemia were used (Table 4-3). Ferritin less than 12 µg/L was used to define iron deficiency. Hemoglobin less than 110 g/L in 3 to 4 year olds, and less than 115 g/L in 5 year olds, was used to define anemia. IDA was defined as the presence of low ferritin coupled with low hemoglobin. A 10 µg/L cut-off value for ferritin in children has been used in similar studies. We also performed statistical analyses using this cut-off. Further, the CRP cut-off for acute inflammation is unclear in children. Some studies have used 8 or 10 ng/mL [51, 52]. Other studies suggest that 2 to 3 ng/mL are more appropriate [50, 53]. In young children, baseline CRP levels should be almost zero and any elevation in CRP could be indicative of acute inflammation occurring in the days leading up to sampling [50]. CRP levels may be slightly elevated as they return to normal after inflammation, but ferritin can still be elevated [50]. We performed statistical analyses using both 8 ng/mL and 3 ng/mL cut-off levels. Finally, because we used two different blood sampling methods, venous puncture and finger prick, we compared the hemoglobin concentration within each type of sample to test for differences.

Usual iron intake from 24-hour recall was estimated from observed intake using Software for Intake Distribution Estimation (SIDE) developed by Iowa State University. SAS version 9.1 was used to run SIDE software. Within-person variability was estimated using information from the 19.8% sub-sample of repeat recalls. The percentage of children below the age-appropriate EAR for iron was determined.

The use of a qualitative FFQ data in examining relationships with health outcomes is still an area of exploration in nutrition research. One approach is to determine nutrient intake from the FFQ by assuming an average portion size, often determined from 24-hour dietary recalls previously administered in the study population [146, 147]. Another
approach was to group people into quantiles of frequency of consumption. A scoring system was created using a combination of NHANES (1971-1975) 24-hour recall dietary data and FFQ data [148]. Subjects in the highest quartile of calcium intake from a 24-hour recall and highest quartile of calcium containing foods from the FFQ were grouped into one category. Similarly, people in both of the lowest quartiles were grouped into one category. Everyone else was grouped in an “All others” category. However, because it is difficult to be sure in this study population that portion sizes are similar for children, the FFQ was only used to provide descriptions of commonly consumed foods.

The outcome variables of interest were IDA and iron deficiency. Risk factor variables, \textit{H. pylori} exposure, household crowding, food insecurity, having a younger sibling and iron intake, as well as age, sex and region are detailed in Table 4-3. Bivariate analyses of outcome and risk factors as well as region, age and sex were performed using Chi-squared test and Fisher’s exact test if there were less than 10 children in a group. Relative Risks and 95% confidence intervals were calculated for each of the exposure variables when p < 0.05. Chi-squared tests were used to examine intercorrelation of independent variables. Multivariate analyses were performed using multiple-logistic regression.

Dietary adequacy analyses were determined using SIDE in SAS version 9.1. All other analyses were performed using Stata 10 (Stata Corp, Texas).
Table 4-1. Nunavut communities, location and population sizes.

<table>
<thead>
<tr>
<th>Community</th>
<th>Pop. size - all ages</th>
<th>Pop. size - 0 to 4 y</th>
<th>% Inuit</th>
<th>Estimated pop. size - 3 to 5 y</th>
<th>20% sample - 3 to 5 y</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kivalliq</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arviat*</td>
<td>2319</td>
<td>345</td>
<td>0.94</td>
<td>290</td>
<td>58</td>
<td>61° 6' N</td>
<td>94° 4' W</td>
</tr>
<tr>
<td>Baker Lake*</td>
<td>1683</td>
<td>175</td>
<td>0.93</td>
<td>147</td>
<td>29</td>
<td>64°18' N</td>
<td>96° 5' W</td>
</tr>
<tr>
<td>Chesterfield Inlet*</td>
<td>366</td>
<td>48</td>
<td>0.93</td>
<td>40</td>
<td>8</td>
<td>63° 21' N</td>
<td>90° 43' W</td>
</tr>
<tr>
<td>Coral Harbour*</td>
<td>780</td>
<td>105</td>
<td>0.97</td>
<td>88</td>
<td>18</td>
<td>64° 11' N</td>
<td>83° 21' W</td>
</tr>
<tr>
<td>Rankin Inlet*</td>
<td>2376</td>
<td>277</td>
<td>0.77</td>
<td>233</td>
<td>47</td>
<td>62° 49' N</td>
<td>92° 7' W</td>
</tr>
<tr>
<td>Repulse Bay</td>
<td>686</td>
<td>107</td>
<td>0.97</td>
<td>90</td>
<td>10</td>
<td>66° 31' N</td>
<td>86° 13' W</td>
</tr>
<tr>
<td>Sanikiluaq*</td>
<td>742</td>
<td>92</td>
<td>0.95</td>
<td>77</td>
<td>15</td>
<td>56° 32' N</td>
<td>79° 15' W</td>
</tr>
<tr>
<td>Whale Cove*</td>
<td>316</td>
<td>42</td>
<td>0.95</td>
<td>35</td>
<td>7</td>
<td>62° 14' N</td>
<td>92° 36' W</td>
</tr>
<tr>
<td><strong>Baffin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arctic Bay</td>
<td>662</td>
<td>68</td>
<td>0.95</td>
<td>57</td>
<td></td>
<td>72° 59' N</td>
<td>85° 0' W</td>
</tr>
<tr>
<td>Cape Dorset</td>
<td>1193</td>
<td>144</td>
<td>0.93</td>
<td>121</td>
<td></td>
<td>64° 13' N</td>
<td>76° 31' W</td>
</tr>
<tr>
<td>Clyde River*</td>
<td>868</td>
<td>133</td>
<td>0.96</td>
<td>112</td>
<td>22</td>
<td>70° 29' N</td>
<td>68° 31' W</td>
</tr>
<tr>
<td>Grise Fiord</td>
<td>180</td>
<td>30</td>
<td>0.91</td>
<td>25</td>
<td></td>
<td>76° 25' N</td>
<td>82° 54' W</td>
</tr>
<tr>
<td>Hall Beach</td>
<td>678</td>
<td>104</td>
<td>0.96</td>
<td>87</td>
<td></td>
<td>68° 46' N</td>
<td>81° 14' W</td>
</tr>
<tr>
<td>Igloolik*</td>
<td>1404</td>
<td>205</td>
<td>0.95</td>
<td>172</td>
<td>34</td>
<td>69° 22' N</td>
<td>81° 48' W</td>
</tr>
<tr>
<td>Iqaluit*</td>
<td>6304</td>
<td>560</td>
<td>0.57</td>
<td>470</td>
<td>94</td>
<td>63° 45' N</td>
<td>68° 33' W</td>
</tr>
<tr>
<td>Kimmirut*</td>
<td>470</td>
<td>60</td>
<td>0.94</td>
<td>50</td>
<td>10</td>
<td>62° 51' N</td>
<td>69° 52' W</td>
</tr>
<tr>
<td>Pangnirtung*</td>
<td>1324</td>
<td>157</td>
<td>0.95</td>
<td>132</td>
<td>26</td>
<td>66° 7' N</td>
<td>65° 42' W</td>
</tr>
<tr>
<td>Pond Inlet*</td>
<td>1298</td>
<td>187</td>
<td>0.94</td>
<td>157</td>
<td>31</td>
<td>72° 40' N</td>
<td>77° 58' W</td>
</tr>
<tr>
<td>Qikitarjuaq</td>
<td>552</td>
<td>69</td>
<td>0.95</td>
<td>58</td>
<td></td>
<td>67° 33' N</td>
<td>64° 1' W</td>
</tr>
<tr>
<td>Resolute Bay</td>
<td>233</td>
<td>31</td>
<td>0.79</td>
<td>26</td>
<td></td>
<td>74° 43' N</td>
<td>94° 59' W</td>
</tr>
<tr>
<td><strong>Kitikmeot</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cambridge Bay*</td>
<td>1387</td>
<td>135</td>
<td>0.79</td>
<td>113</td>
<td>23</td>
<td>69° 6' N</td>
<td>105° 8' W</td>
</tr>
<tr>
<td>Gjoa Haven</td>
<td>1116</td>
<td>147</td>
<td>0.95</td>
<td>123</td>
<td></td>
<td>68° 38' N</td>
<td>95° 51' W</td>
</tr>
<tr>
<td>Kugaaruk*</td>
<td>770</td>
<td>129</td>
<td>0.95</td>
<td>108</td>
<td>22</td>
<td>68° 32' N</td>
<td>89° 48' W</td>
</tr>
<tr>
<td>Kugluktuk*</td>
<td>1324</td>
<td>139</td>
<td>0.92</td>
<td>117</td>
<td>23</td>
<td>67° 49' N</td>
<td>115° 8' W</td>
</tr>
<tr>
<td>Taloyoak</td>
<td>851</td>
<td>120</td>
<td>0.92</td>
<td>101</td>
<td></td>
<td>69° 33' N</td>
<td>93° 35' W</td>
</tr>
<tr>
<td><strong>TOTAL:</strong></td>
<td><strong>29882</strong></td>
<td><strong>3609</strong></td>
<td><strong>3032</strong></td>
<td><strong>469</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Community participated in Inuit Child Health Survey 2007-2008
*Sanikiluaq is a community in Baffin Region, but given its geographical proximity to Kivalliq region, it will grouped with Kivalliq communities for statistical analyses
<table>
<thead>
<tr>
<th>Community</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanikiluaq</td>
<td>August 8, 2007 – August 10, 2007</td>
</tr>
<tr>
<td>Pond Inlet</td>
<td>September 26, 2007 – September 30, 2007</td>
</tr>
<tr>
<td>Igloolik</td>
<td>October 2, 2007 – October 5, 2007</td>
</tr>
<tr>
<td>Clyde River</td>
<td>October 8, 2007 – October 11, 2007</td>
</tr>
<tr>
<td>Pangnirtung</td>
<td>October 13, 2007 – October 18, 2007</td>
</tr>
<tr>
<td>Kimmirut</td>
<td>October 29, 2007 – November 1, 2007</td>
</tr>
<tr>
<td>Rankin Inlet</td>
<td>November 2, 2007 – November 9, 2007</td>
</tr>
<tr>
<td>Coral Harbour</td>
<td>November 9, 2007 – November 13, 2007</td>
</tr>
<tr>
<td>Chesterfield Inlet</td>
<td>November 14, 2007 – November 16, 2007</td>
</tr>
<tr>
<td>Arviat</td>
<td>November 17, 2007 – November 21, 2007</td>
</tr>
<tr>
<td>Cambridge Bay</td>
<td>August 21, 2008 – August 23, 2008</td>
</tr>
<tr>
<td>Kugaaruk</td>
<td>September 1, 2008 – September 3, 2008</td>
</tr>
<tr>
<td>Baker Lake</td>
<td>September 3 – September 9, 2008</td>
</tr>
<tr>
<td>Variable</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
</tr>
</tbody>
</table>
| Anemia<sup>a</sup> | - Ages 3 to 4 years: anemic (Hb < 110 g/L), non-anemic (Hb ≥ 110 g/L)  
- Ages 5 years and older: anemic (Hb < 115 g/L), non-anemic (Hb ≥ 115 g/L) | Binomial: anemic = 1, non-anemic = 0 |
| Iron Deficiency | - WHO cut-off<sup>a</sup>: iron deficient (ferritin < 12 µg/L), non-iron deficient (ferritin ≥ 12 µg/L)  
- Alternative cut-off<sup>b</sup>: iron deficient (ferritin < 10 µg/L), non-iron deficient (ferritin ≥ 10 µg/L) | Binomial: Iron deficient = 1, non-iron deficient = 0 |
| Iron deficiency anemia (IDA) | - IDA: low ferritin coupled with low hemoglobin, no IDA: normal ferritin and/or normal hemoglobin | Binomial: IDA = 1, no IDA = 0 |
| Acute inflammation | - Standard cut-off<sup>c</sup>: acute inflammation (CRP > 8 ng/ml), no inflammation (CRP ≤ 8 ng/ml)  
- Alternative cut-off<sup>d</sup>: acute inflammation (CRP > 3 ng/ml), no inflammation (CRP ≤ 3 ng/ml) | |
| H. pylori exposure | - No exposure to H. pylori, exposure to H. pylori, indeterminate results coded as missing | Binomial: exposure = 1, no exposure = 0 |
| Household crowding | - Crowding (above median people/home), no crowding (below median people/home) | Binomial: crowding = 1, no crowding = 0 |
| Food Insecurity | - Evidence of child hunger (affirmative response on ≥ 5 child-specific questions from the USDA food security module), no evidence of child hunger (affirmative response on ≤ 4 child-specific questions from the USDA food security module)<sup>e</sup> | Binomial: evidence = 1, no evidence = 0 |
| Region | Kivalliq, Baffin or Kitikmeot region | Categorical |
| Age | 5 years (≥ 5.00 years)  
4 years (4.00 – 4.99 years)  
3 years (<4.00 years) | Categorical and Binomial: 5 years = 1, 3 to 4 years = 0 |
| Sex | Male or female | Binomial: male = 1, female = 0 |
| Dietary iron intake | Adjusted iron intake in milligrams from 24-hour and repeat 24-hour dietary recall data | Continuous |
| Anthropometry | BMI-for-age: Overweight (>95<sup>th</sup> %ile), at risk for overweight (85-95<sup>th</sup> %ile), normal weight (5<sup>th</sup>-85<sup>th</sup> %ile), underweight (<5<sup>th</sup>%ile) | Categorical and continuous |

<sup>a</sup> From [28]  
<sup>b</sup> From [44]  
<sup>c</sup> From [51, 52]  
<sup>d</sup> From [50, 53]  
<sup>e</sup> From [144]  
<sup>f</sup> From [145]
5 MANUSCRIPT

Low prevalence of iron deficiency anemia among Inuit children ages 3 to 5 years, living in Nunavut

Angela Pacey and Grace M. Egeland

Centre for Indigenous Peoples’ Nutrition and Environment, School of Dietetics and Human Nutrition, McGill University

Corresponding Author and for Reprints:

Dr. Grace Egeland, PhD

Centre for Indigenous Peoples’ Nutrition and Environment, MacDonald Campus

McGill University

Ste. Anne-de-Bellevue, QC, H9X 3V9, Canada

Telephone: (514) 398-7757 Facsimile: (514) 398-1020

E-mail: grace.egeland@mcgill.ca
Abstract

Objectives: To report the prevalence of iron deficiency and iron deficiency anemia (IDA) and to identify key risk factors among Inuit children living in Nunavut.

Design: In a cross-sectional study, usual iron intake was assessed using a 24-hour dietary recall and a sub-sample of repeat recalls. Interviews were conducted regarding household demographic characteristics, food security and frequency of meat and cereal intake. Anthropometric measurements were taken and blood samples were collected for the measurement of hemoglobin (Hb), ferritin, C-reactive protein (CRP), and antibodies to Helicobacter pylori (H. pylori)

Setting: Sixteen Inuit communities in Nunavut Territory, Canada.

Subjects: Three hundred eighty-eight Inuit children aged 3 to 5 years.

Results: Anemia (3 to 4 years: Hb < 110 g/L, 5 years: Hb < 115 g/L) was prevalent in 20.3% of children. The prevalence of iron deficiency (ferritin < 12 µg/L) was 19.2% of children and IDA was 4.4%. When iron deficiency was defined as ferritin less than 10 µg/L, 10.3% of children were iron deficient and 2.6% were iron deficient anemic. Iron intake was adequate as only 0.3% of children had usual iron intakes below the estimated average requirement (EAR). Exposure to H. pylori, food insecurity and household crowding were not associated with iron deficiency or IDA. However, 5 year olds were less likely to be iron deficient than 3 to 4 year olds (RR = 0.40, 95% CI: 0.19-0.86). Boys were more likely to be iron deficient than girls (RR = 3.5, 95% CI: 1.09-11.2). In a multiple logistic regression model, boys were independently more likely to be iron deficient (Age adjusted OR: 2.87, 95% CI: 1.21 – 6.81).

Key words: iron deficiency, anemia, Aboriginal, Inuit, iron, Helicobacter pylori
Introduction

In infants and children, iron deficiency anemia (IDA) can have serious health consequences including impaired growth and cognitive development and weakened immune defense [15, 28, 30]. Iron deficiency typically exists in three stages: low iron stores, reduced iron delivery to the tissues and IDA characterized by low hemoglobin and reduced erythrocyte size [20]. ‘Aboriginal people’ is a collective name for the indigenous peoples of Canada including Inuit, First Nations and Dene/Métis [4]. It is believed that Inuit children have a higher prevalence of iron deficiency than non-Aboriginal children. However, the most recent estimates for infants and children combined is 24% for tissue iron deficiency and 5% for anemia from all causes [60]. More recent estimates for the prevalence of iron deficiency and IDA among Inuit infants are 36 to 60% for iron deficiency compared to 33% for non-Aboriginal Canadian infants [45, 46, 51]. IDA is thought to affect 26% of Inuit infants compared to 5% for non-Aboriginal infants [45, 46, 51]. Current information on iron deficiency and IDA among Canadian Inuit children in the preschool age group of 3 to 5 years is not available.

Key risk factors for iron deficiency in this group may include low dietary iron intake or infection with the human pathogen Helicobacter pylori (H. pylori). Previous studies show that iron intake is likely adequate among Inuit children [51, 56, 70, 74]. However, a nutrition transition in the Arctic is occurring rapidly so current dietary information for this age group that can be matched with iron status will be useful to obtain. In addition, infection with H. pylori has been recently shown to cause iron deficiency although the exact mechanism remains unclear [51, 56, 70, 74, 109, 110, 119, 121, 123-127]. This pathogen is highly prevalent in most Inuit populations and may increase risk for iron deficiency among children [51, 59, 84, 101]. Understanding the relationship of these risk factors with iron deficiency and IDA among Inuit children may be used to direct future health care planning. Therefore, we completed a cross-sectional survey of Inuit children in Nunavut that examined the iron status in the preschool age group, risk of inadequate iron intake as well as risk factors for iron deficiency.
Experimental Methods

Setting

This study is part of an Inuit Child Health Survey (ICHS) for preschool children in Nunavut, Canada. Sixteen of the 25 communities in Nunavut were selected to participate in the ICHS. Communities were selected based upon region, population size, latitude, and then finally logistical feasibility due to flight routes and financial constraints. Using currently available population census information, we estimated that a sample of 20% of 3 to 5 year olds in each community would achieve the sample size goal of 300 considering that some caregivers would refuse venous puncture. Inuit children, ages 3 to 5, were randomly selected to participate in the survey. Recruiters were instructed to make three attempts to reach households. Written informed consent was obtained from the child’s caregiver and the process was accompanied by a DVD guide, which was a word-for-word reading of the consent form, accompanied by photographs and video-clips relevant to the study.

The ICHS for Nunavut was developed by a steering committee of Inuit organizations and Canadian universities. Steering committee members helped to develop and review questionnaires and clinical methods. They reviewed the informed consent and DVD consent form, as well as coordinated the translations of materials into Inuktitut and Inuinnaqtun. Research agreements were signed between communities and the research centers. Certification of Ethical Acceptability for Research Involving Human Subjects was obtained from the McGill Faculty of Medicine Institutional Review Board. A Scientific Research License was obtained from the Nunavut Research Institute.

Anthropometry

A nurse measured height to the nearest 0.1 cm using a portable stadiometer (Road Rod 214 Portable Stadiometer, Seca, Maryland) and weight to the nearest 0.1 kg using an electronic scale. BMI-for age, height-for-age and weight-for-age were calculated using 2000 Centers for Disease Control (CDC) reference growth curves [145]. Overweight was
classified as being ≥95th%ile. At risk for overweight was classified as being ≥85th%ile and <95th%ile. Underweight was classified as being <5th%ile.

**Iron status and exposure to H. pylori**

Venous or capillary sampling was used to obtain blood samples. When venipuncture was used, 3 mL of blood was collected into sodium heparin Vacutainer® blood (Becton Dickinson and Company, Franklin Lakes, New Jersey). The vacutainer tube was inverted gently 10 times. One drop of whole blood was dispensed onto Parafilm (Pechiney, Chicago, Illinois) using a Diff-Safe® blood dispenser (Alpha Scientific Corporation, Southeastern, PA). Hemoglobin was measured either from this drop or from capillary blood samples using the cyanmethemoglobin method with HemoCueTM 201+ portable photometer (HemoCue, Inc., Lake Forest, California). Blood samples were centrifuged within 6 hours of collection. Separated plasma was stored at minus 20°C during field work and at minus 80°C after completion of data collection.

Serum ferritin was measured from plasma samples using an autoanalyzer (Liason®, DiaSorin, Saluggia, Italy) and a ferritin integral (REF 313551, DiaSorin, Saluggia, Italy). Low, normal and high control samples were tested with each analysis. In the first year of data collection, CRP was measured in plasma samples using a CRP (Human) Enzyme Linked Immunosorbance Assay (ELISA) (2007: Pheonix Pharmaceuticals, Inc., Burlingame, California). One sample and control were plated in triplicate and all CVs were under 10%. In the second year of data collection, plasma samples were sent out for CRP analyses due to time and labour constraints. They were sent to a clinical diagnostic laboratory at the Montreal General Hospital. A qualitative ELISA (Pylori Detect IgG, Calbiotech, Spring Valley, California) was used to analyze plasma samples for the presence of anti-\textit{H. pylori} IgG.

Iron deficiency was defined as low ferritin (< 12 µg/L) and anemia was defined as low hemoglobin (Hb < 110 g/L in 3 to 4 year olds, Hb < 115 g/L in 5 year olds) [28]. IDA was defined as the presence of low ferritin coupled with low hemoglobin. Because the cut-off value for low ferritin in children is unclear, we also conducted analyses using ferritin < 10 µg/L to define iron deficiency [44, 46].
**Dietary intake**

One 24-hour dietary recall was conducted for each child participant by training interviewers using a four stage, multiple pass interviewing technique. Food model kits were used to estimate portion sizes. Depending on the number of days spent in the community, all caregivers from the first or second day of appointments were asked to return to complete a repeat 24-hour dietary recall on a nonconsecutive day. We collected a 20% sub-sample of repeat 24-hour dietary recalls. Interviewers also asked about the child’s mineral and vitamin supplement use. Each caregiver was asked to complete a qualitative food frequency questionnaire (FFQ) for their child. The FFQ was designed to capture past month information about common country foods that are available in the three regions of Nunavut. It also included beef, fish, poultry, pork and breakfast cereal to capture commercially available market food sources of iron containing foods. Caregivers were asked about how often the child ate the foods in the past month. Food frequency information was entered using EpiInfo and data were double verified. Twenty-four hour dietary recall information was entered using CANDAT. Nutrient composition of foods was determined using the Canadian Nutrient File and a USDA Institution file. All 24-hour recalls were double verified.

**The home environment**

Interviewers asked questions about age, sex and relationships of everyone in the child’s household. They also conducted questionnaires for characteristics of the home including the USDA 18-item Household Food Security Survey Module adapted for Inuit populations [69, 144]. From the food security module, homes were classified as having “evidence of children hunger” when 5 or more affirmative responses on child-specific questions were given.

**Statistical analyses**

Prevalence rates and 95% CIs of anemia, IDA and iron deficiency were estimated as the proportion of participating children presenting with the condition. Usual iron intake from 24-hour recall was estimated from observed intake using Software for Intake Distribution
Estimation (SIDE) developed by Iowa State University. Within-person variability was estimated using information from the 20% sub-sample of repeat recalls. The percentage of children below the age-appropriate EAR for iron was determined. The frequency of consumption in number of days per month was calculated, both for consumers only and for all children, for iron containing traditional Inuit foods and commercially available market foods.

Bivariate analyses of outcome and risk factors as well as region, age and sex were performed using Chi-squared test and Fisher’s exact test when cell size were less than 10 children. Relative Risks (RR) and 95% confidence intervals were calculated for each of the exposure variables when p < 0.05. Multiple logistic regression was performed to examine independent effects of risk factor variables. For all analyses, a p-value less than 0.05 was considered significant.

Analyses were repeated using a low CRP cut-off because of the uncertainty of CRP reference values in children [50, 53]. Finally, we compared hemoglobin concentrations from finger prick samples to venous blood samples to assess measurement error from different sampling techniques.

Dietary adequacy analyses were determined using SIDE in SAS version 9.1. All other analyses were performed using Stata 10 (Stata Corp, Texas). Anthropometric analyses were performed in EpiInfo Nutrition Version 6.

**Results**

Of the 644 households that were initially approached, 537 were successfully contacted and overall, while 16.6% of homes were not reached. Of homes that were successfully contacted, 75 (11.6%) refused upon initial contact and 74 (13.8%) accepted, but later cancelled or did not show for the interview. The final participation rate was 72.3% (388) children over the two years of data collection.
**Population characteristics**

Fifty-three percent of the participating children were female and the mean age was 4.4 ± 0.9 years (Table 5-1). Thirty-seven percent of the children were from Kivalliq region, 44% from Baffin region and 19% from Kitikmeot region. Fifty-two percent of the children’s homes were crowded with six or more people per home and the mean number of people per bedroom was 2.1 ± 0.7. Evidence of hunger among children was found in 23.5% of the homes. Forty-three percent of the child participants lived in a home where there was a younger child. Children had high rates of overweight (50.8%) and many were at risk for overweight (27.3%). Underweight was found in 0.5%. Height-for-age and weight-for-age were normally distributed (Shapiro-Wilk test p = 0.07 for both). The median height-for-age was in the 49.6\textsuperscript{th} percentile, which is close to the median for the reference population. However, the median weight-for-age was at the 85.2\textsuperscript{nd} percentile, which is a large upward shift from the reference population, indicating that high weight-for-age and not short height-for-age is behind the high rates of overweight among the sample population. Children’s multi-vitamin and mineral supplements were consumed by 11.9%. Those with added iron were consumed by 2.6% of children.

We obtained venous blood from 289 out of 388 children (74.7%). Capillary blood samples for hemoglobin were obtained for 79 out of 388 children (20.4%). Nineteen children did not undergo venous or capillary blood sampling (4.9%). Reasons included refusal by child or caregiver, skin infection or inability to obtain vein for sampling. Hemoglobin values were obtained for 364 of the 388 participating children. *H. pylori* status was measured for 289 samples and 7 samples with indeterminable results were excluded from analyses. Plasma ferritin was determined for 283 samples and not measured on 7 samples due to low sample volume. CRP was quantitatively determined for 257 children. Twenty-seven children had concentrations below the detection limit (0.05 ng/ml in first year, 0.20 ng/ml in second year) and were thus considered to be below the cut-off. Three samples had CRP concentrations above the detection limit after attempting three assays with different dilutions. These were considered to be above the cut-off for elevated CRP.
Overall, 4.2% (12/286) had CRP concentrations greater than 8 ng/ml. These children were excluded from analyses involving ferritin. The mean CRP concentration was 2.95 ng/ml and the median was 0.80 ng/ml among the participating children (n = 287).

**Iron status**

Mean ferritin concentration for all children was 19.9 µg/L (n = 283). For only children with low CRP, the mean ferritin concentration was 19.3 ± 10.3 µg/L (n = 271, Table 5-2). Mean hemoglobin was 118 ± 9 g/L and 117 ± 8 g/L among the 3 to 4 year olds and 5 year olds respectively. Mean hemoglobin for all ages was 117 ± 9 g/L.

Overall, 19.2% of children were iron deficient (Table 5-3). Using a ferritin cut-off of 10 µg/L, the prevalence of iron deficiency decreased to 10.3%. Anemia from all causes was found in 20.3% of the study population. IDA was found in 4.4% of children but when the lower ferritin cut-off was used, IDA prevalence decreased to 2.6% of children. Iron deficiency explained 30.8% of the observed anemia while the remaining anemia (69.2%) was likely due to other causes. When the lower ferritin cut-off was used, iron deficiency explained only 18.0% of anemia. Among the children with anemia, 17.9% (65/364) had Hb ≥ 100 g/L, but below the age-specific cut-off, otherwise known as mild anemia. Hemoglobin < 100 g/L was observed in 2.5% (9/364). *H. pylori* exposure was found in 45.4% of children (Table 5-3).

**Dietary iron intake**

Iron intake appeared to be normally distributed with a mean of 15.6 ± 10.9 mg/day and median of 13.5 mg/day (Figure 5-1, Table 5-4). Only 0.3% of children had iron intakes below their age-specific EAR. Mean vitamin C intake was 229 ± 329 mg/day and only 0.14% had intakes below the EAR for vitamin C.

Based on analysis of the qualitative FFQ, the most commonly consumed traditional foods were caribou meat (84.2% of children), fish (65.3%) and ringed seal meat (49.5%) (Table 5-5). On average, among those who consumed the food in the past month, children ate caribou on 11.8 days of the month. They ate fish on 7.1 days of the month and ringed seal meat was eaten on 4.4 days of the month. With respect to iron containing market foods,
96.3% of the children ate breakfast cereal and among these, they ate it on 28.9 days of the month on average. Beef was consumed by 82.6% of children on 10.4 days per month on average.

Risk factors for iron deficiency and IDA

Exposure to \( H. pylori \), child hunger and having a younger sibling and household crowding were examined as risk factors for iron deficiency and IDA, using ferritin cut-off values of 10 µg/L and 12 µg/L. Age, sex and region were also examined for possible associations. The proportions of children with iron deficiency or IDA were similar, regardless of exposure to the risk factors investigated (Table 5-6). However, at the lower ferritin cut-off, both age and sex were significantly associated with iron deficiency but not IDA. Boys were more likely to be iron deficient than girls (RR = 2.75, 95% CI: 1.26-6.03). Children aged 3 to 4 years were more likely to be iron deficient (RR: 3.37, 95% CI: 1.05-10.83). We performed post-hoc tests for differences in mean dietary iron intake between sex and age groups. There were no differences in mean intake between boys (15.5 ± 5.8 mg) and girls (15.6 ± 6.1 mg) (p = 0.805). Similarly, there were no mean intake differences between 3 to 4 year olds (15.3 ± 5.4 mg) and 5 year olds (16.3 ± 7.2) (p = 0.164). We noticed that BMI-for-age was significantly higher in 3 to 4 year olds (z = 1.66) than in 5 year olds (z = 1.33) (p < 0.001). However, here were no significant differences in BMI among iron deficiency or IDA groups or based on sex.

In fully adjusted multiple logistic regression models containing all risk factor variables and age and sex, only sex was independently associated with iron deficiency defined as ferritin less than 10 µg/L (Fully adjusted OR = 2.62, 95% CI: 1.08-6.38, p = 0.034). In a post-hoc model containing only age and sex, sex remained associated with iron deficiency (Age adjusted OR: 2.87, 95% CI: 1.21 – 6.81).

Intercorrelation between independent variables

Investigation of intercorrelation between independent variables revealed that household crowding was significantly associated with having a younger child in the home (p < 0.001). Sixty-three of 182 homes had a younger child and were not crowded (34.6%)
whereas 104 of 195 homes had a younger child and were crowded (53.3%). In addition, infection with *H. pylori* was associated with Nunavut region in bivariate analyses. Kitikmeot had a higher prevalence of 62.8% (32/51) than Baffin (39.0%, 53/136) or Kivalliq regions (45.3%, 43/95) ($X^2=8.4587, p = 0.015$). No other independent variables were significantly correlated.

**Differences in capillary and venous blood hemoglobin**

The mean Hb concentration when using capillary blood samples (114.2 g/L, n=79) was significantly lower ($p < 0.001$) than the mean Hb concentration when using venous blood samples (118.3 g/L, n = 285). Because of this, we reassessed anemia and iron status using only the venous blood samples. The prevalence of anemia then decreased somewhat to 16.5%. However, the prevalence of IDA did not change. Bivariate analyses were re-analyzed including only venous blood samples and no change was seen in the results.

**Alternative CRP cut-off value**

The above prevalence rates were determined using CRP < 8 ng/mL to determine valid ferritin measures. We also used a CRP cut-off of 3ng/mL to indicate acute inflammation in children. High CRP was found in 21.3% at this cut-off level. Among the remaining children with low CRP, the mean ferritin concentration was 18.9 µg/L (n = 225). This is similar to the mean ferritin concentration determined from children with CRP less than 8 ng/mL (19.3 µg/L). At the lower CRP cut-off, the prevalence of iron deficiency was 20.9% (95% CI: 15.8-26.8%) and IDA was 5.0% (95% CI: 2.5-8.7%), which is similar to prevalence rates determined using the higher CRP cut-off. Bivariate analyses also did not change at this cut-off, but IDA was no longer associated with any of the risk factor variables.

**Discussion**

The low prevalence rate of IDA among Nunavut’s preschoolers is encouraging. Previous studies have reported high rates of IDA and anemia among Canadian Inuit infants and the current findings reveal that the slightly older age groups are less affected [45, 51, 56]. It is concerning that iron deficiency defined by low ferritin is more common among Inuit
preschoolers and than American preschoolers. In the United States, iron deficiency is prevalent among 4.5% of children aged 3 to 5 years, compared to 10.3% to 19.2% in Nunavut [64]. IDA is found among 0.5% of American children while 2.6% to 4.5% of Nunavut’s preschoolers are affected [64]. More rigorous measures were used to define iron deficiency in the United States. As such, a lower rate of iron deficiency would be expected. However, the gap is quite large and this trend where Inuit children have higher rates of iron deficiency has been observed before in a nutrition survey in Canada, with Alaska Natives as well as with Canadian Inuit infants [45, 46, 51, 59, 60, 64]. The prevalence rates determined from this study would likely be defined as mild according to WHO thresholds for population-level iron deficiency [27]. Since they continue to be higher among Inuit compared to the general population, and improvements are possible.

Iron intake in this population is probably adequate since only 0.3% of children had intakes below the EAR. In addition, beef, breakfast cereals, most of which are iron-fortified and caribou were frequently consumed in this population. Vitamin C intake is also probably adequate suggesting that reduced absorption of non-heme iron is not likely. While over-reporting of portion sizes on 24-hour dietary recalls is possible, the mean and median intake levels are similar in this study compared to others in Inuit children, Dene/Métis children and American children overall [56, 66, 70, 149].

However, given that iron deficiency was found more in children aged 3 to 4 years than in 5 years, perhaps dietary differences between these age groups explain this finding. For example, high consumption of cow’s milk and evaporated milk was an independent risk factor for iron deficiency in Inuit infants [51]. This risk factor may continue to play a role in early childhood but requires further investigation. However, the finding that boys were more at risk than girls is difficult to explain given that there were no differences in dietary intake between the two groups.

One possible explanation for the finding that more boys than girls and more 3 to 4 year old than 5 year olds were iron deficient may be related to BMI-for-age. It has recently been shown that obesity and being overweight is associated with greater risk of iron deficiency, perhaps due to low diet quality, increased iron requirement due to higher
blood volume as well as decreased iron absorption induced by chronic low grade inflammation [150-154]. Although there were no significant associations between iron deficiency and BMI-for-age in the current study, we noticed that more 3 to 4 year olds than 5 year olds were overweight and even though it was not significant, there was a tendency for more boys to have higher BMI-for-age z scores than girls. Perhaps iron deficiency is more prevalent among younger children and boys because of higher rates of overweight and at risk for overweight in these groups. In addition, we may have been limited in our sample size in detecting associations between BMI-for-age and iron deficiency.

The 45.4% prevalence of \( H. \text{pylori} \) exposure observed in this study is high and consistent with other studies with Canadian First Nations and Inuit and Alaskan Native children [51, 59, 84, 101, 102]. Much lower rates of 5.5% to 7.1% have been reported for American and Canadian children [64, 100]. It should be noted that using serodiagnosis to measure \( H. \text{pylori} \) only allows us to estimate previous exposure, and not current infection and may underestimate the prevalence of \( H. \text{pylori} \) infection in this age group [93-95].

\( H. \text{pylori} \) has been previously shown to be independently associated with iron deficiency in other populations including Inuit [59, 64]. In the United States, it was reported that \( H. \text{pylori} \) was independently associated with IDA (OR: 2.6, 95% CI: 1.5-4.6), but not iron deficiency alone for children and adults older than 3 years [59, 64]. Among Alaska Native, \( H. \text{pylori} \) was only independently associated with iron deficiency and not IDA for children in the 9 and older age group, and not in younger age groups [59]. In addition, many case reports from which evidence for this association first emerged involved mostly children in the 9 to 15 year old age group [116, 123-126]. It is perhaps this age-dependent association that may explain why among Inuit preschoolers, there was no association between \( H. \text{pylori} \) and iron status. The mechanism by which \( H. \text{pylori} \) induces iron deficiency is still unclear but may be related to bacterial damage to gastric glandular tissue and iron competition in the stomach [109, 111, 112, 119-121]. If these are indeed the mechanisms, perhaps younger children who are more recently infected, are more protected than older children who have more established infections.
Finally, in the current study, anemia from all causes was found in 16.5% of Inuit children and only 18.0 to 30.8% of anemia was explained by low iron status. Other studies in children and infants have shown similar results where only a portion of the observed anemia is explained by iron deficiency [36, 37, 155]. A possible explanation for this finding is the presence of acute infection, where red blood cell half-life is decreased and the acute phase response block iron export proteins trapping iron inside cells [17, 156, 157]. Other dietary causes of anemia include deficiencies in vitamin A, folate, B12 and riboflavin [35]. Given the high meat and cereal intake from food frequency information, these micronutrient deficiencies are unlikely.

**Limitations**

The present study had a cross-sectional design that allowed us to report observations but not causal relationships. With only a sub-sample of repeat dietary recalls, we can only estimate usual intake in this population and have no measure of each individual’s usual intake. The limitations to using serodiagnosis for *H. pylori* were noted above.

**Conclusion**

Inuit children aged 3 to 5 years, have higher rates of iron deficiency, IDA and anemia than non-Aboriginal children. However, these rates are not so high that they suggest a need for immediate intervention. Instead, we should consider the practices in Nunavut’s communities that are helping to prevent iron deficiency in many children in addition to where public health policies can be improved. This study also revealed that iron deficiency explains only 18.0 to 30.8% of low hemoglobin. As such, low hemoglobin findings among Inuit children likely need additional assessments to better understand the etiology. Finally, young Inuit children have adequate iron intake. Careful work is needed to address the high prevalence of overweight while maintaining these good iron intakes among young Inuit children exposed to the nutrition transition in the Arctic.

**Acknowledgements**

This study was funded though Government of Canada International Polar Year, Canadian Institutes for Health Research and the Government of Nunavut Department of Health and
Social Services. We acknowledge the work of Nancy Faraj, Christine Ekidlak, Laureen Angalik Kathy Morgan, Nelofar Sheikh and Louise Johnson-Down.
Table 5-1. Population and household characteristics.

<table>
<thead>
<tr>
<th></th>
<th>n/N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>204 / 388</td>
<td>52.6</td>
</tr>
<tr>
<td>Male</td>
<td>184 / 388</td>
<td>47.4</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 to 3.99 y</td>
<td>158 / 388</td>
<td>40.7</td>
</tr>
<tr>
<td>4 to 4.99 y</td>
<td>123 / 388</td>
<td>31.7</td>
</tr>
<tr>
<td>5 to 5.99 y</td>
<td>107 / 388</td>
<td>27.6</td>
</tr>
<tr>
<td><strong>Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kivalliq</td>
<td>142 / 388</td>
<td>36.6</td>
</tr>
<tr>
<td>Baffin</td>
<td>171 / 388</td>
<td>44.1</td>
</tr>
<tr>
<td>Kitikmeot</td>
<td>75 / 388</td>
<td>19.3</td>
</tr>
<tr>
<td><strong>BMI-for-age (n=378)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>192 / 378</td>
<td>50.8</td>
</tr>
<tr>
<td>At risk of overweight</td>
<td>103 / 378</td>
<td>27.3</td>
</tr>
<tr>
<td>Normal weight</td>
<td>81 / 378</td>
<td>21.4</td>
</tr>
<tr>
<td>Underweight</td>
<td>2 / 378</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Household characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Younger child in the home</td>
<td>169 / 388</td>
<td>43.6 (38.6 – 48.7)(^a)</td>
</tr>
<tr>
<td>Household crowding (≥ 6 people)</td>
<td>195 / 377</td>
<td>51.7 (46.6 – 56.9)(^a)</td>
</tr>
<tr>
<td>Food insecurity</td>
<td>88 / 374</td>
<td>23.5 (19.3 – 28.2)(^a)</td>
</tr>
</tbody>
</table>

\(^a\) 95% Confidence Interval (%)
Table 5-2. Summary of serum ferritin and hemoglobin concentrations for Nunavut and by region.

<table>
<thead>
<tr>
<th></th>
<th>Nunavut</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean[^a]</td>
<td>95% CI</td>
<td>Min</td>
<td>Max</td>
<td>Median</td>
<td>n</td>
<td>Mean[^a]</td>
</tr>
<tr>
<td>Ferritin (µg/L)[^b]</td>
<td>19.3</td>
<td>(10.3)</td>
<td>18.1 – 20.6</td>
<td>3.2</td>
<td>78.9</td>
<td>16.6</td>
<td>271</td>
</tr>
<tr>
<td>Hb (g/L):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ages</td>
<td>117.4</td>
<td>(9.0)</td>
<td>116.5 – 118.3</td>
<td>82</td>
<td>145</td>
<td>118</td>
<td>364</td>
</tr>
<tr>
<td>5 y[^c]</td>
<td>117.8</td>
<td>(8.1)</td>
<td>116.2 – 119.5</td>
<td>85</td>
<td>137</td>
<td>118</td>
<td>99</td>
</tr>
<tr>
<td>3 to 4 y[^c]</td>
<td>117.2</td>
<td>(9.4)</td>
<td>116.1 – 118.4</td>
<td>82</td>
<td>145</td>
<td>118</td>
<td>265</td>
</tr>
</tbody>
</table>

[^a] Standard deviation shown in brackets
[^b] Ferritin below 10 µg/L or 12 µg/L is considered iron deficiency in children [28, 44].
[^c] Hemoglobin less than 115 g/L is anemia in 5 year olds, and less than 110 g/L is anemia in 3 to 4 year olds [28].
Table 5-3. Prevalence of iron deficiency, anemia, iron deficiency anemia and *Helicobacter pylori* infection among participating children.

<table>
<thead>
<tr>
<th></th>
<th>Nunavut n/N</th>
<th></th>
<th></th>
<th>Regional prevalence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Prevalence</td>
<td>95% CI</td>
<td>Kivalliq</td>
<td>Baffin</td>
<td>Kitikmeot</td>
</tr>
<tr>
<td><strong>Ferritin &lt; 12 µg/L:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>52 / 271</td>
<td>19.2%</td>
<td>14.7 – 24.4%</td>
<td>19.2%</td>
<td>20.2%</td>
<td>18.6%</td>
</tr>
<tr>
<td>Iron deficiency anemia</td>
<td>12 / 267</td>
<td>4.5%</td>
<td>2.3 – 7.7%</td>
<td>6.1%</td>
<td>4.8%</td>
<td>0</td>
</tr>
<tr>
<td><strong>Ferritin &lt; 10 µg/L:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>28 / 271</td>
<td>10.3%</td>
<td>7.0 – 14.6%</td>
<td>10.1%</td>
<td>10.1%</td>
<td>14.0%</td>
</tr>
<tr>
<td>Iron deficiency anemia</td>
<td>7 / 267</td>
<td>2.62%</td>
<td>1.1 – 5.3%</td>
<td>3.0%</td>
<td>3.2%</td>
<td>0</td>
</tr>
<tr>
<td>Anemia</td>
<td>72 / 364</td>
<td>20.3%</td>
<td>16.3 – 24.9%</td>
<td>22.8%</td>
<td>19.2%</td>
<td>18.1%</td>
</tr>
<tr>
<td><em>H. pylori</em> infection</td>
<td>128 / 282</td>
<td>45.4%</td>
<td>39.5 – 51.4%</td>
<td>45.0%</td>
<td>39.0%</td>
<td>62.7%</td>
</tr>
</tbody>
</table>

*a* Presence of anemia coupled with iron deficiency  
*b* Hemoglobin < 110 g/L (3 to 4 years) or < 115 g/L (5 years)
Table 5-4. Mean, median and percentage of individuals with intakes below the EAR, not including supplements, for energy, vitamin C and iron in Inuit children, ages 3 to 5 years (n = 374).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean ± SD intake</th>
<th>Median intake</th>
<th>% individuals below EAR</th>
<th>EAR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RDA&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy – kcal</td>
<td>1875 ± 791</td>
<td>1819</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Vitamin C – mg</td>
<td>229 ± 329</td>
<td>202</td>
<td>0.14</td>
<td>13 – 22</td>
<td>15 – 25</td>
</tr>
<tr>
<td>Iron – mg</td>
<td>15.6 ± 10.95</td>
<td>13.53</td>
<td>0.27</td>
<td>3.0 – 4.1</td>
<td>6 – 10</td>
</tr>
</tbody>
</table>

<sup>a</sup>EAR = estimated average requirements; NA = not applicable; RDA = recommended daily allowance

<sup>b</sup>Nutrient adjusted using SIDE software (Iowa State University 1996). Adjustments made for sequence and day of week. Where appropriate, this software also provides the number below the EAR.
Figure 5-1. Adjusted iron intake distribution for Inuit children, aged 3 to 5 years, in Nunavut. The Estimated Average Requirement (EAR) for children aged 3 years is 3.0 mg and for children 4 to 5 years is 4.1 mg.
Table 5-5. Frequency of consumption of traditional and market food sources of iron among Inuit children, ages 3 to 5 years.

<table>
<thead>
<tr>
<th></th>
<th>Percentage of 3 to 5 year olds who consumed the food in the past month</th>
<th>Average number of days consumed in a month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>n / N</td>
</tr>
<tr>
<td><strong>Traditional foods:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caribou meat (dried, cooked, raw)</td>
<td>84.2</td>
<td>320 / 380</td>
</tr>
<tr>
<td>Fish, all types</td>
<td>65.3</td>
<td>248 / 380</td>
</tr>
<tr>
<td>Ringed seal meat</td>
<td>49.5</td>
<td>188 / 380</td>
</tr>
<tr>
<td>Clams/mussels from the land</td>
<td>15.5</td>
<td>59 / 380</td>
</tr>
<tr>
<td>Goose</td>
<td>14.7</td>
<td>45 / 307</td>
</tr>
<tr>
<td>Ringed seal liver</td>
<td>11.6</td>
<td>44 / 380</td>
</tr>
<tr>
<td>Duck, all types</td>
<td>10.8</td>
<td>41 / 380</td>
</tr>
<tr>
<td>Walrus meat</td>
<td>8.6</td>
<td>29 / 336</td>
</tr>
<tr>
<td>Musk-ox meat</td>
<td>6.0</td>
<td>8 / 133</td>
</tr>
<tr>
<td>Caribou liver</td>
<td>5.0</td>
<td>19 / 380</td>
</tr>
<tr>
<td>Beluga meat</td>
<td>4.5</td>
<td>17 / 380</td>
</tr>
<tr>
<td>Ptarmigan and spruce hen</td>
<td>3.7</td>
<td>14 / 380</td>
</tr>
<tr>
<td>Narwhal meat</td>
<td>2.9</td>
<td>11 / 380</td>
</tr>
<tr>
<td><strong>Market foods:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal, all types</td>
<td>96.3</td>
<td>366 / 380</td>
</tr>
<tr>
<td>Beef, all types</td>
<td>82.9</td>
<td>315 / 380</td>
</tr>
<tr>
<td>Poultry, all types</td>
<td>82.6</td>
<td>314 / 380</td>
</tr>
<tr>
<td>Pork, all types</td>
<td>69.7</td>
<td>265 / 380</td>
</tr>
</tbody>
</table>

*a* Because of limited geographical availability of this food, N is reduced because certain communities were not asked about this traditional food item.
Table 5-6. Bivariate analyses of explanatory factors for iron deficiency and iron deficiency anemia using two different ferritin cut-off values to define iron deficiency.

<table>
<thead>
<tr>
<th></th>
<th>Ferritin &lt; 12 µg/L</th>
<th></th>
<th>Ferritin &lt; 10 µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron deficiency</td>
<td>IDA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Iron deficiency</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>% (n)</td>
<td>p</td>
</tr>
<tr>
<td>H. pylori</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>146</td>
<td>15.3 (18)</td>
<td>0.09</td>
</tr>
<tr>
<td>Negative</td>
<td>118</td>
<td>24.0 (35)</td>
<td>0.07</td>
</tr>
<tr>
<td>Household size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥6</td>
<td>134</td>
<td>17.2 (23)</td>
<td>0.64</td>
</tr>
<tr>
<td>&lt;6</td>
<td>129</td>
<td>20.2 (26)</td>
<td>0.22</td>
</tr>
<tr>
<td>Younger sibling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>151</td>
<td>21.8 (33)</td>
<td>0.36</td>
</tr>
<tr>
<td>Yes</td>
<td>120</td>
<td>16.7 (20)</td>
<td>0.56</td>
</tr>
<tr>
<td>Child hunger</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>64</td>
<td>20.5 (40)</td>
<td>0.47</td>
</tr>
<tr>
<td>No</td>
<td>195</td>
<td>15.6 (10)</td>
<td>0.15</td>
</tr>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kivalliq</td>
<td>99</td>
<td>19.2 (19)</td>
<td>1.00</td>
</tr>
<tr>
<td>Baffin</td>
<td>129</td>
<td>20.2 (26)</td>
<td>0.35</td>
</tr>
<tr>
<td>Kitikmeot</td>
<td>43</td>
<td>18.6 (8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>129</td>
<td>21.7 (28)</td>
<td>0.45</td>
</tr>
<tr>
<td>Female</td>
<td>142</td>
<td>17.6 (25)</td>
<td>0.83</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-4 years</td>
<td>193</td>
<td>22.3 (43)</td>
<td>0.09</td>
</tr>
<tr>
<td>5 years</td>
<td>78</td>
<td>12.8 (10)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

<sup>a</sup> IDA – Iron deficiency anemia
6 DISCUSSION

This is the first study to report population level prevalence estimates of iron deficiency and IDA for Inuit preschoolers in Nunavut, Canada. The prevalence of IDA was low in the study population but overall, it seems that the iron status of Inuit preschoolers is worse than that of North American children. In the United States, iron deficiency is prevalent among 4.5% of children aged 3 to 5 years and IDA among 0.5% [64]. These compare to rates of 10.3% to 19.2% for iron deficiency and to 2.6% to 4.5% of IDA, among Inuit preschoolers observed in the present study.

This trend where Inuit have higher rates was observed in a 1970-1972 national nutrition survey in Canada, albeit only small sample of 29 Inuit children was included [60]. In this study, the prevalence of tissue iron deficiency was 24% for Inuit children 0 to 4 years, compared to 12% for non-Aboriginal Canadian children [60]. Similarly, Alaska natives had 38% iron deficiency and 7.8% IDA compared to 4.5% and 0.5% for American children [59, 64]. Canadian Inuit infants also seem to be more at risk for iron deficiency than Canadian infants. Low ferritin was found in 36.9% of infants from a community in Kivalliq region, 60% of infants from a community in Nunavik and 33.9% of infants from four major Canadian cities [45, 46, 51]. Infants aged 12 months from Nunavik had a 26.3% prevalence of IDA compared to 5.1% of Canadian infants aged 8.5 to 15.5 months [45, 46, 51].

While Inuit preschoolers appear to be more at risk for iron deficiency than American children, the prevalence rates reported here are mild. The WHO estimates that 40% of preschool aged children in lower-income countries are anemic and that 50% of these are specifically iron deficient anemic, or 20% overall. Prevalence rates for Nunavut preschoolers are much lower than this. In addition, the WHO suggested recently that when low ferritin is found in more than 20% of a population, iron deficiency is said to be prevalent [27]. At the WHO recommended ferritin cut-off, Inuit preschoolers are just below this threshold, at 19.2%.

A final approach to interpreting the prevalence findings in this study is to speculate about whether these have improved or increased over time. The 1970-1972 national
nutrition survey in Canada found that 24% of Inuit children 0 to 4 years had tissue iron deficiency, based on transferrin saturation below 16% [60]. Because transferrin saturation gives an estimate of a more severe stage of iron deficiency compared to ferritin, low ferritin will be more common than low transferrin saturation. As such, the prevalence of tissue iron deficiency is likely less than 19.2% among Inuit preschoolers in 2007 and 2008, which is lower than 24% found in 1972. Although comparing in this way is highly speculative, it suggests at least that rates have not increased in the past three decades.

Overall, few Inuit preschoolers experience IDA. The prevalence of iron deficiency continues to be higher among Inuit than in the general population, and as such, improvements are possible.

Iron intake in this population is probably adequate given the finding that only 0.3% of children were eating below the EAR, vitamin C intake was high and many children were consuming traditional and market meats and cereals. On this background of high iron intake, the finding that 10.3 to 19.2% iron deficiency seems contradictory. It is possible that there was some over-reporting of traditional food intake, characterized by the high median and mean iron intake in this young age group. However, iron intake in these children is similar to previously reported intakes. We reported a mean intake of 15.6 mg/day from all sources of food. A study of Dene/Métis children found that mean iron intake was 14 to 16 mg/day [66, 70]. In one Nunavut community among children aged 3 to 12 years, mean iron intake was 20 mg/day from traditional food and 10 mg/day from market food [149]. For all age groups in this study, iron from market food ranged from 8 to 12 mg/day throughout the year and iron from traditional food ranged from 18 to 70 mg/day throughout the year. The 1972 NCNS and a 1995 Keewatin Health Assessment Survey that took place in 1995 also reported high iron intake among children [56, 60]. Finally, intake estimates from the 2000-2001 NHANES were 11.0 to 13.7 mg per day for 3 to 8 year old children [149]. Since iron intake is similarly high in other studies, perhaps either there is a consistent trend for iron intake to be overestimated in children, or perhaps these high intakes are indeed high.

Regardless, there is still a discrepancy between the dietary and biochemical findings, which is perhaps explained by limitations to dietary interviewing tools. Estimating usual
iron intake is challenging in any setting, especially in large epidemiological studies where the number of repeat recalls collected is often limited by time and logistical constraints. In the current study, one recall and a repeat recall on a 20% sub-sample was used. However, it has been suggested that for iron, and other nutrients, a larger number of repeat twenty-four hour recalls is needed to estimate usual intake [22]. For example, using the formula developed by Beaton and colleague, for children 1 to 5 years, if one wanted to estimate usual iron intake within 20% of the real value with 95% confidence, 21 recalls for each individual would be needed [22]. This number increases to 85 in order to obtain estimates that are within 10% of the real intake value [22]. As such, while iron intake for 3 to 5 year old Inuit children in Nunavut seems adequate with a high median intake, these findings cannot rule out diet as a cause of iron deficiency in this population. Overall, the discrepancy between biochemical findings and dietary findings could be explained by the lack of sensitivity and specificity of a limited numbers of 24-hour dietary recalls.

In addition, it was discussed in the previous chapter that high BMI-for-age may explain the observed iron deficiency. It has recently been shown that obesity and being overweight is associated with greater risk of iron deficiency [150-154]. Using data from NHANES 1988-1994, it was found that overweight and at risk for overweight children were 2.0 to 2.3 times more likely to be iron deficient than normal weight children [152]. Similarly, from NHANES 1999-2000 it was found that overweight and at risk for overweight toddlers aged 1 to 3 years were 3.34 times more likely to be iron deficient than normal weight toddlers [150].

Possible explanations for this observation may include lower iron intake among overweight children, especially from consuming too much juice, milk and high-sugar and low-iron foods. As well, overweight children may have increased iron requirement due to larger blood volume and increased basal loss [150, 152, 154]. In addition, it has been suggested that being overweight may induce chronic low-grade inflammation, signalling hepcidin release from the liver and adipocytes, reducing iron absorption into the blood stream from the upper small intestine [154, 158]. Recently, it was discovered that among female adolescents who are greater than 85th percentile for BMI-for-age, the odds ratio
for iron deficiency doubles with each 1 ng/mL increase in CRP [158].

Although there were no significant associations between iron deficiency and BMI-for-age in the current study, we noticed that more 3 to 4 year olds than 5 year olds were overweight and there was a tendency for boys to have higher BMI-for-age z scores than girls. Perhaps iron deficiency is more prevalent among younger children and boys because of higher rates of overweight and at risk for overweight in these groups. In addition, we may have been limited in our sample size in picking up any associations between BMI-for-age and iron deficiency.

The 45.4% prevalence of *H. pylori* exposure that was found in this study is high and consistent with other studies. In Canadian Inuit and northern First Nations infants and children, the prevalence of *H. pylori* has been reported to be 39% to 56% [51, 59, 84, 101]. In Alaska, rates are also high from 32 to 86% [59, 102]. This is compared to low rates of 5.5 to 7.1% among children and adolescents in the United States and Canada [64, 100]. It should be noted that using serodiagnosis to measure *H. pylori* only allows us to estimate exposure, and not current infection. Current infection should be measured with more sensitive measures such as urea breath test, endoscopy or stool antigen test, all of which posed considerable logistical constraints in this study. However, it is believed that in children, serodiagnosis underestimates prevalence of *H. pylori* infection [93-95]. As such, we can estimate that rates are at least 45.4% in this population.

*H. pylori* has been previously shown to be independently associated with iron deficiency in other populations including Inuit [59, 64]. In the United States, it was reported that *H. pylori* was independently associated with IDA (OR: 2.6, 95% CI 1.5-4.6), but not iron deficiency alone for children and adults older than 3 years [59, 64]. Among Alaska Native, *H. pylori* was only independently associated with iron deficiency and not IDA for children in the 9 and older age group, and not in younger age groups [59]. It is perhaps this age-dependent association which may explain why among Inuit preschoolers, there was no association between *H. pylori* and iron status.

The early case series and case reports where this association was first described involved mostly children in the 9 to 15 year old age group [116, 123-126]. A recent
smaller study of risk factors for iron deficiency in Inuit and First Nations infants found that only cow’s milk and evaporated milk consumption were the only independent risk factors, while *H. pylori* was not independently associated [51].

The mechanism by which *H. pylori* induces iron deficiency is still unclear but may be related to bacterial damage to gastric glandular tissue and iron competition in the stomach [109, 111, 112, 119-121]. If these are indeed the mechanisms, perhaps younger children who are more recently infected, are more protected than older children who have more established infections.

One final finding that was interesting was the prevalence of anemia from all causes. In American children aged 3 to 5 years this was estimated to be 1.5% [59, 64]. In the current study, anemia from all causes was much higher at 16.5% of Inuit children. In addition, only 18.0 to 30.8% of anemia was explained by low iron. The WHO estimates that only 50% of anemia is related low iron and our finding is somewhat consistent with this [28]. Other studies in children and infants have shown similar results where only a portion of the observed anemia is explained by iron deficiency [36, 37, 155]. A possible explanation for this finding is the presence of acute infection. In acute infection red blood cell half-life decreases and iron becomes trapped inside cells and is not available for erythropoiesis, both of which explain the mild anemia associated with acute inflammation [17, 156, 157]. Other dietary causes of anemia include deficiencies in vitamin A, folate, B12 and riboflavin [35]. Given the high meat and cereal intake from food frequency information, these micronutrient deficiencies are unlikely.

Future research might focus on the finding that many children were anemic but not iron deficient. This was likely related to acute infection but it would valuable to confirm this, especially since follow-up assessments to low hemoglobin should be done if this were indeed related so another nutrient deficiency. Other studies might further investigate the high prevalence of *H. pylori* exposure by highlighting risk factors or determining which strains are most common in Nunavut, as more virulent strains are associated with more severe health outcomes. Finally, perhaps a more pressing research need from a global health research perspective would include establishing ferritin cut-off values for children. In this study, the prevalence of iron deficiency changed by about 9% between
the two different cut-off values used. With the advent of the HemoCue™ came a method to measure anemia that was accurate and accessible for populations all over the world. Assessing iron status is also important because it helps in deciding how to treat low hemoglobin. Ferritin is promising because it is inexpensive and requires a small sample of blood. However, as mentioned, current cut-offs are unclear.

Overall, it was observed that Inuit children aged 3 to 5 years, living in Nunavut, have low rates IDA and rates of iron deficiency from 10.3% to 19.2%. These are still higher than the general population. However, the etiology of the observed iron deficiency and IDA is still unclear. *H. pylori* was not found to be related iron status, and might be explained by the young age group in this study. The *H. pylori* prevalence was still quite high suggesting that children could be at risk later in life for iron deficiency and other negative health outcomes caused by the bacterium. Dietary iron intake was adequate and even high, although our measure of usual iron intake was limited. Overall the two findings of a low prevalence of IDA and adequate iron intake are encouraging for Nunavut. While public health intervention might aim to bring down the prevalence of iron deficiency, we should bear in mind the other health issues facing Nunavut’s preschoolers such as high BMI-for-age, high *H. pylori* exposure and widespread childhood food insecurity, all of which emerged in this study but were not explored. Addressing these may be more pressing, especially by considering new interventions as well as working to maintain the practices that have led to the overall low prevalence of IDA in Nunavut’s preschoolers.
7 REFERENCES


5. Statistics Canada, Aboriginal Identity (8), Area of Residence (6), Age Groups (12) and Sex (3) for the Population of Canada, Provinces and Territories, 2006 Census - 20% Sample Data, 2006 Census of Population, Statistics Canada catalogue no. 97-558-XCB2006006.


38. Hemocue Hb 201+, Hemocue Hb 201+ Operating Manual, ed. HemoCue AB.


8 APPENDICES
Appendix A. Quality control material for dietary questionnaires.
Qanuippitali? How about us, how are we?
CHILD INUIT HEALTH SURVEY– 2007
Nutrition questionnaire response

To be completed by the Dietary coordinator.

STUDY NO.

Questionnaire Int. No.: ______  Name: __________________________

Table 1: Check List for the 24-HOUR DIETARY RECALL

Recording of the requested information:  Yes  No

1- Time of meal
2- Description in detail of foods eaten
3- Number of servings
4 - Serving models
5- Thickness

Comments:
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________

Corrections to do:
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________

Completion Date: ______/_____/2007  Time: ______/_____

m   d   y   h   m
Table 2: Check List for the Food Frequency Questionnaire

Recording of the requested information: Yes No

**Country Food:**
1- Column Yes or No
2- When is it consumed
   - In season
   - Off season
3- Frequency
   - #
   - D /W/M /S
4- Number of servings
5- Serving models
6- Thickness

**Market Food:**
1- Column Yes or No
2- Frequency
   - #
   - D /W/M /S
3- Number of servings
4- Serving models
5- Thickness

Comments:
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
Corrections to do:
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

Completion Date: ____/____/2007
Time: ____/____
       h     m
Appendix B: Clinical protocols and quality control procedures for clinical equipment
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).
Weight Protocol

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

**Equipment Required**
Weight scale

**Time Required**
Approximately 5 minutes

**Measurement Procedure**

1. Ask the participant to remove his/her shoes but keep socks.
2. Ask the participant to step on the platform of the scale and to remain steady while you record the number.
3. The measurement should be taken to the nearest half kilogram.
Hemoglobin Measurement using HemoCue® 201+
Adapted from: USING THE HEMOCUE® by Ibrahim Parvanta (CDC, 1999)

Purpose

The HemoCue® is a machine that measures the amount of hemoglobin in whole blood (g/L). Hemoglobin tells us about anemia and a child’s risk for iron deficiency (weak blood). Low hemoglobin can result in poor health and poor ability to function in school.

Errors in hemoglobin assessment occur if appropriate procedures and techniques are not followed. Use of inappropriate procedures/techniques may lead to wide variations in hemoglobin values. This then leads to erroneous estimates of anemia prevalence in the population tested. The following steps are recommended to help ensure reliable testing using the HemoCue® photometer.

Equipment Needed

Hb 201+ Hematology controls (low, medium and high)
Hb 201+ kit (Hemoglobin analyzer and microcuvettes)

Vacutainer with whole blood
Piece of paraffin film
200µL transfer pipette
OR
OneTouch UltraLight lancet
Alcohol wipe and Kim Wipes

Measurement Procedure

At the beginning of each survey day, check instrument accuracy using the three hematology controls (Quality Assurance). If readings are in question, clean the cuvette holder. If readings continue to be outside the correct range, do not use the instrument. It should be serviced or replaced.

Start up

1. Connect power supply to the HemoCue®.

2. Pull out the cuvette holder. Press the left button. Wait until the display shows three flashing dashes (– – –). The HemoCue® is now ready for use.

Quality Assurance
3. Remove control vials from the refrigerator and allow to warm to room temperature for 15 minutes before mixing.

4. To mix, hold vial horizontally between palms of hands. Do not use mechanical mixer.
   a. Roll the vial back and forth for 20-30 seconds, occasionally inverting the vial. Mix vigorously but do not shake.
   b. Continue to mix in this manner until the red cells are completely suspended. Vials stored for a long time may require extra mixing.
   c. Gently invert the vial 8 – 10 times immediately before sampling.

5. Remove cap from vial. Dispense drop of control on parafilm or other appropriate material. Recap immediately.

6. Remove cuvette from container and reseal. Fill cuvette by touching tip of cuvette to blood drop. Wipe outer edges of the cuvette with a Kim Wipe. Do not touch the cuvette opening with the tissue. Take a new sample if you see any air bubbles in the optical eye of the cuvette.

7. Place the cuvette in the holder and slide into the analyzer. Record results in appropriate column of the Quality Assurance log.

8. Discard cuvette in bio-hazard sharps container.

9. Measure Level 1 (Low), Level 2 (Normal) and Level 3 (High) controls. Place back in refrigerator after use.

---

**HemoCue® Procedure**

Only do finger prick if venipuncture is not possible. If using blood from venipuncture, follow Venipuncture Blood Collection protocol and use blood from vacutainer. Invert tube containing whole blood 10 times gently. Pipette 10µL of blood from the capillary tube onto paraffin film. Skip to step 7.

1. Seat the child comfortably. If child is not comfortable, ask caregiver to come to help. Ensure that the patient's hand is warm so that blood circulates freely before sampling. Rubbing or wrapping it in a warm towel will help warm the hand.

2. The patient's fingers should be relaxed but not bent to allow for maximum blood flow. Use only the middle finger or ring finger for sampling; remove rings from the finger before testing.

3. Clean the puncture site with alcohol wipe and dry it completely using a gauze pad.

4. Using a rolling movement of your thumb, lightly press the finger from the top knuckle towards the tip. This stimulates the flow of blood towards the puncture site.

5. When the thumb has reached the fingertip, maintain gentle pressure and puncture the side of the fingertip with a sharp, quick motion. Using the side of the finger causes less pain and produces the best flow of blood. Dispose the lancet immediately into a bio-hazard sharps container.
6. Using a dry gauze pad, wipe away the first two drops of blood to stimulate spontaneous blood flow. If necessary, gently press the finger until another drop of blood appears. Avoid "milking" the finger. Ensure that the third drop of blood is big enough to fill the cuvette completely in one attempt.

7. Fill the cuvette by touching its pointed tip to the middle of the blood drop - the cuvette fills automatically by capillary action. This helps avoid trapping air in the cuvette. Never “top off” the cuvette after the first filling. If the cuvette is not filled with the attempt, discard it in the bio-hazard sharps container and use a second cuvette. If you see air bubbles in the optical eye of the cuvette, take a new sample.

8. If a second sample is taken from the same puncture site, it should be collected before clotting has occurred. Wipe away the remains of the previous blood sample, apply gentle pressure to form another adequate blood drop and collect the sample as described above.

9. Wipe off any excess blood on the sides of the cuvette with a Kim Wipe. Ensure that no blood is sucked out of the cuvette when wiping it.

10. Place the cuvette in its holder and gently push the holder into the photometer. The cuvette should be read within 10 minutes after being filled. It takes 15-45 seconds for a reading. Record reading in child’s clinical sheet. Discard the cuvette into a bio-hazard sharps container immediately.

11. Results will be displayed as long as the holder is in the machine unless using batteries only in which case machine will shut off after 5 minutes.

12. To shut off machine, press the left button until the machine is OFF. Push the holder into the analyzer.
Common Problems to Avoid

The following are some important points related to the use of the HemoCue® and capillary sampling procedures that should be stressed:

1) **Keep the instrument clean, especially the cuvette holder.**

   A swab dabbed with alcohol can be used to clean away any dirt or dried blood. This should be done at least once a day or when there is a visible build-up of dirt or blood. Be sure the cuvette holder is dry before re-inserting it in the machine.

2) **Ensure instrument accuracy**

   Check the accuracy of the instrument daily, or when performance is questioned, using the control cuvette which comes with each HemoCue® instrument. Keep a daily log of accuracy readings. If the accuracy readings are outside the range of the control cuvette, and the HemoCue® is clean, then the instrument needs to be replaced. Operating temperature for HemoCue® analyzer is 15-30°C. The HemoCue® measures hemoglobin concentrations from 0-256 g/L. Results above 256g/L will be displayed HHH.

3) **Keep cuvettes clean, dry and away from heat**

   Cuvettes in unsealed containers are good for 3 months after opening. Keep the container lid closed when not being used to avoid unnecessary exposure of the cuvettes to air, especially in humid conditions. Heat and moisture will denature the chemicals in the cuvette which can lead to inaccurate measurements.

4) **Make sure the finger stick is adequate**

   Wide variations can occur in hemoglobin measurements if the finger stick is inadequate (basically equated to the finger stick not being deep enough to allow adequate flow of blood and a representative concentration of red blood cells). In most cases if the finger stick is done poorly, hemoglobin values will be underestimated and the prevalence of anemia will be overestimated.

5) **Avoid poor technique**

   - **Milking the finger** (usually related to an inadequate finger stick) to obtain proper blood flow which will underestimate hemoglobin readings.

   - **Not waiting for at least the 3rd drop of blood to sample**. It is recommended that after wiping away the first two drops of blood using a dry wipe, the third or subsequent drops should be measured. This helps allow for the collection of blood with a representative concentration of red blood cells.

   - **Mixing alcohol with the blood**. The patient’s finger should be totally dried before the finger prick is performed. Use alcohol to clean the finger before the prick is made and then wipe away each drop of blood with a dry wipe to avoid any mixing of the blood with alcohol. Wiping away the first few drops of blood
also will minimize the mixing of sweat with blood in hot, humid climates. This error usually underestimates the hemoglobin reading.

Avoid removing a cuvette from its container when your fingers are wet with alcohol. Alcohol coming in contact with the cuvette can denature the needed chemical in the cuvette selected, as well as, other cuvettes still in the container.

- **Obstructing blood flow to the puncture site.** Do not hold the subject’s hand so tightly as to obstruct blood flow to the fingers.

6) **Adequately fill the cuvette**

   The cuvette needs to be filled with a drop of blood in one continuous motion. Again this depends on the flow of blood and the size of the drop formed; if it is not adequate, the cuvette will not fill adequately. Do not “top off” the cuvette that is not completely filled. This results in erroneous hemoglobin readings; usually too high.

   Any signs of air-bubbles means that the cuvette has not been filled adequately and should be discarded and a new cuvette used. The presence of bubbles will usually underestimate the hemoglobin reading.

7) **Do not “slam” the cuvette holder into position for reading.**

   This will avoid spraying blood droplets which can hamper the scanner.

---

**How the HemoCue® works:**

Sodium deoxycholate haemolyses the erythrocytes and hemoglobin is released. Sodium nitrate converts hemoglobin to methaemoglobin which together with sodium azide, gives azidemethaemoglobin. The absorbance of azidemethaemoglobin is measured at 570nm and 880nm. The absorbance reading is converted to give a measure of total hemoglobin in the sample.
Summary of common problems and solutions related to capillary sampling and use of the HemoCue™ photometer.

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not preparing all needed materials before testing a subject.</td>
<td>Place cuvette, alcohol swab, gauze pad, and lancet on work surface; turn on photometer; pull out the cuvette holder to &quot;locked&quot; position so that digital screen reads &quot;READY&quot;; put on latex gloves.</td>
</tr>
<tr>
<td>Selecting a cuvette from its jar with fingers wet with alcohol (the alcohol denatures the chemicals inside the cuvette; thus, the selected cuvette as well others inside the jar can be denatured).</td>
<td>Take cuvette out of its container before handling a wet alcohol swab.</td>
</tr>
<tr>
<td>Not drying finger completely after disinfecting with alcohol (since the HemoCue cuvette only hold 10 µL of blood, its volume can be easily affected by even a trace of alcohol on the puncture site).</td>
<td>Firmly wipe the finger using a dry gauze pad. Firm wiping can also help stimulate blood flow to the finger tip.</td>
</tr>
<tr>
<td>Inappropriate and shallow finger puncture.</td>
<td>An appropriately deep puncture done with a &quot;quick stab&quot; will result in a better blood flow and more rapid completion of the test. (A number of brands of high quality single-use lancets are now available in the market).</td>
</tr>
<tr>
<td>Restricting blood flow to finger tip following the finger-stick.</td>
<td>Release the subject’s finger after the stick to allow blood flow; also hold the subject’s hand without squeezing and restricting blood flow to the finger tip.</td>
</tr>
<tr>
<td>Milking the finger (this will lead to mixing of interstitial fluids with the blood drop leading to an inaccurate Hgb reading...usually too low).</td>
<td>A good finger-stick should result in spontaneous blood negating the need to apply pressure to the finger. If stimulating blood flow is needed, apply gentle pressure with the thumb on the opposite side of the finger from the puncture site.</td>
</tr>
<tr>
<td>Not appropriately wiping off the first 2 blood drops (this may result in collection of &quot;unrepresentative&quot; blood sample being tested).</td>
<td>Firmly wipe off the first 2 large blood drops. Firm wiping will stimulate blood flow. Discarding the first 2 large drops will allow flow of &quot;representative&quot; blood sample after initial constriction of capillary bed following the finger-stick.</td>
</tr>
<tr>
<td>Holding cuvette in inverted position (slit facing down) during filling (this can lead to air bubbles being trapped resulting in erroneous result).</td>
<td>Hold the cuvette with the slit facing up and the pointed tip touching the blood drop.</td>
</tr>
<tr>
<td>&quot;Topping off&quot; a partially filled cuvette with repeated blood collection (the reagents in the cuvette are denatured upon contact with the initial amount of blood; red cells of blood introduced later will not be adequately analyzed).</td>
<td>Allow a large blood drop to form on the finger so that it will completely fill the cuvette in one motion. Once filled, hold the cuvette in place for about 2-3 seconds longer to ensure complete filling.</td>
</tr>
<tr>
<td>Not cleaning off blood on outside of cuvette before testing (can result in erroneously high Hgb reading).</td>
<td>Wipe off excess blood from sides of cuvette using a &quot;butter knife&quot; motion to ensure that blood from inside the cuvette is not removed.</td>
</tr>
<tr>
<td>&quot;Slamming&quot; the cuvette holder into place (can lead to blood drops spattering inside the reading chamber).</td>
<td>Push the cuvette holder gently into position. Once or twice a day clean the cuvette holder with alcohol swab and completely dry before testing. Periodically clean the reading chamber with dry gauze.</td>
</tr>
</tbody>
</table>
Venipuncture Blood Collection

Purpose

Blood will be used to measure various aspects of health such as vitamin D, infection with *Helicobacter pylori*, iron deficiency, and fatty acids.

Equipment Needed

Alcohol wipes
Tourniquet
Butterfly
6ml Vacutainer (with heparin) labeled with child’s study number
2x2 gauze
Band-Aid

Blood Collection Procedure

1. Wipe collection area with alcohol wipes and air dry.
2. Collect 3 ml blood with butterfly needle and vacutainer.
3. Place Vacutainer on ice. Test a 10-microliter sample with HemoCue™ within 10 minutes of blood collection (refer to HemoCue™ Protocol)
4. If venipuncture is too difficult for the child, refer to HemoCue™ protocol for instructions for finger prick and hemoglobin measurement.
RBC and Plasma Sample Preparation

Materials

Lab coat and goggles
Styrofoam Ice Bucket
6 labeled cryovials (3 red caps, 3 green caps)
2 labeled cryovials (2 brown caps)
1000µL pipetor
200µL pipetor
Fine and broad tipped transfer pipettes
2000xg Mini centrifuge
2 mL micro-centrifuge tubes
Ice cold Saline
Ice cole MeOH:Water:BHT solution
Cryovial freezer boxes
Timer and BioHazard Bin

Methods

1. Collect 3 ml blood into green-top vacutainer tubes (containing heparin). Invert tube gently at least 5 times to mix blood and heparin thoroughly.

2. Keep sample on ice at all times.

3. Invert tube gently at least 5 times immediately before dividing blood into tubes. Using a broad tipped transfer pipette, divide blood equally into 2 micro-centrifuge tubes. Cap securely.

4. Record volume of blood collected, roughly.

5. Centrifuge sample at 2000 x g for 15-20 minutes. Longer is better, 15 minutes is a minimum. (The mini-centrifuge automatically operates at 2000 x g)

6. With a fine tipped transfer pipette, remove plasma from each micro tube and combine in one micro-centrifuge tube.

7. Aliquots plasma into individual cryovials according to the table below. Cap with red caps and place vials in a cryovial box. Freeze at -20C immediately. Record tubes collected and record freezer temperature twice daily. This step does not need to be completed immediately by plasma should be kept over ice until it is completed.
8. Very carefully remove the layer of white blood cells (i.e. the “buffy” coat) from the top of the remaining RBC pellet and discard into biohazard bin.

9. Add enough cold saline to RBC pellet to fill the cryovial (leave sufficient headspace), mix RBC and saline gently by inverting the tube. It may be necessary to stir the cells very gently. Do flick or shake the tube.

10. Centrifuge again for 15-20 minutes, remove and discard the saline layer and any remaining buffy coat. Avoid removing RBCs as much as possible but you may need to remove some to completely remove the buffy coat.

11. Add cold BHT solution to the RBC in a 1:1 ratio. (FYI - BHT solution is equal parts water/methanol + 42 mg/L BHT). Mix by gently inverting tube several times. It may be necessary to stir the cells very gently with the transfer pipette. Do flick or shake the tube.

12. Carefully transfer all the RBC solution to two 1.5 ml cryovials and cap with a brown cap, place vial in cryovial box, and freeze at -20 immediately.

<table>
<thead>
<tr>
<th>Sample type</th>
<th># aliquots</th>
<th>Volume</th>
<th>Cap</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>1</td>
<td>50 µL</td>
<td>Red</td>
<td>H. pylori</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>500 µL</td>
<td>Red</td>
<td>Vitamin D/PTH/SF</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>50 µL</td>
<td>Red</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>300 uL</td>
<td>Red</td>
<td>Remainder</td>
</tr>
<tr>
<td>RBC</td>
<td>1</td>
<td>Remainder</td>
<td>Red</td>
<td>Remainder</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>Brown</td>
<td>Fatty acid</td>
</tr>
</tbody>
</table>

13. Store at -20 C until shipped in a cooler with ice packs to Montreal. Upon arrival in the lab, flush tubes with N₂ gas and store at -80 C for up to 4 months.

(Note: blood volumes of 400 - 500 µl will yield enough RBC for fatty acid analysis as long as high quality solvents are used. Volumes of 75 µl can be used for singlet analyses.)