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Iron Deficiency, Iron Deficiency Anemia, and Infectious Disease in Calgary, Alberta

Giacobbo, Olivia

UNIVERSITY OF CALGARY

Iron Deficiency, Iron Deficiency Anemia, and Infectious Disease in Calgary, Alberta

by

Olivia Giacobbo

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Abstract

An estimated 30% of the global population suffers from iron deficiency anemia (IDA) and previous studies have suggested that iron deficiency (ID) and IDA are associated with adverse health outcomes. However, some research suggests that ID and IDA may be adaptive in areas with high levels of endemic infectious diseases. The present study examined the association between serum iron levels and four infectious diseases in a sample of 55,437 individuals in Calgary, Alberta. Associations between sociodemographic variables (SDVs) and iron and infection were also tested to explore ID's complex etiology. This study evaluates two hypotheses: H_{A1}: Low baseline serum iron predicts a lower risk of infection up to one year out, and H_{A2}: Sociodemographic variables (higher median income, more postsecondary education, non-immigrant status, and non-Indigenous status) will be associated with higher serum iron levels. Cox regression analyses found that the lowest levels of iron were predictive of greater risk for infection in sepsis (blood) and urinary tract infections. Iron level was not associated with fungal sepsis and strep throat. Multiple regression analyses found no significant relationships between infection and SDVs and found that greater median household income and postsecondary education level were associated with higher mean serum iron levels. This research emphasizes the importance of context when evaluating the adaptiveness of a trait, in addition to calling for further investigation into individual-level associations of sociodemographic variables and infection throughout the city of Calgary.

Keywords: Iron Deficiency, Infectious Disease, WEIRD Countries

Preface

This thesis is original, unpublished, independent work by the author, O. Giacobbo. The methods and analyses reported in Chapters Three and Four were covered by Ethics Certificate REB 18-0607_REN1, issued by the University of Calgary Conjoint Faculties Ethics Board for the project "Iron deficiency, anemia, and susceptibility to infection" on February 11, 2020.

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List of Abbreviations

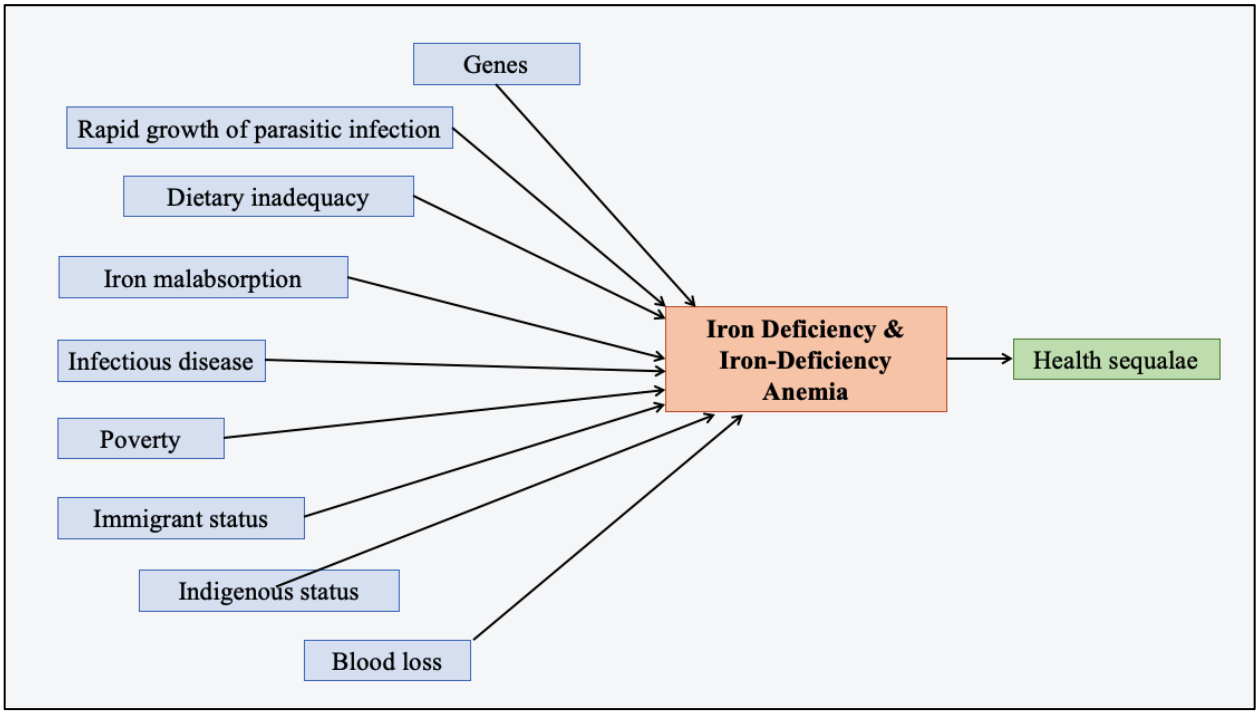
ID – iron deficiency
IDA – iron deficiency anemia
SDV(s) – sociodemographic variable(s)
LMIC(s) – low-middle income country/countries
HIV/AIDS – human immunodeficiency virus/acquired immunodeficiency virus syndrome
ATP – adenosine triphosphate
UV – ultraviolet
HbS – sickle cell allele
Fe^{2+} – soluble ferrous iron
Fe^{3+} – insoluble ferrous iron
DYCTB – Duodenal Cytochrome B
DMT1 – Divalent Metal Transporter 1
RBC(s) – red blood cell(s)
AMP – antimicrobial peptide
APPR – acute phase protein response
HFE – hepcidin gene
HAMP – hepcidin anti-microbial peptide
H. pylori – *Heliobacter pylori*
WEIRD – Western, educated, industrialized, rich, democratic
HI – hypoferremia of infection
TfR – transferrin receptor
CRP – C-reactive protein
ZPP:H – zinc protoporphyrin to heme ratio
APL – Alberta Precision Laboratories
CLS – Calgary Lab Services
PHN – provincial health number
MB – microbiology
FSA – forward sortation area
UTI(s) – urinary tract infection(s)
GAS – group A streptococcal infection
CQCI – Charlson-Quan comorbidity index
CRG – Clinical Risk Group
ANOVA – analysis of variance
SES – socioeconomic status
HR – hazard ratio
CDA – census dissemination area
NHS – National Household Survey
SANDS – Spatial and Numeric Data Services
NW – northwest
SW – southwest
SE – southeast
NE – northeast

Chapter One: Introduction

The most prevalent micronutrient deficiency worldwide and leading cause of anemia in both low-middle income and high-income countries is iron deficiency (ID) (Pasricha & Drakesmith, 2016; Powers & Buchanan, 2014): 30% of the global population, 2.3 billion people, suffer from iron deficiency anemia (IDA) (World Health Organization, 2019). Given their relatively high iron requirements, pre-term and low birthweight infants, children up to five years of age, women of reproductive age, pregnant and post-partum women, and those over 65 years of age are disproportionately affected by ID and IDA (Goonewardene et al., 2011; Shavelle et al., 2012; Abbaspour et al., 2014). Studies conducted on North American populations have concluded that the highest prevalence of ID and IDA are found in individuals of African, African American, or Hispanic descent (Barton et al., 2020; Le, 2016).

Iron deficiency has a complex etiology that varies from person to person and place to place, indicative of environmental risk factors for this phenotype (Figure 1.1). Risk factors for ID and IDA include genetic variation (Finberg et al., 2008; Fairweather-Tait et al., 2013), blood loss (chronic and/or acute), dietary iron inadequacy, iron malabsorption (e.g., Crohn's disease, Celiac disease), rapid growth of parasitic infections (Kumar & Coltran, 2014), poverty (WHO, 2001), Indigenous status in Canada (Christofides et al., 2005a; Willows et al., 2000; Hodgins et al., 1998), immigrant status (Pottie et al., 2011; Hayes et al., 1998; Bindra & Gibson, 1986), and, as noted below, viral, bacterial, and fungal infectious diseases.

Figure 1.1 Sociodemographic factors and iron deficiency



The prevalence of ID and IDA is alarming as the health sequalae can be devastating. For example, iron deficiency during pregnancy is associated with adverse outcomes for both the mother and the infant, including increased risk of sepsis, maternal mortality, and perinatal mortality (CDC, 2010). Maternal depression has also been listed as a consequence of IDA during pregnancy (Black et al., 2011). Furthermore, infants who are born with insufficient iron stores are likely to suffer from cognitive impairment, given that iron uptake is maximal during this period due to the rapid brain growth and development that occurs (Georgieff, 2011). Moreover, iron is a critical component of immuno-surveillance, immuno-activation, and immuno-regulation in the body (Cherayil, 2010). Low iron levels can lead to impaired immune function and thereby increase susceptibility to infection and exacerbate infection-related outcomes. Even mild-to-moderate forms of ID/IDA may therefore decrease host fitness and can be maladaptive in environments with endemic infectious disease (Mendoza et al., 2019; Harju, 1988).

Iron deficiency has also been associated with decreased work capacity (Haas & Brownlie, 2001); for example, Li et al. (1994) found that iron deficiency affected energy expenditure and productivity in both strenuous and non-strenuous physical occupations (e.g., physical labour vs.

factory production-line work) in a sample of iron-deficient females in Beijing, China. Male rubber tree tappers or weeders in Indonesia who received iron supplements had a 17% increase in productivity output compared to their placebo-fed colleagues (Basta et al. 1979).

The results of these studies are unsurprising given that one of the most common consequences of ID and/or IDA is the fatigue caused by a lack of oxygen in the body's muscles and tissues (Horton & Ross, 2003). The ability of ID/IDA to impact economic productivity is undoubtedly concerning, especially in lower-middle income countries (LMICs) where physical labour is required to feed one's family and is the engine of economic development (Balarajan et al., 2011; Horton & Ross, 2003). In fact, Horton & Ross (2003) devised a formula to calculate the annual per capita loss associated with anemia in blue collar work from 10 developing countries[a]. Shockingly, the authors concluded that when the results were expressed in absolute dollars, the productivity losses in Bangladesh, India, and Pakistan combined equalled close to $4.2 billion annually (Horton & Ross, 2003). Finally, in addition to their economic impacts, ID and IDA may also exacerbate infections such as malaria, hookworm, human immunodeficiency virus/acquired immunodeficiency virus syndrome (HIV/AIDS), schistosomiasis, and tuberculosis, placing even larger burdens on healthcare systems in areas where these conditions are prominent (Miller, 2013; Joynson et al., 1972).

Hence, ID and IDA have traditionally been viewed as deleterious conditions that result in adverse developmental and health outcomes for the host (Georgieff, 2011; Monga et al., 2010; Abbaspour et al., 2014). However, recent research suggests that we might view ID/IDA differently; that is, ID/IDA may be beneficial in environments with high levels of infectious disease (Denic & Agarwal, 2007; Falkenberg, 2016; Pietrangelo, 2015; Scrimshaw & SanGiovanni, 1997). Iron is an essential nutrient[b] for almost all pathogens; however, pathogenic organisms lack the necessary physiological structures that can facilitate consumption and absorption of iron from the environment. Instead, pathogens must acquire (steal) iron from their

[a] Horton & Ross' (2003) formula is as follows: (5% x WS x BC Share x GDP/cap x Pr(adult)) + (12% x WS x HML x GDP/cap x Pr(adult)), where WS = wage share in GDP; HML = heavy manual labor share in GDP; BC Share = share of blue-collar employment in total employment; Pr(adult) = prevalence of anemia in adults.

[b] Essential nutrients are those that must be obtained from the environment (e.g., through food consumption) as the host organism cannot make it in sufficient quantities to fulfill its needs. Iron is an essential nutrient as both humans and pathogens must acquire the element from their environment in some manner.

hosts (Cassat & Skarr, 2013; Pietrangelo, 2015). Interestingly, infection and inflammation trigger systemic decreases in iron availability in the host via multiple pathways, such as the hypoferremic response, reduction of iron uptake, and sequestration of iron at infectious loci (Cassat & Skaar, 2013). This decrease in iron availability appears to be a transient, phenotypically plastic adaptation as low iron levels would deprive pathogens of iron, thereby enhancing host resistance or tolerance to pathogenic attack (Denic & Agarwal, 2007; Wander et al., 2009). It is therefore hypothesized that ID and IDA are highly conserved, adaptive by-products of the co-evolution between hosts and pathogens (Denic & Agarwal, 2007; Falkenberg 2016; Pietrangelo, 2015; Schrimshaw & SanGiovanni, 1997).

But, what exactly is meant by adaptive? Mazess (1975) defines an adaptation as a characteristic of an organism that facilitates physiological homeostasis (discussed in Chapter Two) and, consequently, survival and reproduction in a given environment. An adaptation must, consequently, be considered in light of the context in which it exists. As well, the potential range of phenotypic variation is constrained by phylogenetic inertia; that is, in the process of speciation, organisms do not start from scratch, but can only build upon existing traits (Darwin, 1859; Huber, 1939).

While research has been conducted on the relationship between iron and infection in regions with high endemicity for infectious disease, there are few studies that explore this association in large, urbanized populations with low infectious disease endemicity. As such, the purpose of this study was to elucidate the relationship between host iron levels and susceptibility to infections in an urban Canadian environment. Specifically, this study was designed to address two questions:

1) Is serum iron predictive of infection up to one year out?

And, because variation in serum iron levels and infection has a complex etiology, it was pertinent to examine what, if any, influence sociodemographic variables had on these variables. Indeed, a robust body of literature suggests that health disparities are largely explained by sociodemographic differences (Darin-Mattsson et al., 2017; Irwin et al., 2006; Marmot, 2015), so this study additionally asked:

2) Is sociodemographic context associated with (1) mean iron level and (2) infection across Calgary?

Further, this study was designed to evaluate two hypotheses:

1) H_{A1}: Low baseline serum iron predicts a lower risk of infection up to one year out.
2) H_{A2}: Sociodemographic variables (higher median income, more postsecondary education, non-immigrant status, and non-Indigenous status) will be associated with higher serum iron levels.

These questions and their hypotheses are considered from the perspective of biological anthropology, a discipline that seeks to understand human variation in light of human physiology, local context, and evolutionary biology.

Chapter Two elaborates on the body's need for physiological homeostasis and how stressors such as iron deficiency or infectious disease can disrupt it. Chapter Three describes the methods used and Chapter Four provides the results. Finally, Chapter Five summarizes and interprets the study's findings and suggests some implications for future research.

Chapter Two: Background

2.1 Humans Must Maintain Physiological Homeostasis

A fairly constant or steady state, maintained in many aspects of the bodily economy even when they are beset by conditions tending to disturb them, is a most remarkable characteristic of the living organism.

Walter B. Cannon, in Walter B. Cannon, Science and Society[c]

2.1.1 What is Physiological Homeostasis?

All living organisms must maintain a state known as physiological homeostasis to survive. While the road to defining homeostasis was a slightly convoluted one, with contributors ranging from the ancient Greeks (Adolph, 1961) to Voltaire (Langley, 1973), from French physician Claude Bernard (Bernard, 1927) to Russian Nobel laureate Ivan Pavlov (Smith, 2008), it was ultimately American physiologist Walter Cannon who labelled the 'steady state' maintained by the living organism as physiological homeostasis in 1925 (Wolfe et al., 2001).

To achieve homeostasis, the biochemical, thermal, and physical conditions within a host operate within a narrow range of 'normal', thereby facilitating a state of dynamic equilibrium, or stability (Canon in Cooper, 2008). For example, human body temperature fluctuates between 36.4°C to 37.6°C, with an average temperature reading of approximately 37°C (Lu & Dai, 2008). If a physiological component (e.g., body temperature, blood pressure, heart rate, etc.) exists outside of the normal range an organism can experience a suite of negative consequences, including death. For example, should an individual's core temperature surpass this normal range (reaching values of 38°C or higher) an onset of a fever and/or fever-like symptoms such as chills, body aches and pains, or, more severely, damage to the central nervous system, can occur (Walter & Carraretto, 2016). At the other extreme, when body temperatures fall below normal, individuals can enter states of mild to severe hypothermia. While mild hypothermia can generally be treated with at-home remedies, severe cases in which body temperature drops below 32°C may require medical intervention (Turk, 2010). How does homeostasis become disrupted in the first place, however? To answer this question the concepts of stress and stressors will be discussed.

[c] Wolfe et al., 2000: p. 152

2.1.2 What Disrupts Physiological Homeostasis?

Cannon (1926) first used the term stress to refer to the external factors that could disrupt homeostasis. In the 1930s, endocrinologist Hans Selye (1950) argued for a broader definition of stress; that is, he stated that stress was a sum of all the 'non-specific' changes caused by the body's function or damage to the body. For this study, the definition of stress is 'the reaction of organisms to stressors', and stressors are therefore defined as 'any environmental challenge that has the potential to disrupt physiological homeostasis' (Wilson & DeCaro, 2013). For example, an ambient temperature below 0ºC may be a stressor that could reduce one's core temperature below 37°C. Another example of a stressor can be the presence of a predator around prey. Xu and colleagues (2019) studied the physiological effects of predator exposure and predator avoidance between a group of prey, qingbo fish, and their natural predators, catfish. Using the hormone cortisol as a metric for stress, their study found significantly elevated cortisol levels in qingbo who were exposed to the catfish as compared to when the predators were absent (Xu et al., 2019). A further example of a stressor is inadequate nutrition. When an individual cannot consistently meet appropriate caloric and essential nutrient intake values they can become nutritionally stressed. Physical manifestations of nutritional stress in children may be evident as wasting, in which a child has a low weight for their height, or stunting, in which a child is of a low height for their age.

Regardless of the type of stressor faced by an organism, the body's internal reaction is comprised of a common, integrated set of responses dubbed the *stress response*. The stress response can be thought of as an organism's response to homeostatic imbalance as caused by the effects of a stressor(s) (Selye, 1976; Schneiderman et al., 2005; Sapolsky, 2004). Throughout the stress response, an individual's immune defences and cognition are enhanced, their pain perception is blunted, and sensory thresholds are sharpened (Foy et al., 2005). How is this achieved? The stress response is controlled by the sympathetic nervous system, with significant contributions from the adrenal glands, the hypothalamus, and the pituitary gland. Upon encountering a stressor, the sympathetic nervous system is triggered to release epinephrine and norepinephrine from the adrenal glands and nerve endings, respectively. These increase an individual's heart rate and blood pressure. Within minutes, the hypothalamus triggers a cascade of hormones[d] down

[d] Corticotrophin releasing hormone (CRH) and adrenocorticotropic hormone (ACTH)

through the pituitary gland leading to the eventual release of cortisol into the blood. Cortisol functions to ensure that there are adequate nutrients for ATP (adenosine triphosphate) synthesis to occur [e]. Once the perceived stressor is identified and the stress response is initiated, an organism's systems must work to retain homeostasis. The maintenance of physiological homeostasis in the face of stressors is termed an adaptation.

2.1.3 Adaptations Return the Body to Physiological Homeostasis

While the term adaptation has been subject to debate (e.g., Gould & Lewontin, 1979), adaptation is defined here as a characteristic of an organism that facilitates physiological homeostasis and, consequently, survival and reproduction in a given environment (Mazess, 1975). For example, the production of antibodies by the immune system in the face of infection is an adaptation that facilitates survival. Or, upon exposure to cold ambient temperature, a mammal may shiver. Shivering, a stress response, can elevate the mammal's core temperature to maintain thermal homeostasis. Shivering may therefore be considered an adaptation as it may ensure survival.

On an individual organism level, Mazess (1975) lists nine separate adaptive domains in which potentially adaptive traits can be assessed, including: [1] reproduction; [2] health (i.e., morbidity, mortality, and disease resistance); [3] nutrition (i.e., nutrition requirements, utilization, and efficiency); [4] nervous system (i.e., sensory, motor, and neural function); [5] growth and development (i.e., physical and mental progressions in the rate of attainment); [6] resistance and cross-tolerance (i.e., generalized stress resistance); [7] physical performance (i.e., exercise and motor abilities); [8] affective function (i.e., happiness, tolerance, and sexuality); and [9] intellectual ability (i.e., learning and expression). Mazess (1975) contends that traits that are beneficial (i.e., can return the body to physiological homeostasis) in one or more of these domains can be considered adaptive to an individual. It is important to note, however, that an adaptation in one domain may prove to be detrimental in another (i.e., adaptations are not perfect), which is why it is crucial to consider adaptations in light of their environment (Darwin, 1859; Mazess, 1975).

[e] ATP is the source of energy at the cellular level

If a trait is deemed to be adaptive, it can fall into one of three broad and often overlapping categories: genetic, phenotypically plastic, or behavioral. Genetic adaptations can generally be defined as variants that confer adaptive benefits to the host at the level of the genome that arise by conventional Darwinian mechanisms. Human skin colour is an example of a genetic adaptation to ultraviolet (UV) radiation. It is a product of two clines[f] that were produced in response to the variation in UV radiation levels in different parts of the world (Jablonski & Chaplin, 2010).

Phenotypically plastic adaptations are the changes in an organism's morphology or physiology that mould its phenotype to the prevailing environmental conditions (Fusco & Minelli, 2010; Sultan & Stearns, 2005). There are two categories of phenotypically plastic adaptations: acclimatizations and developmental. Acclimatizations represent an adaptive response to the changing environment and as such, are reversable (Mazess, 1975). Acclimatizations can be further subdivided into structural, functional (physiological or neurological), and psychobehavioral (affective, cognitive, or behavioral) categories. To revisit the body temperature example, shivering is considered a functional-physiological acclimatization because it is a change in the organ system's functioning in response to a cold environment (Mazess, 1975). Conversely, developmental phenotypically plastic adaptations are non-reversible. Children who experience chronic undernutrition will undergo growth faltering – a one-time developmental adaptation that has molded their morphology to suit their environment at a given period.

Behavioral adaptations are activities (or behaviors) organisms engage in to survive and reproduce. For example, birds migrating south for the winter to escape the cold is a behavioral adaptation. Hibernation is another example, as animals shelter themselves during the coldest months of the year. Being nocturnal is also a behavioral adaptation and can provide environments with fewer predators or competitors for food or mates. Human-specific behavioral adaptations often find their roots in technology. The use of fire for cooking or warmth, for example, is a behavioral adaptation. Calgarians repeatedly experience below-zero temperatures throughout the winter months; therefore, it is essential that humans have access to both proper

[f] A cline is a measurable gradient in a single character of a species across its' geographical range; coined by Julian Huxley in 1938.

heating technology for our buildings and weather-appropriate clothing to stay warm when commuting around the city.

Evidently, humans and other organisms alike exhibit a suite of adaptations to the pervasive stressors experienced in everyday life. Yet how might the concepts of physiological homeostasis, stressors, stress, and adaptation apply to infectious disease? Before answering this question, it is imperative to define and describe infectious disease, and discuss why they have always been an important problem for humankind.

2.2 Infectious Disease as a Stressor

> Humanity shares a common ancestry with all living things on Earth. We often share especially close intimacies with the microbial world. In fact, only a small percentage of the cells in the human body are human at all. Yet, the common biology and biochemistry that unites us also makes us susceptible to contracting and transmitting infectious disease.
>
> Brenda Lerner, Infectious Diseases in Context[g]

2.2.1 Health and Disease; Relevant Disease Terminology

In order to define disease, it is necessary to first define health. The World Health Organization's (WHO) (2020a) definition, and the definition used in this thesis, defines health as the "state of complete physical, mental, and social well-being, not merely the absence of disease or infirmity." It is relevant to note, however, that while the WHO's definition is frequently cited, it is not without debate. Defining who is or is not healthy is difficult as notions of health are highly context dependent. Humans live in varied cultural settings in which perceptions of illness and disease can change with constructs of class, gender, and ethnicity (Scully, 2004). Furthermore, technological advancements and changing perceptions of health through time affect how individuals define health and subsequently, disease. For example, osteoporosis was classified by the WHO in 1994 as a disease. This declaration shifted cultural perceptions away from the belief that osteoporosis was a condition to be expected alongside the process of aging (WHO, 1994, as seen in Scully, 2004). Our expanding expectations of health, such as increased longevity, and

[g] Infectious Diseases in Context (2009, p.1)

social and economic factors can greatly alter how society perceives cultural and pathological conditions.

For the purposes of this project, disease is defined as "a physiological alteration that impairs function in some way" (Wiley & Allen, 2017: p.15)[h]. There are two categories of disease: non-communicable and communicable. Non-communicable diseases cannot be transmitted directly from one person to another[i]. Non-communicable diseases are not directly related to the goal of this project. They are defined here primarily to avoid confusion with communicable diseases. Communicable, or infectious, diseases can either be spread directly from person to person or indirectly (through a person-vector-person approach). Communicable diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites, or fungi (WHO, 2019). Examples of infectious disease include malaria, tuberculosis, and the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). Occasionally vector-borne or vector-borne diseases are referred to as 'infectious non-communicable' because they require a vector or intermediate host to transmit the disease. For example, mosquitos in the case of malaria and snails in the case of schistosomiasis[j]. Vector-borne diseases account for approximately 17% of all deaths caused by infectious disease and are especially prevalent in tropical and subtropical areas (WHO, 2020b).

2.2.2 Epidemiological Transitions and Infectious Diseases Throughout History

It is likely easiest to think about the significant and deadly role of infectious diseases on human history in three stages, or 'Epidemiological Transitions', as coined by Abdel Omran in 1971. While evidence on disease prevalence prior to ~10,000 BC is sparse, research on contemporary hunter-gatherer societies indicates that our early ancestors likely dealt with communicable diseases such as lice, pinworms, and salmonella (Armelagos et al., 2005; Sprent, 1969). Authors such as Armelagos et al. (2005) and McKeown (2009) additionally argue that low population

[h] With 'function' being equivalent to physiological homeostasis.
[i] There are four sub-categories of non-communicable diseases: cardiovascular (e.g., heart attacks), cancer, chronic respiratory diseases (e.g., chronic obstructed pulmonary disease), and diabetes (WHO, 2013).
[j] Schistosomes (i.e., flatworms), the organisms that cause schistosomiasis, are known as macroparasites. Macroparasites can also cause infectious disease, although these infections tend to be chronic and accumulate slower than microparasites.

density, minimal contact with outside groups, high mobility rates, and a varied diet likely kept epi- and pandemic[k] rates low in Pre-Neolithic Cultures.

In contrast, the agricultural revolution in ~10,000 BC was a significant catalyst for the consistent presence of infectious diseases in human society and is considered the first epidemiological transition (Harper & Armelagos, 2010; Armelagos et al., 2005; Omran, 1971) The agricultural revolution introduced sedentary lifestyles and larger populations with greater density, replacing the highly mobile lifestyles of hunter-gatherers (Armelagos et al., 2005; McKeown, 2009). Individuals with domesticated animals were also dwelling within agricultural settlements, often in close contact with the animals themselves. Collectively this meant that people were in closer proximity with one another, their animals, and each other's waste, creating breeding grounds for both communicable and zoonotic diseases such as malaria, smallpox, measles, and tuberculosis. Other prominent disease episodes in this first transition include the highly virulent Bubonic Plague that moved through Asia and west into Europe in the 1300s (Armelagos et al., 2005). Eventually the plague would kill approximately one-third of the global population due to the bacterium known as *Yersinia pesits,* commonly spread by fleas. Also disastrous was the first cholera pandemic in 1817 that decimated populations across Russia, India, Spain, the African continent, Indonesia, China, Japan, Italy, Germany, and America (Harris et al., 2012).

The agricultural revolution was further significant in that it introduced new food sources into human diets. Specifically, the advent of cereal and maize-based crops likely would have decreased the reliance on iron-rich foods such as meat, given that they could be grown, harvested, stored, and processed into different food products for future use. Since agricultural efforts effectively provided more food than previous hunter-gatherer efforts, population growth increased, which in turn put a demand on food production, facilitating a positive feedback loop with a greater reliance on grains in the diet (Denic & Agarwal, 2007). And, despite the presence of domesticated animals, authors like Larsen (2003) state that the amount of meat obtained from the livestock could not have kept up with the growing demands of the larger settlements. Grains,

[k] An epidemic is an occurrence of an illness or specific health-related behavior within a specified region or community that exceeds normal expectancy (e.g., the 2019 Chikungunya epidemic in Congo) (Porta, 2014). A pandemic is a disease spread across a large region (possibly multiple continents) and affects a large number of people (Porta, 2014). For an infective agent to cause a pandemic, it must be able to infect humans, cause disease in humans, and spread easily from human to human (Porta, 2014).

therefore, became a staple in the diet (Denic & Agarwal, 2007). This dietary shift is significant as cereal crops (e.g., wheat, barley, sorghum, etc.) do contain iron, but in smaller, harder-to-absorb amounts compared to the iron found in red meats (section 2.3.1). Further, the phytate content found in cereal grains would have hindered iron absorption from other foods, given that phytates bind to minerals such as iron or zinc before the body can absorb them. As such, authors such as Larsen (2003, 1995), Poskitt (2003), and Denic & Agarwal (2007) state that the agricultural revolution would have been accompanied by a 'pandemic' of iron deficiency.

These conclusions may be supported by the presence of skeletal lesions like cribra orbitalia or porotic hyperostosis. Larsen (1995) writes that cribra orbitalia (lesions in the eye sockets) and porotic hyperostosis (characterized by spongy or porous bone tissue in the cranial vault) are mostly attributed to iron deficiency anemia in archaeological remains. However, iron deficiency anemia has a complex etiology (Chapter 1), and therefore while iron-poor diets and iron deficiency could have led to these lesions, they may have also occurred due to parasitism or other chronic stressors that can affect iron homeostasis (Larsen, 1995). With this caveat in mind, it is still reasonable to conclude that iron deficiency and iron deficiency anemia plagued populations during the Neolithic because of the drastic dietary shifts that occurred and continue to plague human populations today.

The second epidemiological transition occurred from the Early Modern times to the 20th century[l] and was characterized by a decrease in infectious diseases and a rise in chronic and degenerative diseases (Armelagos et al., 2005). In contrast to the high-fertility, high-mortality rates of the first transition, the second epidemiological transition witnessed an initial climb and subsequent decline in fertility rates and a decline in the mortality rate. During this transition, population sizes and lifespan expectancies both increased, largely due to the discovery of antibiotics, creation of vaccines, improved hygiene, and nutrition – all contributing to better standards of living (Harper & Armelagos, 2010; Armelagos et al., 2005). At this point in time, many believed that infectious diseases were a thing of the past, given the effectiveness of antibiotic and vaccine treatments against infection.

[l] Armelagos et al. (2005) argue that this second transition has not been observed globally, rather, the first transition is still characteristic of many low-to-middle income countries today that suffer from marked social inequalities and a lack of public health infrastructure.

Moving from the end of the 20th century to present-day, society has entered the third epidemiological transition (Harper & Armelagos, 2010; Armelagos et al., 2005). Unlike its predecessor, however, the third transition has seen a rapid increase in the same infectious diseases that were once believed to be under control (e.g., malaria, tuberculosis) as well as the emergence of "new" diseases [m] (Harper & Armelagos, 2010). Notably, epidemiologists like Armelagos (2005; 2010) address the pressing issue of antibiotic resistance observed in many of these previously eradicated infections as a reason for potentially declining life expectancy in this third transition. Furthermore, rapid globalization fueled by constant travel, in addition to large-scale agricultural endeavours and industrial impacts on the environment mean that infectious diseases can spread around the world. Perhaps the most relevant example is the SARS-CoV-2 virus, or Covid-19 pandemic, that has dispersed worldwide since December 2019 (Sridhar et al., 2021).

It is evident that infectious diseases disrupt a host's physiological homeostasis and can therefore be classified as a stressor. They are among the leading causes of death in adults and children under the age of five (WHO, 2016). Yet infectious disease stressors are different from that of the presence of a predator, or a change in temperature, or an increase in UV exposure. These differences will be explored in the following sections and will elucidate why infectious diseases are so negatively impactful.

2.2.3 How Infectious Disease Differs from Other Stressors: Red Queens and Evolution

Infectious diseases differ from other stressors because pathogens have the capability to evolve and adapt to new environments, just as their hosts (e.g., humans) can. Cold ambient temperatures can certainly be a stressor, but the temperature is not going to change, as do infectious diseases, and circumvent our adaptations to it. The 'evolvability' of biological agents, which range from microscopic, intracellular viruses to large, structurally complex helminthic parasites, is arguably the most significant component in understanding how infectious diseases have affected human

[m] These 'new' or 'emerging' diseases have been criticized by some epidemiologists and scientists. Paul Farmer (1996) argues that we only 'discover' new diseases when they reach the minds of the wealthy; otherwise, they are not deemed significant enough to be a credible threat to our livelihoods.

life (Inhorn and Brown, 1990). In populations faced with endemic[n] infectious disease, individuals who are better adapted to handle the negative effects of an infection are more likely to survive and reproduce; that is, they have the potential to produce more offspring as a result of natural selection (Cagliani & Sironi, 2013; Fumagalli et al., 2011). Conversely, those who lack the necessary adaptations (whether these are phenotypically plastic, genetic, or behavioral) are at a higher risk of mortality and therefore less likely to successfully pass their genes into subsequent generations. The individual genetic factors that conferred protection from a specific disease may eventually be selected for in populations where infectious disease is prevalent (Fumagalli et al., 2011). Diverse, unstable environments with intense selective pressure from highly virulent pathogens can drive rapid adaptation from hosts; however, this creates an ongoing arms race between host and pathogen evolution as neither species can afford to be outcompeted (Inhorn and Brown, 1990). This co-dependent evolution is often referred to as the Red Queen Hypothesis, one of many pertinent concepts that should be discussed to better conceptualize pathogen persistence and examine the associations between iron and infection.

In 1973, evolutionary biologist Leigh Van Valen borrowed the idea of "running to stay in the same place" from Lewis Carrol's *Through the Looking Glass* (1871) when he coined the Red Queen Hypothesis (RQH). Van Valen (1973) sought to explain patterns of extinction of paleontological species and proposed that coevolved species were involved in omnipresent competitive interactions that shaped their genotypes and phenotypes. Over time, this competition did not lead one species to be better than coevolving species in a competitive sense as improvements in one species were met by improvements in another, resulting in a zero-sum expectation (Strotz et al., 2018). As regards infectious disease, the RQH offers an explanation for why infectious diseases persist and why host species survives in the face of these biotic stressors: both the pathogens and hosts are "running", that is, evolving, to ensure survival. An attack by a host is parried by evolution in the pathogen and vice-versa.

Likely one of the best examples to conceptualize the arms race between pathogens and hosts is with the phenomena known as antibiotic resistance. The misuse of antibiotics in hosts (humans

[n] Endemic is defined as "the constant occurrence of a disease, disorder, or noxious infectious agent in a geographic area or population group", such as the Tay Sachs condition within the Ashkenzai Jewish population in Southern Quebec (Porta, 2014, p. 92)

or animals) has been found to accelerate bacterial resistance because pathogens are adapting in response to repeated use of the medicines (WHO, 2018). The WHO (2018) lists a number of diseases including, but not limited to, pneumonia, tuberculosis, and gonorrhoea as infections that are becoming "harder, and sometimes impossible" to treat due to their antibiotic resistance. Furthermore, the WHO (2018: p. 1) argues that although we have new antibiotics under development, "none of them are expected to be effective against the most dangerous forms of antibiotic resistant bacteria." This capability of infectious diseases to evolve and adapt to host environments places them among the strongest selective pressures driving human phenotypic and genetic variation (Cagliani & Sironi, 2013; Fumagalli et al., 2011). How then, do humans keep "running"? The following section will explore some of the genetic, phenotypically plastic, and behavioral adaptations humans have undergone in the face of infectious diseases.

2.2.4 Examples of Adaptation to Infectious Disease

As noted above, humans adapt to infectious disease at three levels: genetic, phenotypically plastic, and behavioural. Biological statistician J. B. S. Haldane (1949: pg. 1) suggested that humans "adapted to infectious disease at the most basic level – the level of the gene." A commonly cited example of genetic adaptation to infectious disease is the association between the sickle-cell allele (HbS), a mutation found in the hemoglobin Beta gene on chromosome 11, and malaria. This HbS allele did not originate in response to the malaria, however individuals who are heterozygous fair better in environments where malaria is endemic (Allison, 1954). The life cycle of the malaria parasite (e.g., *Plasmodium* spp.*)* requires a period of development in the red blood cells (RBCs) of its host. Hosts that are heterozygous for HbS (e.g., HbAS)[o] have RBCs that generally are too unstable for the parasite to utilize, rendering the pathogen less virulent in these hosts (Allison, 1954). As an aside, HbS is a balanced polymorphism; individuals homozygous for HbS (HbSS)[p] die at an early age from sickle-cell disease if they receive no medical intervention. Some evidence also suggests that HbAS individuals may not be completely out of harm's way, either. In other words, HbAS may represent a gene-environment mismatch, where a trait evolved in an organism in one environment can be disadvantageous in a different environment. Nelson and colleagues (2016) found that American soldiers with the HbAS trait

[o] HbAS indicates an individual who has the sickle cell trait ('S') but is not infected. 'A' represents hemoglobin A, or normal hemoglobin.
[p] HbSS indicates an individual who has sickle cell anemia

were more at-risk for developing external rhabdomyolysis[q]. Additionally, Austin and colleagues (2007) reported that HbAS in Black individuals is a risk factor for venous thromboembolism. Against these dire outcomes, HbAS individuals are more likely survive infection by *Plasmodium* spp. and, consequently, to pass their genes on to subsequent generations; thus, selection drove increases in the frequency of the HbS allele in regions endemic for malaria (Allison, 1954).

A second type of genetic adaptation to disease, albeit, far more complex than HbS, is the innate immune system: the non-specific defenses that react immediately or shortly thereafter to the presence of an antigen[r], a foreign substance or toxin, in the body (Alberts et al., 2002a). The skin and other epithelial cells in humans help comprise the innate immune system. In the presence of an infectious disease, the proteins and phagocytic cells within the system recognize features of the antigen and work quickly to help destroy these 'invaders' (Alberts et al., 2002a). Casals and colleagues (2011: pg. 1) state that innate immune functions are one of several physiological responses "enriched with genes under positive or balancing selection," supporting the classification of innate immunity as a genetic adaptation. Innate immunity has low developmental costs on the host – it does not require extended investment by the host to maintain – but it has high operating costs; when functioning, the innate immune system is energetically costly for the host to maintain, and can even 'over-respond' in certain cases, causing a cytokine storm to occur (Georgiev et al., 2016). Complementing innate immunity is the secondary component of the immune system, known as adaptive immunity.

Adaptive (i.e., acquired) immunity is an example of a phenotypically plastic adaptation to infectious disease. As the name suggests, the acquired immune system develops over time. Unlike the innate immune system, the adaptive immune system does not immediately respond to antigens. Instead, the T and B lymphocytes must first be exposed to an antigen in order to 'memorize' how to defeat it (Alberts et al., 2002b). 'Memorization' entails the production of antibodies which should 'recognize' and effectively defend against subsequent exposure to the pathogen. An example of the adaptive immune system in action concerns *Varicella*, more

[q] Rhabdomyolysis is a condition resulting from the death of muscle fibres and the release of their contents into the bloodstream. If not treated, it can lead to kidney failure.

[r] Antigens are any substances that cause the immune system to produce antibodies. Antigens are found on the surfaces of pathogens. The term antigen is used specifically when discussing the innate and adaptive immune systems, as they respond to the antigens brought into the host via a pathogen.

commonly known as ‘chickenpox’. Varicella is a virus spread through respiratory droplets or direct contact with lesions on the skin (WHO, 2015). Thankfully, however, individuals who have had chickenpox once in their lifetime are less likely to experience the virus again because the adaptive immune system has memorized how to defend against it[s]. It is relevant to note that the adaptive immune system is more developmentally costly than the innate immune system. Therefore, Georgiev and colleagues (2016) argue that during early development, individuals who live in pathogen and resource abundant environments with higher average life expectancies are more likely to ‘invest’ in acquired immunity.

Humans have a wide array of behavioral adaptations to infectious disease. For example, actions such as washing our hands with soap and warm water to prevent the spread of microbes, or sneezing into the crook of our elbow, constitute behavioral adaptations. Another example of a behavioral adaptation is demonstrated by individuals residing in Northern Vietnam. Malaria is endemic to the area; however, mosquitos have a 10-foot flight ceiling (Inhorn and Brown, 1990). Individuals have taken to elevating their homes on poles so that they are above that 10-foot ceiling, thereby preventing the insects from reaching their living quarters and reducing the possibilities of being bitten (Inhorn and Brown, 1990). Advancements in medical technology, such as antibiotics and vaccinations, also comprise behavioral adaptations (Gaynes, 2017; Riedel, 2005).

Clearly humans exhibit a suite of adaptations to the pervasive stress of infectious disease. The following section will explore the significance of iron within our immune systems and its role as a potential adaptation to infectious disease.

[s] Approximately 1 in 3 Americans will experience a secondary varicella infection known as *Varicella-zoster*, or shingles. After the initial chickenpox fades, the virus remains dormant in the skin and can be reactivated later on in life as shingles (CDC, 2019)

2.3. Iron

Ancient stars in their death throes spat out atoms like iron which this universe had never known.
... Now the iron of old nova coughing's vivifies the redness of our blood.
Howard Bloom

2.3.1 Iron Metabolism in the Body

Iron is one of the most abundant elements on earth and a biologically essential component of almost every living organism on the planet. Given its importance it might not be surprising that there are multiple types of iron (Table 2.1). Despite iron's relative geologic abundance, however, the element does not exist in a soluble form that can be utilized by living organisms. This inconsistency is due to iron's significant affinity for redox reactions – that is, the process of gaining or losing electrons which causes the composition of an element, such as iron, to be altered. In the presence of oxygen, soluble ferrous iron (Fe^{2+}) changes into insoluble ferric iron (Fe^{3+}) (Abbaspour et al., 2014). To successfully meet iron requirements, therefore, several organisms, including humans, have evolved mechanisms to successfully obtain iron in three main steps: iron intake, iron absorption, and iron storage/recycling.

Table 2.1 Types of Iron

Term	Definition
Heme iron	One of two forms of dietary iron, it is easily absorbed by the body and found predominantly in meat, poultry, and fish.
Nonheme iron	One of two forms of dietary iron, it is not soluble and must be broken down by the small intestine to be absorbed into the body. It is commonly found in plant protein (legumes, fruits, vegetables). Exists as either Fe^{2+} or Fe^{3+}
Ferrous iron	Soluble iron; exists as Fe^{2+}
Ferric iron	Insoluble iron, the most common form found in our food; exists as Fe^{3+}
Apoferritin	A protein commonly found within the intestinal mucosa membrane that combines with Fe^{2+} to create Fe^{3+}
Ferritin	A protein that acts as the primary form iron storage (Fe^{3+}) inside the body; found within the liver, spleen, and bone marrow
Apotransferrin	A protein that binds to Fe^{3+}, forming transferrin
Transferrin	Transferrin is a transport protein, designed to carry Fe3+ iron throughout the bloodstream and into cells
Ferroportin	A transmembrane protein that exports iron from within a cell into the bloodstream; it is the only known iron *exporter*

Iron is obtained in food in two forms: heme and nonheme (Abbaspour et al., 2013; Zimmermann & Hurrell, 2007). Heme iron is predominantly found within the hemoglobin and myoglobin of meat, fish, and poultry, whereas nonheme iron is sourced from plant proteins. Heme iron has a high bioavailability: 15-35% of heme iron consumed is absorbed by our bodies. Nonheme iron absorption is significantly influenced by the presence of other food components in the digestive system and has a bioavailability ranging from 2-20% (Hurrell & Egli, 2010). Despite its reduced bioavailability, however, the quantity of nonheme iron in most human diets is found to be much greater than heme iron and it generally contributes more to human iron-nutrition than its counterpart (Monsen et al., 1978). Alberta Health Services (2018) recommends 8 mg of dietary iron per day for adult males (19-50) and 10mg of dietary iron per day for females, however daily iron intake recommendations vary by geographic area and health provider.

The primary sites of iron absorption are the intestinal mucosal cells, or enterocytes, of the duodenum and upper jejunum in the small intestine (Zimmermann & Hurrell, 2007; Hasundungan, 2014). When nonheme iron is released into the intestinal lumen it predominantly exists as insoluble Fe^{3+} ions[t]. A ferrireductase enzyme attached to the surfaces of the enterocytes, Duodenal Cytochrome B (DCYTB), is responsible for catalyzing Fe^{3+} into soluble Fe^{2+} ions. After catalyzation occurs, the ferrous iron moves from the lumen into the enterocytes via cotransporter proteins called Divalent Metal Transporter 1 (DMT1). Cotransporter proteins send two different molecules into cells at the same time; in this case, Fe^{2+} ions are accompanied by hydrogen ions (H^+). However, any free iron inside a cell is dangerous to the host because of its redox potential (Tansarli et al., 2013). If not bound to another molecule, free ferrous iron and hydrogen can react and form hydroxyl (free) radicals that will damage the body (Tansarli et al., 2013). The ferrous iron must either be stored or exported out into the bloodstream. A molecule known as apoferritin will bind with some of the ferrous ions, causing them to oxidize back into ferric iron (Abbaspour et al., 2014). Together they form ferritin, which can be safely stored in the enterocytes. Alternatively, the Fe^{2+} ions are exported out of the enterocytes via a transmembrane protein known as ferroportin (Abbaspour et al., 2014; Hussain, 2018). Ferrous iron is not

[t] Heme iron absorption is less understood than that of nonheme iron but there appear to be two prevailing hypotheses for this process (West & Oates, 2008). The first hypothesis proposes that heme is engulfed by receptor-mediated endocytosis (Grasbeck et al., 1979), whereas the second hypothesis proposes a possible heme transporter that would take heme iron from the small intestinal lumen directly into the cytoplasm of the enterocytes (Shayeghi et al., 2008).

transportable; therefore, these ions are oxidized back into ferric iron via the hephaestin enzyme. The ferric iron ions bind to apotransferrin to form transferrin and will be transported to other tissues and cells in the body via the bloodstream. The iron bound to transferrin accounts for less than 0.2% of all iron in the body; the majority of iron can be found stored throughout various tissues (Zhang & Enns, 2009; Schmaier & Petruzzelli, 2003).

After leaving the enterocytes, iron is predominantly found within the bone marrow, the liver, and the spleen. Approximately 75% of the body's absorbed iron is utilized in erythropoiesis[u]. An estimated 10-20% of an individual's iron is stored within the liver as ferritin (Abbaspour et al, 2014; Johnson-Wimbley & Graham, 2011). As red blood cells (RBC) reach the end of their life cycle (~120 days), they are engulfed by macrophages in the spleen and liver. The RBCs will be broken down into free iron ions, which are then either stored as hemosiderin or ferritin, or released back into the blood stream to be recycled by the body. A constant balance between iron uptake, transport, storage, and utilization is required in order to maintain iron homeostasis in the host. Low iron can lead to iron deficiency and iron deficiency anemia, whilst excess iron can cause iron overload or haemochromatosis (Rishi et al., 2015). The only physiological mechanism in which mammals are able to excrete excess iron from their systems, however, is blood loss (i.e., pregnancy, menstruation, or chronic/acute bleeding). To prevent the toxic effects of excess iron, therefore, mammalian iron levels are predominantly regulated at the point of absorption by a hormone known as hepcidin (Johnson-Wimbley & Graham, 2011).

Hepcidin is an evolutionarily conserved circulating peptide hormone commonly referred to as the 'master regulator' in iron homeostasis (Segat et al., 2008; Rishi et al., 2015; Abbaspour et. al., 2014). Hepcidin is produced and secreted by the liver and at the most basic level, the hormone serves to regulate iron levels by causing the internalization and degradation of ferroportin (Sheftel et al., 2012). More specifically however, hepcidin maintains a dual role in humans; that is, hepcidin is a part of the acute phase protein response (APPR) in addition to contributing to an individual's innate immune response. The APPR occurs immediately during infection and inflammation and usually lasts around 30 days (Stadnyk & Gauldie, 1991). During this time the

[u] Otherwise known as red blood cell production.

presence of inflammatory cytokines[v] causes the upregulation of hepcidin, which then will decrease plasma iron levels by inhibiting ferroportin function (Rishi et al., 2015). Hepcidin's other role in innate immunity comes from its classification as an antimicrobial peptide (AMP). AMPs are an assorted group of naturally occurring molecules that can be found in organisms ranging from prokaryotes to humans. They are produced as the first line of defense against invading pathogens; hepcidin specifically can bind to and destroy an invading bacteria's membrane, thereby facilitating the degradation of the target (Zhang & Gallo, 2016). In sum, therefore, infections in most organisms cause the upregulation of hepcidin, which serves to kill pathogens while simultaneously downregulating iron release. In humans, hepcidin's dual functions are linked in one molecule, however other animals such as mice have two hepcidin molecules, and certain suborders of fish, such as the Nototheniodei[w], have been found with up to four (Xu et al., 2008). The duality of hepcidin in humans may be considered adaptive, given that both functions are involved in the immune response and provide a degree of protection against infection.

Certain studies involving mice have found that by supressing erythropoiesis, hepcidin levels dramatically increased, suggesting that RBC production may be a negative regulator for this hormone (Vokurka et al., 2006). Given that hepcidin plays such a crucial role in iron regulation and immune defense, any changes to its expression can be dangerous for the host. Mutations in the HFE gene[x] as well as in the hepcidin anti-microbial peptide[y] (HAMP) prohibit hepcidin function and are known to cause hereditary haemochromatosis, in which an individual suffers from the effects of excess iron accumulation in the tissues (Rishi et al., 2015). In contrast, constant hepcidin expression can lead to conditions such as anemia. Both iron-overload and iron-deficiency can significantly impact the host's immune system.

2.3.2 Conditions of Iron Deficiency - Iron Deficiency and Iron Deficiency Anemia

As noted in Chapter One, ID and IDA are global health crises. ID is defined as a "condition in which there are no mobilizable iron stores and in which signs of a compromised supply of iron to

[v] Cytokines are small proteins responsible for cell-signalling throughout the body
[w] The Notothenioidei are a suborder of fish that primarily include Antarctic fish and Subantarctic fish.
[x] Codes for the haemochromatosis protein
[y] Codes for hepcidin itself

tissues, including the erythron[z], are noted" (WHO/UNICEF/UNU, 2001: p.19). ID occurs when iron absorption cannot keep pace with the body's utilization of iron over an extended period of time (Johnson-Wimbley & Graham, 2011). Conversely, *anemia* is clinically defined as a decrease in the total amount of hemoglobin or the number of RBCs (Johnson-Wimbley & Graham, 2011). ID and anemia are not always found together; iron deficiency without anemia can occur when a person has normal hemoglobin levels, but below normal serum ferritin and/or transferrin saturation (CDC, 1998). Iron deficiency anemia (IDA) specifically occurs when the levels of iron needed for erythropoiesis are insufficient for more than three months (Zimmerman & Hurrell, 2007). As such, IDA is characterized by microcytic, hypochromic RBCs[aa] and low iron stores (see Table 2 below for exact clinical measurements). An individual who has prolonged, insufficient daily iron requirements will progress from depleted iron stores to iron-deficiency erythropoiesis, and finally to iron deficiency anemia. Individuals with ID and IDA can suffer from conditions such as dizziness, fatigue, shortness of breath, and reduced immune function while afflicted (National Heart, Lung, and Blood Institute, 2020).

One cause of iron deficiency is an inadequate dietary intake of iron. This can occur because of an insufficient caloric intake, improper nutrition (e.g., not eating enough iron-dense foods), or dieting. Furthermore, diets rich in iron inhibitors, such as phytates and polyphenols, can place individuals at risk for iron deficiency (Skikne & Hersho, 2012). Iron deficiency may also occur when an individual has unmet increased iron requirements, as seen in adolescent males and females throughout puberty, or in pregnant women. In addition to increased iron requirements during puberty, adolescents experience an increase in their total blood volume, lean body mass, and the start of menstruation in females. The increase in blood volume alone raises daily iron requirements by 0.14 mg in females and 0.18 mg in males (Skikne & Hersho, 2012). Furthermore, greater lean body mass increases iron requirements by another 0.33 mg in females and 0.55 mg in males (Skikne & Hersho, 2012). Females also specifically require an extra 0.56 mg per day to replace blood lost during menstruation. Pregnant women are additionally subjected to increased iron requirements. Skikne and Hersho (2012) state that pregnant women need (on average) an additional 2.5 mg of iron per day over the 1 mg base requirements. Women with

[z] Erythron are the red blood cells and the surrounding marrow
[aa] Small and less-pigmented, respectively

depleted iron stores (ferritin levels below 50 micrograms/litre) prior to conception are more likely to develop iron deficiency anemia during pregnancy (Skikne & Hersho, 2012).

Other risk factors for ID and IDA include increased iron losses, infection, and stress. Frequently blood loss (and subsequent iron depletion) occurs within the genito-urinary, gynecological, or gastrointestinal tracts (Zimmerman & Hurrel, 2007). Infection may also contribute to iron deficiency anemia. Studies have demonstrated possible links between *Helicobacter pylori* (*H. pylori*) infection and conditions of IDA (Hershko et al., 2005; Hershko et al., 2006). Individuals who had both IDA and were positive for *H. pylori* were more than twice as likely to fail to respond to oral iron supplementation than those without *H. pylori* (Hershko et al., 2005; Hershko et al., 2006). Once the infection was successfully treated, patient's hemoglobin levels increased to those of the non-infected, iron-deficiency anemia patients (Hershko et al., 2005; Hershko et al., 2006). Annibale and colleagues (2003) argue that the reason *H. pylori* infections affect IDA treatment is because of their effect on the host's gastric juice. Normal iron absorption requires a specific pH level in addition to ascorbate[bb] content, both of which are adversely impacted by *H. pylori* (Annibale et al., 2003). Once eradicated, pH and ascorbate levels return to normal, and iron absorption resumes. Finally, and perhaps most interestingly, studies have shown that stress may contribute to conditions of ID and/or IDA. Wei and colleagues (2008) reported that, in addition to physical stressors such as electric shock exposure, rats placed under pure psychological stress had a significant decrease in their hemoglobin and red blood cell levels.

Both ID and IDA can exacerbate conditions such as malaria, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), schistosomiasis, and tuberculosis due to iron's crucial role in the immune system (Joynson et al., 1972; Cherayil et al., 2010). Conversely, however, maintaining normal or even elevated iron levels in the presence of a pathogen can be detrimental to the host, as pathogens, like their hosts, require iron to survive.

[bb] Ascorbic acid is also known as Vitamin C

Table 2.2 Laboratory diagnoses used for IDA (adapted from Johnson-Wimbely & Graham, 2011)

Laboratory Measure	Definition and Values
Mean Corpuscular Volume (MCV)	Measure of average RBC volume; normal reference range is 80-100fL
Mean Corpuscular Hemoglobin	Measure of concentration of hemoglobin in given volume of packed RBCs; normal reference range is 320-360g/l
Red Cell Distribution Width	Measure of variation of red blood cell width and used in combination with MCV to distinguish an anemia of mixed cause from that of a single cause; normal reference range is 11-14%
Hemoglobin	Protein responsible for transporting oxygen; <7.7mmol/L in men and <7.4mmol/L in women is considered abnormal
Serum Iron	Measures amount of circulating iron that is bound to transferrin; <7.1µg/L is abnormal
Serum Ferritin	Measures of iron storage; <30ng/l is abnormal
Transferrin Saturation	Value of serum iron divided by the total iron-binding capacity of the available transferrin to indicate how much iron is bound; <15% is abnormal
Total Iron-Binding Capacity	Measures the blood's capacity to bind iron with transferrin; >13.1µmol/L is abnormal

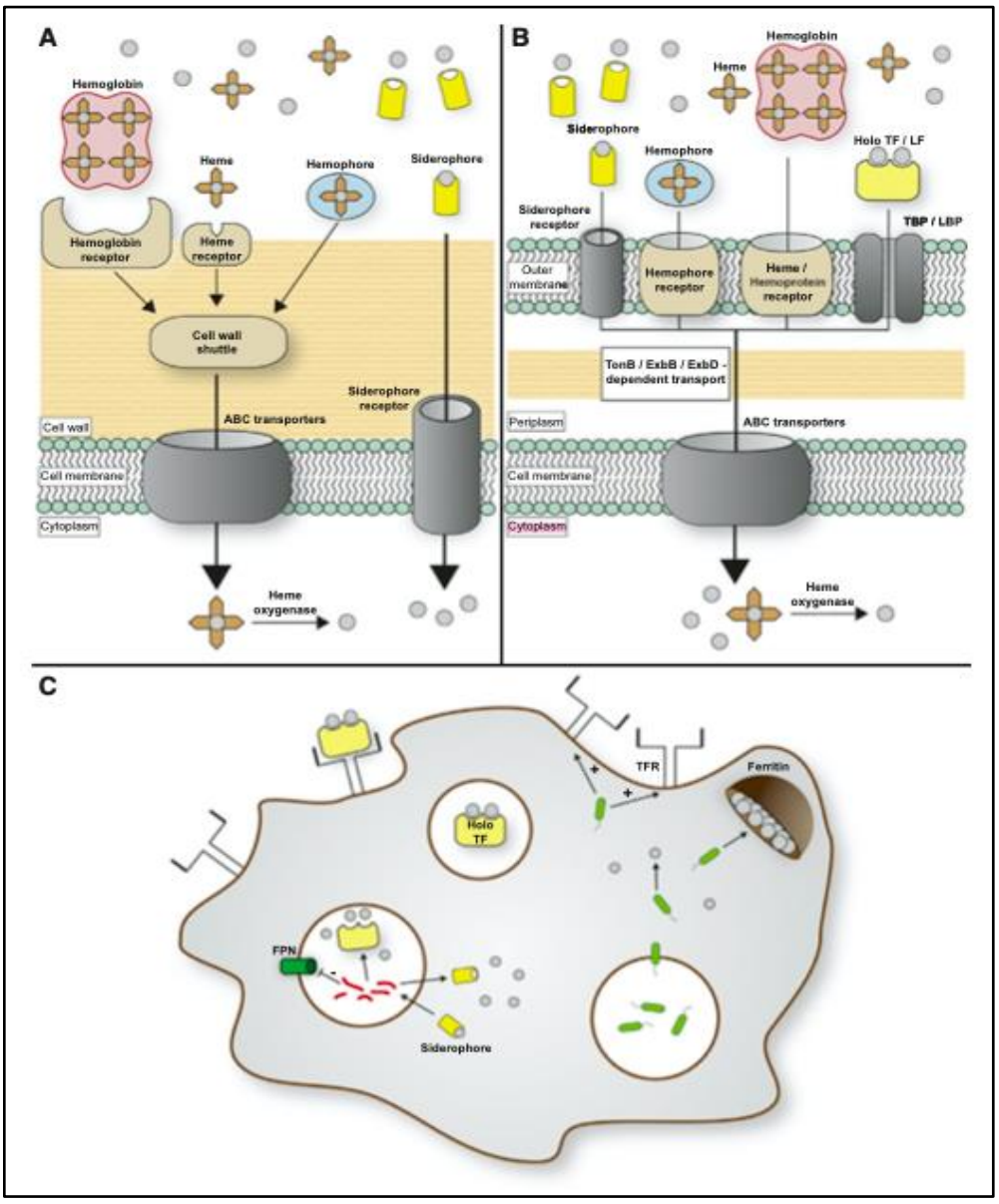
2.3.3 Iron and Infectious Diseases

Similar to other living organisms, iron is an essential nutrient for almost all pathogens (Cassat & Skarr, 2013). Pathogenic organisms, however, lack the necessary physiological structures (i.e., a gastrointestinal tract) that can facilitate the consumption and absorption of ferric iron from the environment (Pietrangelo, 2015). Pathogens have therefore evolved mechanisms that allow them to assimilate iron from their hosts. For example, bacterial species such as *Escherichia coli* produce siderophores, which are small, ferric iron chelators used to scavenge iron from the host (Hagan, 2017; Cassat & Skarr, 2013). Siderophores specifically compete with unsaturated transferrin[cc], binding directly to ferric iron and effectively transporting it back into its host bacterial cells (Hagan, 2017; Cassat & Skarr, 2013). Other bacterial species such as *Neisseria meningitidis* use siderophores to directly absorb iron from transferrin or lactoferrin complexes (Hagan, 2017; Cassat & Skarr, 2013). These iron-absorption methods have allowed pathogens to

[cc] Otherwise known as apotransferrin

effectively compete with their hosts for iron and subsequently establish infections in the body (see Figure 2.1 for examples).

Figure 2.1 Methods of bacterial iron acquisition (Cassat & Skaar, 2013)



Methods of bacterial iron acquisition (Cassat & Skaar, 2013). (**A)** represents how gram-positive organisms can obtain iron through heme and hemoprotein cell surface receptors or through the secretion of hemophores (*B. anthracis*). Heme is moved across the cell wall before transport into the cytoplasm by ABC-type transporters. After it is in the cytoplasm, heme can be degraded to release iron. Alternatively, gram-positive pathogens can secrete siderophores that capture iron and re-enter the cell via specific transporters. (**B)** is an example of gram-negative organisms utilizing siderophores and heme/hemoprotein receptors to steal host iron. Certain gram-negative pathogens express transferrin or lactoferrin binding proteins that can utilize transferrin- or lactoferrin-bound iron. **(C)** demonstrates intracellular pathogens producing siderophores. In this instance, the tuberculosis pathogen (Mtb, depicted in red) can diffuse out of the phagosome to capture cytoplasmic iron.

2.3.4 Host Adaptations to Iron Piracy

The ongoing arms race between vertebrates and pathogens is reflected in the competition for iron: vertebrates and most of their pathogens require iron, but it is in the interest of hosts to reduce iron levels because iron availability increases pathogenic proliferation and virulence[dd] (Hennigar & McClung, 2016; Kortman et al., 2012; Ding et al., 2014; Marx, 2002). Recall that the majority of iron is found sequestered in ferroproteins such as hemoglobin, stored in iron-complexes such as ferritin, or bound to high-affinity carrier proteins like transferrin. This virtually iron-free environment is hypothesized to be an ancient evolutionary defense mechanism against infectious disease, as almost all living organisms appear to limit the bioavailability of their iron stores via the methods discussed above (Barber & Elde, 2014; Cassat & Skarr, 2013; Lee et al., 2012).

Another way of reducing iron bioavailability comes from the master-regulator, hepcidin. As above, a trigger for hepcidin synthesis is the presence of inflammatory cytokines, which are released upon the onset of infection. Hepcidin will inhibit ferroportin function, thereby reducing serum iron concentrations in the body by approximately 30% (Kossiva et al., 2013). These reduced serum iron levels force the host into a functional state of iron deficiency known as hypoferremia of infection (HI) (Kossiva et al., 2013). Hepcidin is effective to a fault, however, and prolonged infections can reduce RBC production (a condition known as restricted erythropoiesis) and lead to anemia of chronic infection / anemia of inflammation (Kossiva et al., 2013). HI (and consequently anemia of inflammation) have been shown to limit the viability and proliferative capacities of extracellular pathogens within the host (Collins et al., 2008; Stefanova et al., 2016), but long term-exposure can actually contribute to the growth and severity of intracellular pathogens (Spottiswoode et al., 2014; Portugal et al., 2011). In environments where intracellular pathogens exhibit high selective pressure, therefore, HI and anemia of chronic disease/inflammation may be maladaptive to the host.

If hepcidin only provides short-term protection against infectious disease before causing additional harm to the host, then what other mechanisms have humans formed to defend against

[dd] This concept is often referred to as 'Nutritional Immunity' – a process by which a host organism sequesters trace minerals, such as iron or zinc, in an effort to limit the proliferation of pathogens during an infection (Hennigar & McClung, 2016).

iron piracy? Iron deficiency, and consequently iron deficiency anemia may be the answer to this question. For example, mild forms of ID and IDA may increase resistance to acute infections (Wander et al., 2009). Additionally, ID and IDA can reduce microbial virulence and even decrease the risk of disease recurrence in environments with high levels of endemic disease (Goheen et al. 2016; Senga et al. 2011; Jonker et al., 2012). Furthermore, low iron bioavailability may increase the phagocytic effectiveness of host immune cells[ee] (Bullen et al., 2006). Other studies have shown that low host iron levels can prevent or inhibit intra- and extracellular pathogenic proliferation (Chlosta et al., 2006; Portugal et al., 2011). Low iron levels have also been found to decrease or reduce the production of biofilm[ff] (Hindre et al., 2008; Hunter et al., 2013; Lin et al., 2012). Finally, some researchers have found that low iron increases the effectiveness of antibiotics in multi drug-resistant infections (Grenier et al., 2000).

2.3.5 Iron's Conflicting Roles

Although low iron levels could be favorable for humans, iron remains an essential component for immuno-surveillance, immuno-activation, and immuno-regulation in the body (Cherayil, 2010; Collins, 2003). Therefore, opposing lines of evidence have demonstrated that even mild-to-moderate forms of ID and IDA are maladaptive for the host. Adverse health effects such as ineffective inflammatory responses, altered cytokine expression, impaired growth and maintenance of B and T lymphocytes, and increased susceptibility to infection may all be experienced by hosts with low iron (Cherayil, 2010; Srikantia et al., 1976). So, iron is an essential nutrient, a shortage of which is a risk factor for elevated morbidity and mortality. Opposingly, the presence of iron may exacerbate infectious disease. What might we make of these potentially conflicting roles of iron? The reason for these discordant results could potentially be a by-product of *where* and *why* this research has been conducted, in that many studies on ID and IDA in relation to infectious diseases have occurred in low-to-middle income countries with both high levels of infectious disease and high levels of ID/IDA.

[ee] The ability for immune cells to engulf invading pathogens like bacteria
[ff] Multi-cellular communities formed by bacteria as protection from antibiotics and host defenses

2.3.6 Previous Studies on IDA and IDA in Relation to Infectious Disease

The majority of studies on ID and IDA in relation to infectious diseases have been conducted in low-to-middle income countries where infectious diseases such as malaria (Senga et al., 2010; Sazawal et al., 2006; Mebrahtu et al., 2004; Menendez et al., 1997; Smith et al., 1989;), tuberculosis (Gangaidzo et al., 2001; Devi et al., 2003) or HIV/AIDS[gg] (Wiskansa et al., 2011; Meda et al., 1998) are prevalent in the region. Furthermore, many of these studies have focused on the relationship between iron deficiency and infection in children or have examined the effects of iron supplementation on children specifically and excluded other age groups (e.g., Hadley & DeCaro, 2015; Lind et al., 2003; Stoltzfus et al., 2001; Dijkhuzien et al., 2001; Mitra et al., 1997; Thibault et al., 1993; Angeles et al., 1993). Collectively, results of these studies appear to be somewhat of a mixed bag.

For example, Senga et al. (2011) examined the relationship between placental malaria and iron deficiency in pregnant women from Southern Malawi, Philippines. Using serum ferritin and transferrin to quantify iron levels and placental tissue to identify malaria, Senga et al. (2011) concluded that iron deplete, pregnant women were less likely to have placental malaria than those who were iron replete. Similarly, Nyakeriga et al. (2004) examined iron deficiency and its association with malaria in Kenyan children from 2001 to 2003. Their study utilized measures of transferrin saturation and plasma ferritin concentration to define iron deficiency ($\leq$ 10% and $\leq$ 12μ/mL, respectively) and concluded that iron deficiency was associated with a small but significant degree of protection from episodes of clinical malaria[hh] (Nyakeriga et al., 2004).

Examples of studies that examined the association of other infectious diseases and iron levels include Wander et al. (2009), who measured infection via the C-reactive protein (CRP), and iron status (as reflected by the transferrin receptor, TfR, and zinc protoporphyrin to heme ratio, ZPP:H) in 270 school-aged Kenyan children. TfR was selected given its robustness as a biomarker of iron status (i.e., it does not alter with infection); however, the authors did have to define their own range of TfR in order to identify elevated ZPP:H, as TfR cut-offs can differ by

[gg] Relevant to note that of the studies focusing on HIV/AIDS and iron levels, many more were conducted in high-income countries such as the United States (compared to malaria/tuberculosis).

[hh] In non-malaria-endemic areas, malaria is often diagnosed with a positive blood film and the presence of a fever. In areas such as Kenya, however, malaria can be diagnosed through the presence of increased IgE antibodies (Nyakeriga et al., 2004)

population (Wander et al., 2009). Wander et al. (2009) found that an iron replete condition (defined as TfR $\leq$ 4.5 mg/l and ZPP:H $\leq$ 60 µmol/mol) increased the odds of infection (OR = 2.0, p = 0.11) and determined that clinical iron deficiency may protect against infection (OR = 0.50, p = 0.11). Further, Wander et al. (2017) explored the associations between iron status and both pre-existing and new infections (those that occurred after iron status was characterized) in a cohort of Tanzanian children. Using transferrin as a biomarker for iron level, blood hemoglobin to characterize anemia, and CRP and α_1-acid glycoprotein as markers for inflammation, Wander et al. (2017) collected data from 314 children. Their study reported a protective effect of mild-to-moderate iron deficiency against respiratory infections among the children sampled.

Regarding iron supplementation, research conducted in Bangladesh by Mitra et al. (1997) found that iron supplementation in children under a year old resulted in a 49% risk increase in the number of dysentery cases. In Zanzibar, Tanzania, Sazawal and colleagues (2006) found a 12% (95% CI: 2%, 23%) greater risk of severe illness leading to hospitalization due to malaria associated with iron and folic acid supplementation in children with high rates of malaria and other infectious diseases. Conversely, a previous systematic review of 28 studies conducted by Gera and Sachdev (2002) examining the effects of iron supplementation in children and their resulting susceptibility to infection concluded that iron supplementation in these children did not appear to significantly increase the risk of overall infection, which suggests that increasing iron levels is not detrimental to an individual's immune system[ii]. Similarly, Angeles et al. (1993) reported that fever, respiratory infections, and diarrhea were reduced in children aged 2-5 who had received iron supplementation.

In the studies that have taken place in Western, educated, industrialized, rich, and democratic countries (WEIRD; Henrich et al., 2012), the focus appears to be on infections such as *H. pylori* (Cardenas et al., 2011). Moreover, Cardenas et al. (2011) cite several other authors who had examined the interactions between *H. pylori* and iron levels (e.g., Kurekci et al., 2005; Sarker et al., 2008; Choe et al., 2000; Choe et al., 1999) – however each of these studies was conducted using children as the sample demographic, and only one study other than their own was

[ii] Of the 28 studies included in this review, 11 were from Africa, eight were from Asia, five were conducted in the Americas, two were conducted in Europe, and the remaining two were from Australia and New Zealand.

conducted in America (Fagan et al., 2009). Therefore, although there is ample literature on the interactions between iron and infectious disease, the limitations of these studies include their geographic location, the severity and scale of infectious diseases present, and the chosen sample demographics.

2.3.7 Possible Associations of Sociodemographic Variables with Iron and Infection

Variation in serum iron levels and infection has a complex etiology (as noted in Figure 1.1 in Chapter One, and previously throughout Chapter Two). Part of this variation can likely be explained by sociodemographic variables (SDVs), with a robust body of literature supporting the claim that health disparities can largely be explained by sociodemographic differences (Darin-Mattsson et al., 2017; Irwin et al., 2006; Marmot, 2015). In the present study, four SDVs were considered: 1) socioeconomic status, 2) education level achieved, 3) Indigenous status, and 4) immigrant status.

Socioeconomic status (SES) has strong predictive power: lower SES is predictive of worse health outcomes in Canada (Bayoumi et al., 2020; Health Quality Ontario, 2016; Humphries & Doorslaer, 2000; Health Promotion and Chronic Disease Prevention in Canada: Research, Policy, and Practice, 2016; Canadian Population Health Initiative, 2008) and lower SES has been linked to higher rates of anemia, globally (WHO, 2001). Following Lightman et al. (2008) and Geyer et al. (2006), in this study, SES is assessed via median household income. Moreover, median household income was chosen as a marker of SES because income is ranked as the first 'determinant of health' by the Public Health Agency of Canada (Government of Canada, 2016)[jj]. Postsecondary education level is also considered a determinant of health by the Public Health Agency of Canada (Government of Canada, 2016). More broadly, lower education levels have been linked to high levels of infectious disease and chronic disease, poorer self-rated health, reduced survival rates when sick, and shorter life expectancy around the world (Ahmmad et al., 2021; Manor et al., 2004; Silventoinen & Lahelma, 2002; Ross & Wu, 1995).

[jj] The government of Canada defines determinants of health as "the broad range of personal, social, economic and environmental factors that determine individual and population health." (Government of Canada, 2016, para. 2).

Indigenous status was selected as relatively high levels of iron deficiency and iron deficiency anemia have been documented in Canadian Indigenous children and women (Christofides et al., 2005a; Willows et al., 2000; Hodgins et al., 1998). The prevalence of IDA in Canadian children among the general population is relatively low (approximately 3.5% – 10.5%). However, these levels have been recorded as varying anywhere between 14% – 50% in Canadian Indigenous populations (Christofides et al., 2005b; Willows et al., 2000; Hodgins et al., 1998). For example, in their study on two northern Ontario First Nations communities and one Inuit community, Christofides et al. (2005b) found that anemia (diagnosed by hemoglobin levels < 110 g/L) was present in 36.0% of the sample and approximately 53.3% of the sample had depleted iron stores (assessed via serum ferritin levels < 12 µg/L). Similarly, recent immigrant status was considered because newly arriving immigrants and refugees to Canada have been found to have higher levels of iron deficiency and iron deficiency anemia compared to that of North American-born residents (Pottie et al., 2011; Hayes et al., 1998; Bindra & Gibson, 1986). In fact, Pottie and colleagues' (2011) Canadian study concluded that immigrants coming from regions with limited iron availability in foods (e.g., East Indian origins), higher rates of infectious disease (e.g., Southeast Asia or the Mediterranean), and higher birth rates (e.g., populations with African ancestry) are at risk for iron deficiency and should be screened upon entering the country to offset these conditions and the potential for related morbidity.

Collectively, each of the four chosen sociodemographic variables have been linked to iron levels and/or infections in Canada, which makes them relevant to the present study's second research question and hypothesis.

2.4 Chapter Summary

In conclusion, all organisms must maintain a narrow range of operating conditions or physiological homeostasis. To survive organisms must adapt to environmental perturbations or stressors. Humans respond to these stressors via a mix of genetic, phenotypically plastic, and behavioral adaptations. One of the most potent environmental perturbations faced by any organism is infectious disease, a stressor which triggers all three levels of adaptation. Notably, humans required iron to maintain physiological homeostasis, but, utilizing an adaptation to infection, will limit the availability of iron in the face of infection. While this appears to render

the host's environment less hospitable for the pathogen, it also extracts a toll on other aspects of physiological function and is thus a delicate balancing act. While there is some understanding of this balancing act among peoples in low-and-middle income countries where the infectious disease load is high, relatively little is known about this in WEIRD countries, hence the goal of this paper. The present study is designed to provide new insights to our understanding of the association between iron and infection by conducting research on a large sample of an urbanized population that does not suffer from high levels of endemic infectious disease. Furthermore, the present study sought to gain valuable insight into the potential interactions between sociodemographic measures such as household income and postsecondary education level and serum iron, to parse out why variations in mean iron level across the City of Calgary may be observed. In the next chapter the methodology will be discussed for this research.

Chapter Three: Methods

3.1 Study Design

3.1.1 Ethics Statement

Ethics approval for this study was obtained from the University of Calgary Conjoint Faculties Research Ethics Board (REB 18-0607_REN1).

3.1.2 Context of Study Site

Province of Alberta

Alberta is the second-most Western province in Canada, bordered by British Columbia to the west and Saskatchewan to the east. Alberta is also flanked by the Northwest Territories along its northern border and the state of Montana to the south. The 2011 Canadian Census data reported Alberta as the third-most densely populated region in Canada at 5.7 persons per squared kilometre, falling behind Quebec (5.8 persons/km^2) and Ontario (14.1/km^2). Alberta had a population of 3,645,257 individuals in 2011 with a 10.8% increase from its recorded population of 3,290,350 in 2006 (Statistics Canada, 2012a). As of 2011, 80.6% of the population resided within a Census Metropolitan Area (CMA) (Statistics Canada, 2012a).[kk] The two largest CMAs in Alberta are Calgary and Edmonton, with Calgary's population surpassing Edmonton's at 1,214,839 to 1,159,869 (Statistics Canada, 2012a).

City of Calgary

Geography

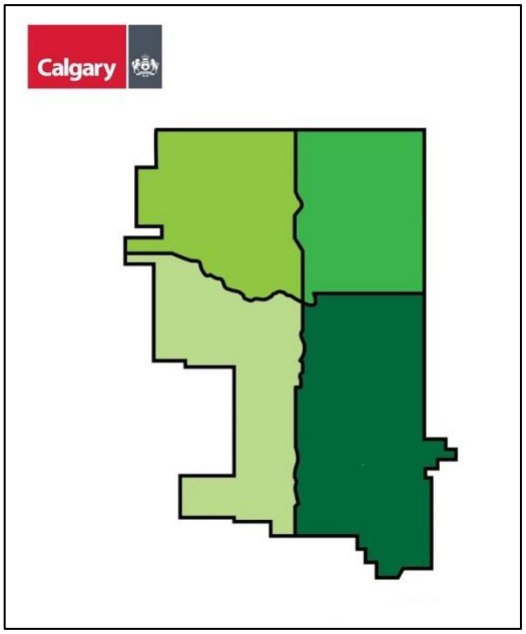
Calgary consists of 198 communities which are divided into one of four quadrants based on their geographical location: Northeast, Northwest, Southwest, and Southeast [ll] (Figure 3.1A). Furthermore, Calgary is parceled out into 14 wards, each represented by a city councillor. Calgary's civil census in 2011 revealed that Ward 3, located in the Northeast, had the largest population with 89,863 residents. Ward 3 is comprised of eight communities: Country Hills,

[kk] A CMA is an area consisting of one or more neighboring municipalities situated around a core. The total population must be at least 100,000 individuals to receive the designation.

[ll] The quadrants of Calgary differ from the eight electoral districts that were in place at the time of the 2011 National Household Survey, which was used to discern median household income as a proxy for socioeconomic status. The eight districts are approximately divided within the four quadrants, and for the purposes of this study, were considered to be reliable reflections of SES throughout Calgary (Figure 3.1B).

Country Hills Village, Coventry Hills, Harvest Hills, Panorama Hills, Hidden Valley, Carrington and Livingston. In contrast, Ward 14, located in the Southeast, had the smallest population at 71,251 individuals. Communities in Ward 14 include Bonavista Downs, Chaparral, Deer Ridge, Deer Run, Diamond Cove, Douglasdale/Douglasglen, Lake Bonavista, Legacy, McKenzie Lake, Midnapore, Parkland, Queensland, Sundance, Walden, and Wolf Willow. The following link will provide access to view the quadrants, wards, and community layers of Calgary: https://www.calgary.ca/citycouncil/Pages/WardBoundaryMap.aspx.

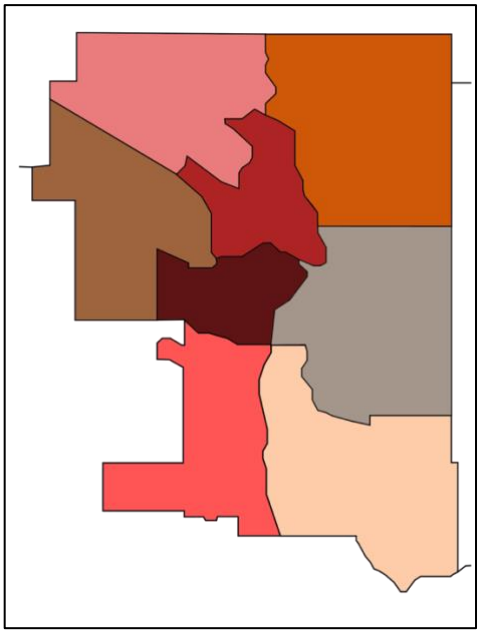
Figure 3.1A Calgary quadrants



Legend

Northwest	Northeast
Southwest	Southeast

Figure 3.1B Calgary regions[mm]



Legend

Nose Hill	Northeast
West	Centre North
Centre	East
Southwest	Southeast

[mm] Quadrant map is adapted from City of Calgary (2017) maps. Regions are demarcated based on major roadways and/or the Bow River. The east and west quadrants are divided by Centre Street in the north and Macleod Trail in the south (approximately). The Bow River is typically considered the dividing line between north and south in the western half of the city, whereas Centre Avenue and Memorial Drive divides the two in the east.

Age and Sex Demographics

Calgary had a population of 1,214,839 individuals in 2011, with a 17.8% increase in population size from 2006 (988,812 individuals). As such, Calgarians comprised approximately 30% of the total population of Alberta. Census data indicated that 547,475 individuals in Calgary identified as male and 549,360 individuals identified as female (Statistics Canada, 2012b). The age brackets with the highest number of residents were 24-29 years (8.5%), 30-34 years (8.2%), and 35-39 years (7.9%)[nn]. Statistics Canada (2012b) reported the median age of Calgarians as 36.4 years[oo]. Statistics Canada (2012c) reported that 9.8% of Calgary's population in 2011 was comprised of seniors (i.e., individuals aged 65 and above).

Indigenous Status and Ethnic Origins

In 2011, 28,905 people residing in Calgary self-identified as a member of an Indigenous group[pp]. The majority of these individuals identified as Métis, while 44.5% identified as First Nations and <1% as Inuk (Statistics Canada, 2013). The three most common ethnic origins[qq] in Calgary were identified as English (23.3%), Canadian (19.4%), and Scottish (17.9%). Irish and German origins were listed at 14.9% and 14.8%, respectively, whereas French and Ukrainian origins ranked at 9.0% and 6.6%. Individuals of Chinese (7.8%), East Indian (5.9%), and Filipino (4.6%) origin rounded out the remaining spots in the top 10 list (Statistics Canada, 2013).

Immigrant Populations and Visible Minorities

In the 2011 census, 27.6% of Calgarians reported immigrant status[rr] and 2.1% reported that they were non-permanent residents[ss] (Statistics Canada, 2013). Of Calgarians, 30.1% self-identified as belonging to a visible minority (Statistics Canada, 2013)[tt]. Of the 30.1%, South Asians (7.5%), Chinese (6.8%), and Filipino (4.4%) comprised the top three visible minority groups (Statistics

[nn] Age refers to the age at the last birthday before the reference date – May 11, 2011 (Statistics Canada, 2012b)
[oo] Median age is reported as age 'x', such that exactly ½ the population is older than 'x' and ½ is younger than 'x' (Statistics Canada, 2012)
[pp] The Government of Canada (2015) defines Aboriginal Status as: "whether the person reported being an Aboriginal person, i.e. First Nations, Métis or Inuk (Inuit) and/or being a Registered or Treaty Indian (defined by the *Indian Act* of Canada) and/or being a member of a First Nation or Indian band." However, for the purposes of this report, I will be using the term Indigenous to refer to these individuals as it is a more modern term reflective of our political and cultural climate.
[qq] Defined as: "the ethnic or cultural origin of the respondent's ancestors" (City of Calgary, 2013)
[rr] Defined as: "a person who is or has ever been a landed/permanent resident (City of Calgary, 2013)
[ss] Defined as: "a person from another country who has a work or study permit or who is a refugee claimant, and any non-Canadian-born family member living in Canada with them" (City of Calgary, 2013)
[tt] Defined as: "whether a person belongs to a visible minority group as defined by the *Employment Equity Act,* and, if so, the visible minority group to which the person belongs."

Canada, 2013). As of 2011, Statistics Canada (2013) reported that 18.6% of all recent immigrants in Calgary were born in the Philippines, 12.5% were born in India, and 8.7% were born in China.[uu]

3.1.3 Source of Data

Alberta Precision Laboratories (APL) is the consolidation of diagnostic lab services that were previously provided by Calgary Lab Services (CLS), Covenant Health, and Lamont Health Care Centre. Prior to the consolidation, CLS serviced over 1.4 million individuals in the Calgary and Southern Alberta area annually. Access to Cerner Millennium, the software utilized by APL, provided diagnostic data on blood tests collected within the established time frame for this study (January 1, 2010 – December 31, 2010). Cerner Millennium also provided microbiology test data for each individual within the timeframe. Lastly, a handful of databases linked by a unique patient identifier, the Alberta Provincial Health Number (PHN), were used to generate the patient demographics. The PHN was matched to the postal code using the provincial registry for use as a geographical control.

3.1.4 Case Inclusion

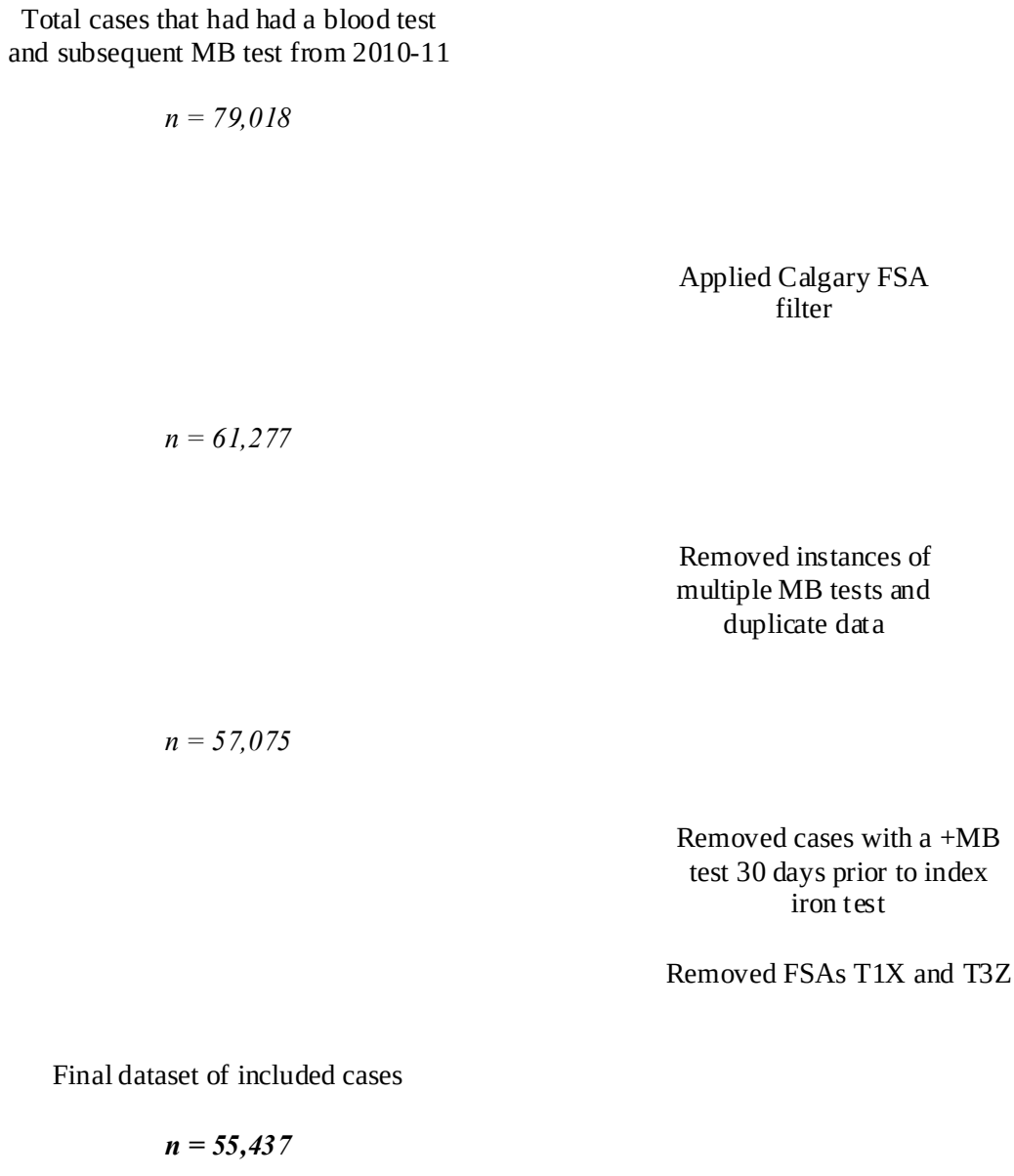
Inclusion of patient data was restricted to those residing in the city of Calgary who had received a blood and microbiology (MB) test between January 1, 2010, and December 31, 2010. One thousand one hundred and twenty-eight cases under Forward Sortation Areas (FSAs) 'T1X' and 'T3Z' were removed from the sample as they are geographically outside of the City of Calgary. T1X includes the City of Chestermere, hamlet of Indus, and locality of Bennett, Alberta. T3Z represents the 'Redwood Meadows' region, which encompasses sections of the Tsuut'ina First Nations land. Individuals who had had a positive MB test 30-days prior to their index iron test were also identified and subsequently removed from the sample (n = 510)[vv]. This resulted in a sample size of 55,437 (Figure 3.1). Patients could have received their blood and microbiology tests while in hospital, at a clinic, or through an APL laboratory services center. Only an

[uu] Recent immigrants were defined as those who had immigrated between 2006 and 2011

[vv] These individuals were specifically identified and then removed from the sample due to the body's response to infection, known as the Acute Phase Protein Response (APPR). During the APPR an individual's iron levels are decreased due to the upregulation of hepcidin in an attempt to fight the invading bacteria. As a result, these 510 individuals were likely experiencing systemically lowered iron levels at the time of their blood test, therefore affecting the results.

individual's first blood test (i.e., the index test) and first MB test within 2010-2011 were utilized for analyses.

Figure 3.2 Study flow diagram.



3.2 Research Question #1: Does Baseline Serum Iron Predict Infection Up To 1 Year Out?

3.2.1 Variables

Independent Variable: Baseline Serum Iron Level

Baseline serum iron level, hereafter referred to as serum iron level, is considered as a predictor of infection up to one year after the serum iron level was determined. Normal serum iron levels range between 14-32 µmol/L for males and 11-29 µmol/L for females. Rather than treat the serum iron levels as continuous data, patients were separated into quartiles according to serum iron levels, following the standard technique in epidemiology (Ward et. al., 2018; Charles et al., 2005; Hager et al., 2005) (Table 3.1).

Table 3.1 Quartiles by serum iron levels

Quartile	Serum Iron (µmol/L)
1	0.5-10
2	15-20
3	15-20
4	20-77

For reference, the date of both the index iron test and subsequent microbiology test were recorded to calculate the time-to-event variable, measured in days.

Dependent Variable – Subsequent Infection

The dependent variable of interest is the presence or absence of infection in an individual up to one year after baseline serum iron. A span of one year (2010-2011) was chosen as there was full access to both lab (blood) and microbiology tests in addition to the follow-up year aligning with a census year. The presence of infection was determined via a microbiology test for sepsis (blood and fungal), urinary tract infections (UTIs), and strep throat. Sepsis, UTIs, and strep throat were used as markers of infection because they are the most common tests ordered by community physicians in Alberta (Ma et al., 2019). Sepsis is a potentially fatal systemic inflammatory response syndrome. While any microorganism can trigger sepsis, the most frequently identified pathogens are *Escherichia coli, Staphylococcus aureus,* and *Streptococcus*

spp. (CDC, 2018). Sepsis affects roughly 30,000 Canadians each year and was the 12th leading cause of death in Canada in 2011 (Navaneelan et al., 2016). Additionally, one in 18 deaths in Canada involved sepsis in 2011 and the infection contributed to more than half of all deaths from infectious diseases in Canada between 2009-2011 (Navaneelan et al., 2016). UTIs are one of the most common bacterial infections worldwide, particularly amongst women and the elderly (Bennett et al., 2015; Franco, 2005). In Canada, UTIs are the 5th most common reason for emergency room visits and 8th most common reason for ambulatory clinic visits (CIHI, 2012; Touchie, 2013). The most common cause of UTIs is uropathogenic *E. coli*, followed by *Klebsiella pneumoniae* (Bennett et al., 2015). *Streptococcal pharyngitis*, or strep throat, is an infection of the oropharynx[ww] most commonly caused by Group A streptococcal infections (GAS) (CDC, 2018)[xx]. GAS infections can be classified as either invasive or non-invasive. Strep throat is considered non-invasive, meaning it is less severe but more contagious than an invasive GAS infection (CDC, 2016). Invasive GAS infections can cause conditions such as *Streptococcal* toxic shock syndrome to occur if not treated appropriately. Strep throat is responsible for between 20-30% of all sore throats in children, and between 5-15% of sore throats in adults (CDC, 2016).

Control Variables

This analysis controls for patient age, sex, and their associated Charlson-Quan Comorbidity Index (CQCI) and Clinical Risk Groups (CRG) scores.

Age

Age was controlled for given that increasing age has been linked to an increase in infectious diseases (e.g., urinary tract infections, sepsis) (Bender, 2003). While the reasons behind these elevated risks appear to be debated, contributing factors are likely due to the anatomical and physiological changes that accompany the ageing process, such as an impaired immune system (Bender, 2003). As regards differences in iron levels by age group, a literature review indicated that there is evidence to suggest both that hemoglobin levels do decrease with age (Milman et al., 2008; Nilsson-Ehle et al., 2000; Salive et al., 1992); and that they do not decrease with age

[ww] The oropharynx is the middle part of the throat, behind the mouth.
[xx] A 'Group A' streptococcal infection is one caused by *Streptococcus pyogenes*, a species of gram-positive bacteria.

(Raisinghani et al., 2019; Fairweather-Tait et al., 2014; Timiras and Brownstein, 1987). In the studies where it was concluded that iron decreased with age, it was typically seen in individuals aged 80 or above. Notably, it has been suggested that conditions such as malnutrition or an increase in circulating hepcidin leading to chronic inflammation may be behind the decreasing iron levels, rather than age itself (Raisinghani et al., 2019; Fairweather-Tait et al., 2014).

Sex

Sex was controlled for as females are known to have lower iron levels on average as compared to males. Women of reproductive age (~12 years to ~49 years) as well as pregnant and post-partum women are disproportionately affected by iron deficiency and iron deficiency anemia due to menstrual blood loss and the complications that can arise during pregnancy (Goonewardene et al., 2011; Shavelle et al., 2012; Abbaspour et al., 2014). In fact, a study conducted from 2009-2011 on the Canadian population with the intent of measuring iron levels and iron deficiency found that mean hemoglobin concentrations were significantly higher among men aged 3-79 than women (Cooper et al., 2012).

Charlson-Quan Index

CQCI was controlled for as it provides insight to an individual's health status based on the presence or absence of comorbidities[yy]. The presence of comorbidities is associated with worse health outcomes and increased health care costs (Valderas et al., 2009); therefore, it is of value for healthcare providers to utilize comorbidity indexes to accurately describe the health status of individuals (de Groot et al., 2003; 3M Health Information Systems, 2016). The CQCI is a modified version of the Charlson Comorbidity Index, which aims to predict mortality by classifying or weighting comorbid conditions (Quan et al., 2011). The CQCI assigns a score to an eligible Alberta resident based on the amount of healthcare resources used within a designated time period. The CQCI encompasses 16 medical conditions, which are then weighted on a scale of 1-6 to reflect the patient's relative risk of mortality (Hall et al., 2004).

Clinical Risk Groups

Similarly, CRGs are a population classification system that assign individuals to a severity-adjusted group based on their inpatient and ambulatory diagnoses/procedure codes, pharmaceutical data, and functional health status (3M Health Information Systems, 2016) and was controlled for as they provide insight to an individual's health status. While there are two methods used for compiling CRG scores, this study utilizes Method 1 given that it is most recommended method by the Alberta Health Services Data Integration Management and Reporting (DIMR) database when examining results over time. Method 1 includes data from inpatient, emergency department ambulatory, and physician claims. It does not include questionable secondary diagnoses or cancelled procedures for inpatients/ambulatory emergency departments. Both methods will produce a score that corresponds to an individual's CRG that references their status level. There are nine status levels, with '1' indicating an individual is healthy (i.e., absent of any primary chronic diseases, episode diagnostic categories, and episode procedure categories) and '9' indicating the highest severity (i.e., catastrophic conditions) (Alberta Health Services, 2014).

[yy] The definition of 'comorbidity' is debated throughout scientific literature (Meghani et al., 2013; Valderas et al., 2009; de Groot et al., 2003) however for the purposes of this paper, a comorbidity is the simultaneous presence of two or more diseases or medical conditions within an individual.

3.2.2 Statistical Analyses

Summary statistics were performed to measure baseline and demographic characteristics. Chi-squared tests for independence were run to assess associations between sex and infection, sex by city quadrant, and infection rate by city quadrant. Two one-way, between-groups analyses of variance (ANOVA) tests were run to explore the differences in mean age and mean iron level across quadrants. Tests of homogeneity of variances were violated for both age and iron level analyses ($p < 0.001$) therefore Welch's ANOVA with the Games-Howell post-hoc tests were used. Cases were divided into four groups based on their postal codes (Group 1: Northwest; Group 2: Northeast; Group 3: Southwest; Group 4: Southeast) (Figure 3.1A). This division was completed to statistically assess variation in mean iron level and infection that may have occurred due to differences in socioeconomic status (SES) across the city. While specific data was not available for median household income by quadrant of Calgary, median income data was recorded for each of the eight electoral districts that were in place for the 2011 census (Table 3.2, Figure 3.1B). These areas can roughly be approximated into the quadrants to gain an overview of variations in SES across Calgary (further explored in section 3.3).

Associations between iron level and infection were then determined by performing Cox regressions with time-varying covariates and the development of infection as the dependent variable. Cox regressions are a form of survival analysis technique. More broadly, survival analyses are frequently used in medical research, especially when researchers are interested in comparing the risks for death or another event that is associated with different treatments or groups (Bewick et al., 2004). Cox regressions were chosen as they are used to explore the relationship between the 'survival' of a subject and several explanatory values; in the present study, survival is equated to the development of infection (Luke & Homan, 1998). Given that the current study wished to assess the individual contributions of the covariates sex, age, CQCI, and CRG, in addition to the overall effect of iron level on infection, the cox regression was a better choice for survival analysis than Kaplan-Meier or a log rank test (Bewick et al., 2004). Furthermore, because time was a factor in the analysis, the cox regression model was selected over logistic regression. Cox regression analysis also provides researchers with a hazard ratio (HR) and confidence intervals for said HR. Hazard ratios indicate the effect of a covariate on the event (in this case, infection). If a HR is greater than 1, this indicates that a covariate is positively

associated with the event probability and therefore negatively associated with survival. If the HR is less than one, the opposite is true (Luke & Homan, 1998). For the categorical covariate of sex, females were used as the reference category, therefore interpretations were made based on the hazards of males in the sample. Similarly, quartile 1 was the reference group for iron levels as the study wished to compare infection rates of individuals with low iron against those with higher iron levels. Continuous variables, such as age, are referenced as a one unit (e.g., year) increase or decrease as regards the value of the hazard ratio. Additionally, cox regression analysis considers censoring; in other words, there were individuals in our sample who did not experience the event of interest (infection), and/or some individuals who died before the time which omits the use of a logistic regression model for this research question. All data analyses and calculations were done in IBM SPSS Statistics (version 26).[zz]

Table 3.2 Median household income in 2011 by Calgary region (Statistics Canada, 2015).

Region	Median Household Income
Nose Hill	$101,191
Centre	$63,224
Centre-North	$68,953
East	$63,264
Northeast	$75,942
Southeast	$96,744
Southwest	$88,320
West	$99,824

3.3 Research Question #2 – Is sociodemographic context associated with (1) mean iron level and (2) infection across Calgary?

3.3.1 Sociodemographic Variables, Iron, and Infection

As noted in Chapters One and Two, the variation seen in both serum iron levels, and rates of infection is multifaceted and likely influenced by sociodemographic variables. Indeed, a robust body of literature suggests that health disparities are largely explained by sociodemographic

[zz] Appendix 1 (page 108) explains each column in the Cox Regression output and how to interpret the data.

differences (Darin-Mattsson et al., 2017; Irwin et al., 2006; Marmot, 2015). As such, four SDVs were considered based on their associations with iron and infection (section 2.3.7): 1) socioeconomic status, 2) education level achieved, 3) Indigenous status, and 4) immigrant status. In this study, SES is assessed via median household income, following the work of Lightman et al. (2008) and Geyer et al. (2006) (Table 3.2). Education level achieved refers to the frequency of individuals who reported having at least some postsecondary education. Indigenous status included all individuals who self-identified as First Nations, Métis, or Inuit. Immigrant status was restricted to recent immigrants, or those who identified as coming to Canada within the last five years prior to the 2011 census.

While the Cerner Millennium Database provides PHNs that could then be linked to postal codes for each individual in this study, it does not provide their SDV data. As such, to explore the possible association between SDVs and serum iron level, as well as the association between SDVs and infection, this study considered the association between postal code and serum iron and infection, with postal code serving as a proxy for sociodemographic context. While such an approach assuredly masks variation within each postal code, it is the finest grained approach available with these data. The SDV data were collected by Statistics Canada for each Census Dissemination Area (CDA) (2011 National Household Survey) (NHS). CDAs are geographical groupings consisting of 400-700 people[aaa].

3.3.2 ArcGIS and Geomapping

Postal codes of each individual in the sample were linked to their corresponding CDA (*n* =1,589) using validated conversion tables provided by Census Canada and the Academic Data Center at the University of Calgary. These data were then used to determine the mean serum iron level of each CDA available from the 2011 Canadian Census file. Following Naugler et al. (2013), de Koning et al. (2014), and Ma et al. (2018), the SDVs were linked to the primary data file in ArcGIS Pro and plotted onto maps to draw visual inferences in the variation between mean serum iron level by CDA, infection by CDA, and SDVs by CDA. Geomapping and geocoding[bbb]

[aaa] Multiple CDAs exist within a census tract, a larger area containing between 2,500 and 8,000 people. There are 249 census tracts within the census metropolitan area of Calgary, each with a range of CDAs. Our dataset had information for 1,589 CDAs but not every variable had information (e.g., information on Median Household Income was only available for 1,548).
[bbb] NHS data files were accessed through the SANDS website with the help of Paul Pival, a research librarian at the University of Calgary.

[ccc] were conducted by a Renna Truong, a Data & Geospatial Resources Specialist at the Spatial and Numeric Data Services (SANDS) department at the University of Calgary.

3.3.3 Statistical Analyses

Standard multiple regression analyses were used to assess the associations between SDVs and mean iron level and infection throughout Calgary. Standard multiple regression is used to explore the relationship between one continuous, dependent variable (i.e., mean iron level or percentage of the CDA population infected) and a set of independent variables (i.e., selected SDVs).

[ccc] Geocoding involves assigning latitude and longitude coordinates to an item (in this case, an individual) as described in terms of its postal address. This was achieved using a software known as GeoPinpoint so that the data could be displayed as points in ArcGIS.

Chapter Four: Results

4.1 Descriptive Statistics

Fifty-five thousand four hundred and thirty-seven individuals were identified, with a mean age of 45.90 years (± 21.397), who resided within Calgary and had received a blood test between January 1, 2010, and December 31, 2010 (Table 4.1) and subsequent microbiology test within the year[ddd]. Females composed 63% (n = 34,924) of the sample and were younger than the males (t = -19.478, $p < 0.001$)[eee].

Table 4.1 Age and sex of sample

Sex	*n*	Mean Age	SD Age	Min. Age	Max. Age	% of Total *n*
Female	34,924	44.51	20.558	0	103	63.00%
Male	20,513	48.26	22.56	0	103	37.00%
Total	**55,437**	**45.9**	**21.397**	**0**	**103**	**100.00%**

Infection was documented in 3,451 (6.23%) cases with females comprising more of those infected (72.5%) than males (27.5%) (χ^2 (1, n = 55,437) = 142.12, $p < 0.001$). The mean age of infected individuals, 57.22 ±24.24 years, was higher than that of the non-infected group, 45.15 ±20.99 years (t = -28.557, $p < 0.001$). Among females, 84% of the infections were UTIs, while the next common was strep throat, found in 8.19% of the sample (Table 4.2, Figure 4.1). Among males, UTIs comprised 60% of the infections, while the next common was sepsis (blood), found in 22% of the sample (Table 4.2, Figure 4.2). Sepsis (fungal) infections made up the lowest number of infections for both females and males.

The Northwest (NW) quadrant had the highest overall population, comprising 28.28% (n = 15,678) of the sample. The Southeast (SE) had the smallest overall population, comprising 20.7% (n = 11,473) of the sample. As well different quadrants of Calgary had different frequencies of males and females (χ^2 (3, n = 55,437) = 65.38, $p < 0.001$, Table 4.3). Different quadrants also had different rates of overall infection (χ^2 (3, 55,437) = 50.13, $p < 0.001$) (Table

[ddd] Not all individuals had both a blood test and a microbiology test within 2010. Some individuals may not have had their microbiology test until 2011 (i.e., the follow-up year).
[eee] Equal variances condition not assumed, p = 0.000

4.4, Figure 4.3). Individuals living in the Southwest (SW) quadrant of Calgary comprised 30.9% of the infected subsample. Specifically, the SW had the most blood-borne (28.74% of all cases) and fungal-borne sepsis infections (32.85% of all cases) as well as the most UTIs (31.4% of all cases). Conversely, the NW had 29.5% of the infected cases and had the most strep throat infections (34.01% of all cases).

4.2 Comparison of Mean Age and Mean Iron Level by Quadrant

Calgary's quadrants differed by age (*Welch's F* (3, 29,819.6) = 448.91, $p < 0.001$, Table 4.5). The quadrant with the highest average age was the SW at 49.32 ±21.66 years. Conversely, the lowest average age was found in the Northeast (NE) at 40.26 ±20.77 years (Figure 4.4). Despite reaching statistical significance, the actual difference in mean age between the groups was quite small (effect size, calculated using an adjusted omega squared formula = 0.02). The mean iron level also differed across Calgary's quadrants (*Welch's F* (3, 29,810.6) = 23.17, $p < 0.001$, Table 4.5). However, the effect size was negligible at 0.001.

Figure 4.1 Infection type in females

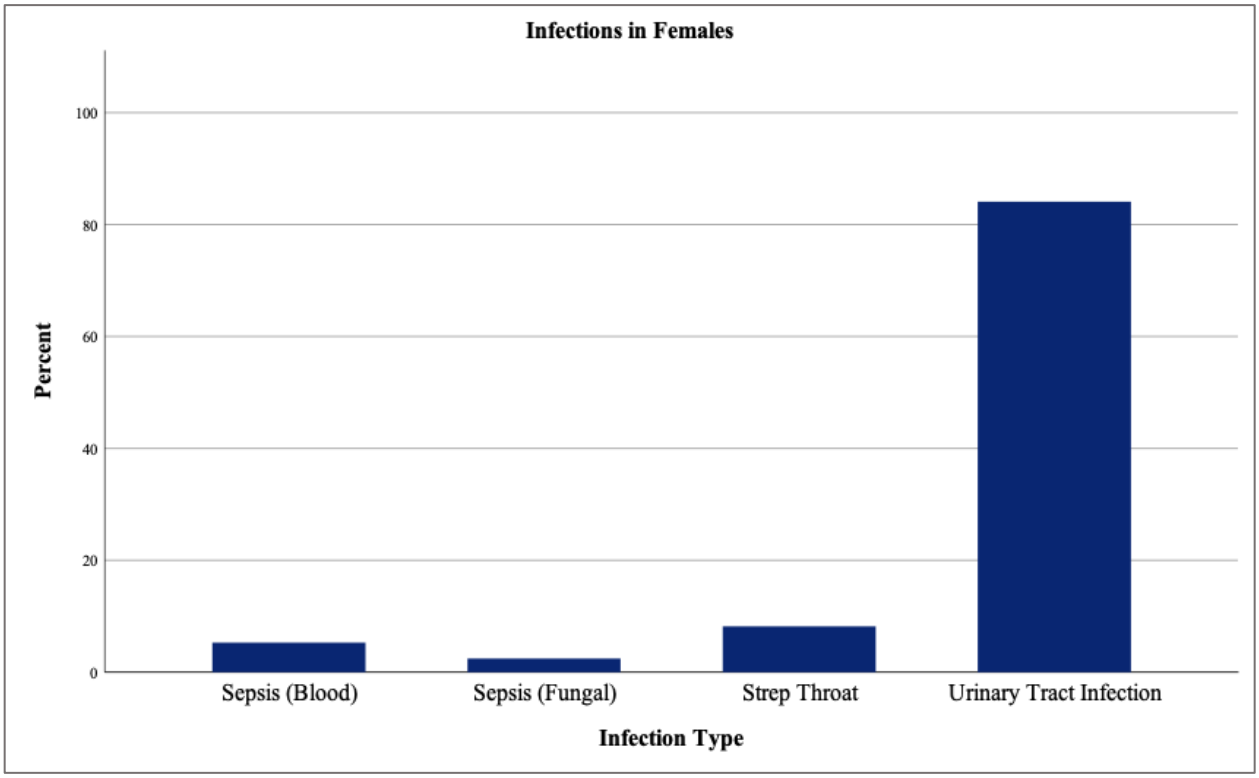


Figure 4.2 Infection type in males

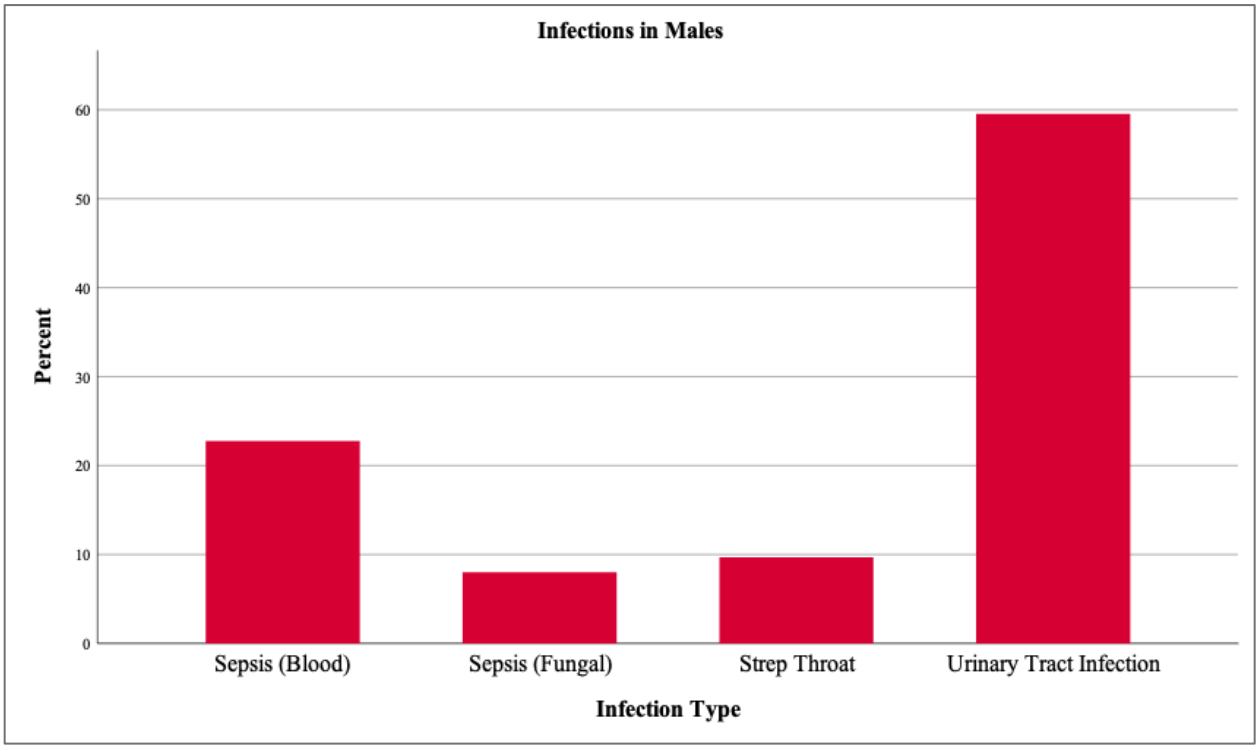


Table 4.2 Infection frequency by sex

Infection Type	Female (%)	Male (%)	Overall (%)
Blood	132 (5%)	216 (23%)	348 (10.1%)
Fungal	61 (2%)	76 (8%)	137 (4.42%)
Throat	205 (8%)	92 (10%)	297 (10.01%)
Urine	2,104 (84%)	565 (60%)	2,669 (77.34%)
Total	**2,502 (72.50%)**	**949 (27.5%)**	**3,451 (100%)**

Figure 4.3 Infection by Calgary quadrant

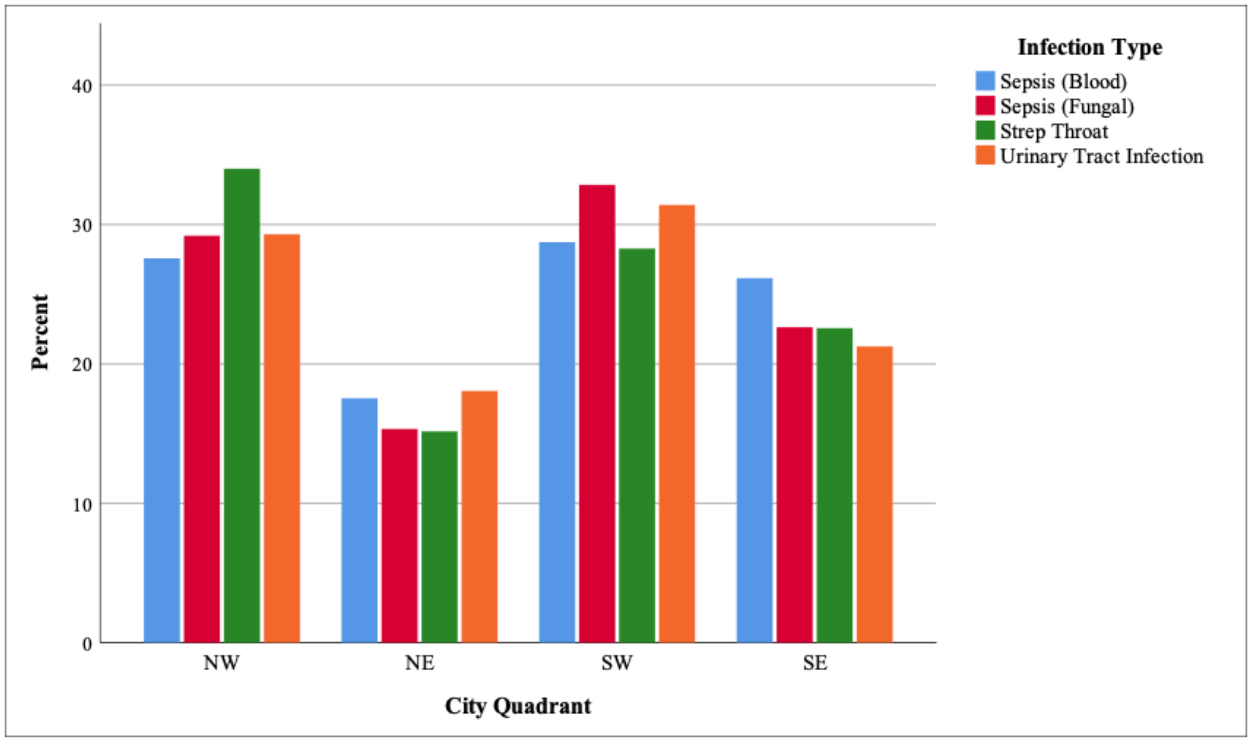


Table 4.3 Sex frequency by city quadrant

Quadrant	*n* Female	*n* Male	**Total *n***
NW	10,164 (29.1%)	5,514 (26.9%)	15,678 (28.3%)
NE	8,018 (23.0%)	4,444 (21.7%)	12,462 (22.5%)
SW	9,672 (27.7%)	6,152 (30.0%)	15,824 (28.5%)
SE	7,070 (20.2%)	4,403 (21.5%)	11,473 (20.7%)
Total	**34,924 (100%)**	**20,513 (100%)**	**55,437 (100%)**

Table 4.4 Infection frequency by city quadrant

Infection Type	**NW**	**NE**	**SW**	**SE**	**Total *n***
Blood	96 (27.59%)	61 (17.53%)	100 (28.74%)	91 (26.14%)	348 (10.1%)
Fungal	40 (29.2%)	21 (15.33%)	45 (32.85%)	31 (22.62%)	137 (4.42%)
Strep Throat	101 (34.01%)	45 (15.15%)	84 (28.28%)	67 (22.56%)	297 (10.01%)
Urinary Tract	782 (29.3%)	482 (18.06%)	838 (31.4%)	567 (21.24%)	2,669 (77.34%)
Total	**1,019 (29.52%)**	**609 (17.65%)**	**1,067 (30.92%)**	**756 (21.91%)**	**3,451 (100%)**

Figure 4.4 Age range distribution of females and males throughout quadrants of Calgary

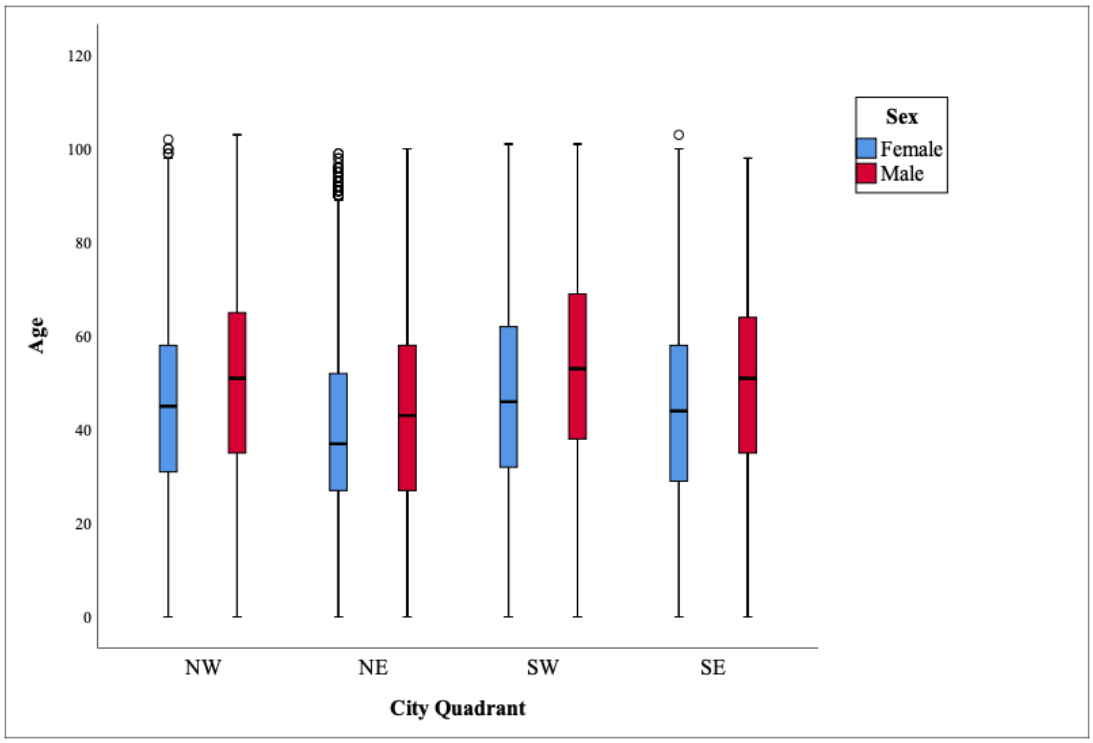


Table 4.5 One-Way Analysis of Variance comparing age and iron level across Calgary quadrants (using Welch's robust test of equality of means)

	NW	NE	SW	SE	*df*	Statistic	*p*
Variable	***n*** **| M | SD**	***n*** **| M | SD**	***n*** **| M | SD**	***n*** **| M | SD**			
Age	15,678 | 46.86 | 21.07	12,462 | 40.26 | 20.77	15,824 | 49.32 | 21.66	11,473 | 45.99 | 20.93	3, 29,819.6	448.91	.000
Iron Level	15,678 | 15.38 | 7.20	12,462 | 14.89 | 7.11	15,824 | 15.54 | 7.42	11,473 | 15.07 | 7.19	3, 29,810.6	23.17	.000

4.3 Cox Regressions[fff]

4.3.1 Iron and Infection Overall

A cox regression examining the risk of overall infection (i.e., development of sepsis (blood or fungal borne), strep throat, and urinary tract infections) based on serum iron level found that individuals in quartile 1, those with the lowest iron levels, were at a significantly greater risk for developing an infection over a 1-year period as compared to individuals in quartiles 2, 3, and 4 ($p < 0.001$; Figure 4.5). Individuals in quartile 4 were 39.2% less likely to develop an infection compared to those in quartile 1 (HR = .608, 95% CI .549 – .674; Table 4.6). Furthermore, increasing age, being a female, higher CRG, and CQCI were all significant risk factors for the development of infection ($p < 0.001$; Table 4.6).

4.3.2 Cox Regressions by Infection Type

Further cox regressions were completed to examine the risk of specific types of infection based on serum iron level. Serum iron level was a significant predictor of both sepsis (blood) and UTIs. As regards sepsis (blood) infection, individuals within quartile 4 were 59.8% less likely to develop an infection compared to those in quartile 1 ($p < 0.001$; HR = 0.402, 95% CI .277 – .584; Figure 4.6). While being a male, higher CRG, and higher CQCI levels were all significant risk factors for sepsis (blood) infections ($p < 0.001$), age was not ($p = 0.641$; Table 4.7). As regards UTIs (Figure 4.7), individuals in quartile 4 were 36.2% less likely to develop an infection compared to those in quartile 1 ($p < 0.001$, HR = 0.594, 95% CI .532 – .663). Older age, being a female, higher CRG, and higher CQCI were all significant risk factors for the development of a UTI ($p < 0.001$; Table 4.8).

Conversely, baseline serum iron level was not predictive of sepsis (fungal) or strep throat infections (Figures 4.8 and 4.9). As regards sepsis (fungal) infection, male sex ($p = 0.012$), higher CRG and higher CQCI ($p < 0.001$) were significantly associated with higher rates of fungal infection. Males were 55.6% more likely to develop a fungal infection compared to females (Table 4.9). As regards strep throat infections, individuals in quartile 2 and quartile 4 appeared to be the most likely to develop a strep throat infection over a one-year period (Figure 4.9), although the associations were not statistically significant ($p = 0.519$ and $p = 0.641$,

[fff] Appendix 1 explains how to interpret a cox regression output.

respectively). Individuals who were older had a 4.9% decreased risk of infection, while being a female was the only covariate associated with a higher risk of a strep throat infection ($p < 0.001$; Table 4.10).

Figure 4.5 Graphical output of the risk of infection overall based on serum iron level; *n* = 55,160

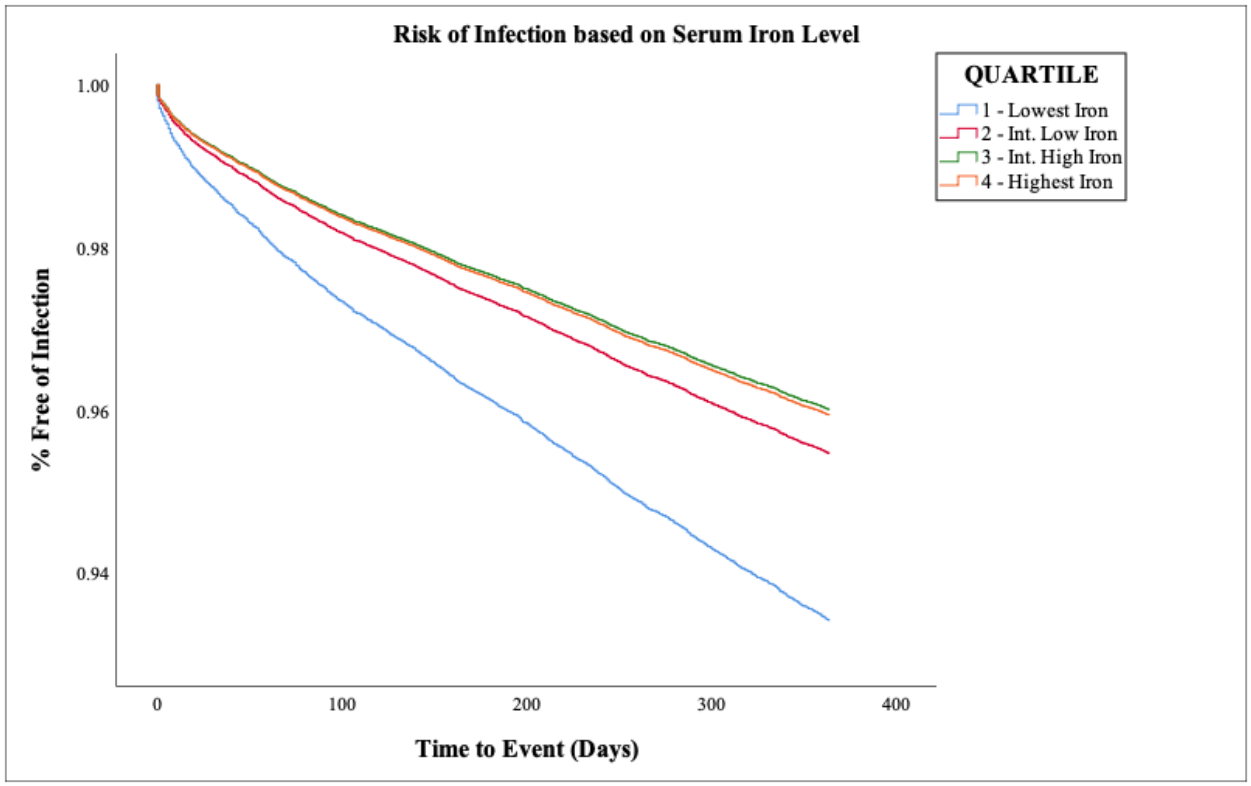


Table 4.6 Output displaying the contribution of covariates to the risk of infection overall; α = 0.05

							95.0% CI for Exp(B)	
	B	SE	Wald	df	Sig.	Exp(B)	Lower	Upper
Age	.130	.001	172.726	1	.000	1.013	1.011	1.015
Sex	-.646	.039	275.714	1	.000	.524	.486	.566
CRG Numeric	.141	.010	199.153	1	.000	1.151	1.129	1.174
Charlson Index	.208	.007	823.523	1	.000	1.231	1.214	1.249
Quartile			165.961	1	.000			
Quartile (2)	-.385	.044	75.385	1	.000	.681	.624	.742
Quartile (3)	-.514	.049	109.327	1	.000	.589	.543	.659
Quartile (4)	-.497	.052	90.031	1	.000	.608	.549	.674

Figure 4.6 Graphical output examining the risk of sepsis (blood) infections based on serum iron level; *n* = 52,063

Table 4.7 Output displaying the contribution of covariates to the risk of sepsis (blood) infections; α = 0.05

							95.0% CI for Exp(B)	
	B	SE	Wald	df	Sig.	Exp(B)	Lower	Upper
Age	-.001	.003	.217	1	.641	1.001	.996	1.007
Sex	.525	.114	21.310	1	.000	1.690	1.353	2.112
CRG Numeric	.648	.037	298.828	1	.000	1.911	1.776	2.057
Charlson Index	.203	.018	127.346	1	.000	1.225	1.183	1.269
Quartile			47.288	3	.000			
Quartile (2)	-.757	.146	26.732	1	.000	.469	.352	.625
Quartile (3)	.686	.162	17.991	1	.000	.504	.367	.691
Quartile (4)	-.911	.190	22.968	1	.000	.402	.277	.584

Figure 4.7 Graphical output examining the risk of urinary tract infections based on serum iron level; *n* = 54,385

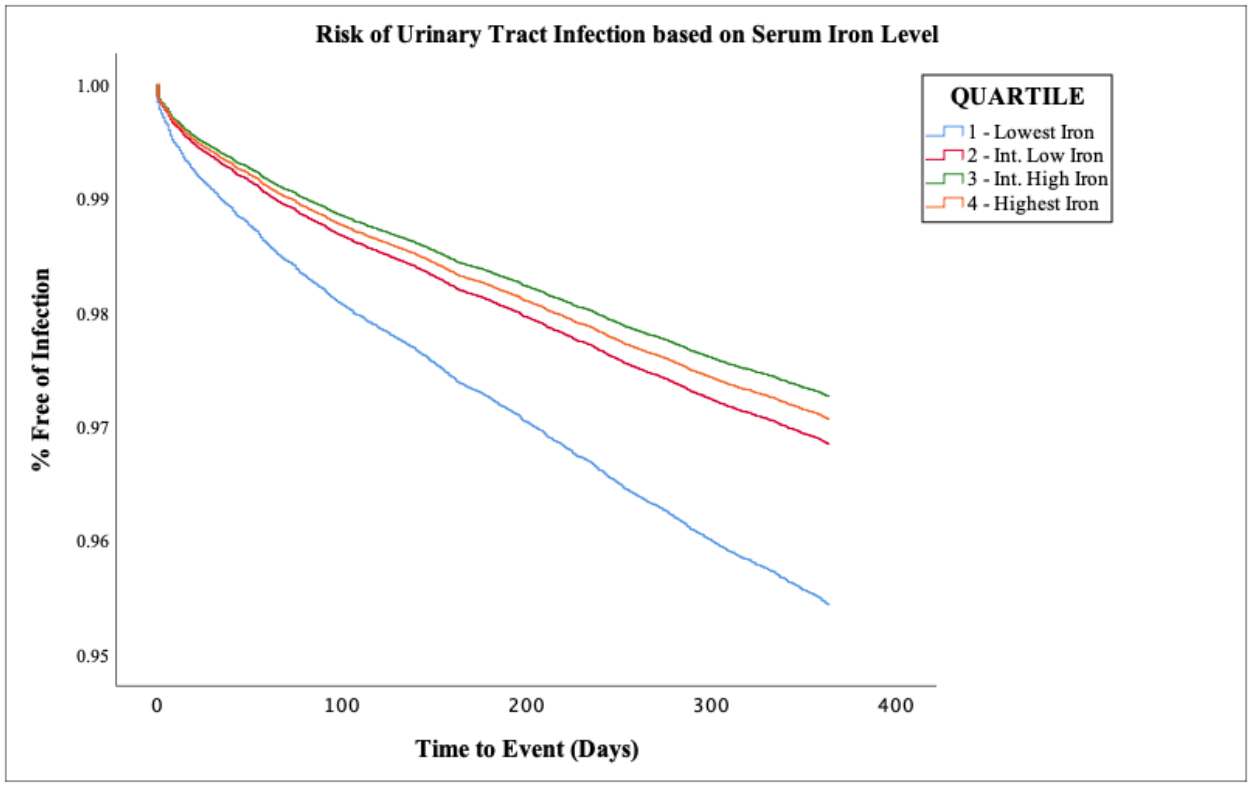


Table 4.8 Output displaying the contribution of covariates to the risk of urinary tract infections; α = 0.05

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	.023	.001	413.064	1	.000	1.023	1.021	1.025
Sex	-.995	.048	428.083	1	.000	.370	.336	.406
CRG Numeric	.107	.012	82.266	1	.000	1.113	1.087	1.138
Charlson Index	.200	.008	557.732	1	.000	1.221	1.201	1.242
Quartile			121.286	3	.000			
Quartile (2)	-.377	.050	56.7171	1	.000	.686	.622	.756
Quartile (3)	-.521	.056	85.827	1	.000	.594	.532	.663
Quartile (4)	-.450	.060	56.832	1	.000	.638	.567	.717

Figure 4.8 Graphical output examining the risk of sepsis (fungal) infections based on serum iron level; *n* = 51,859

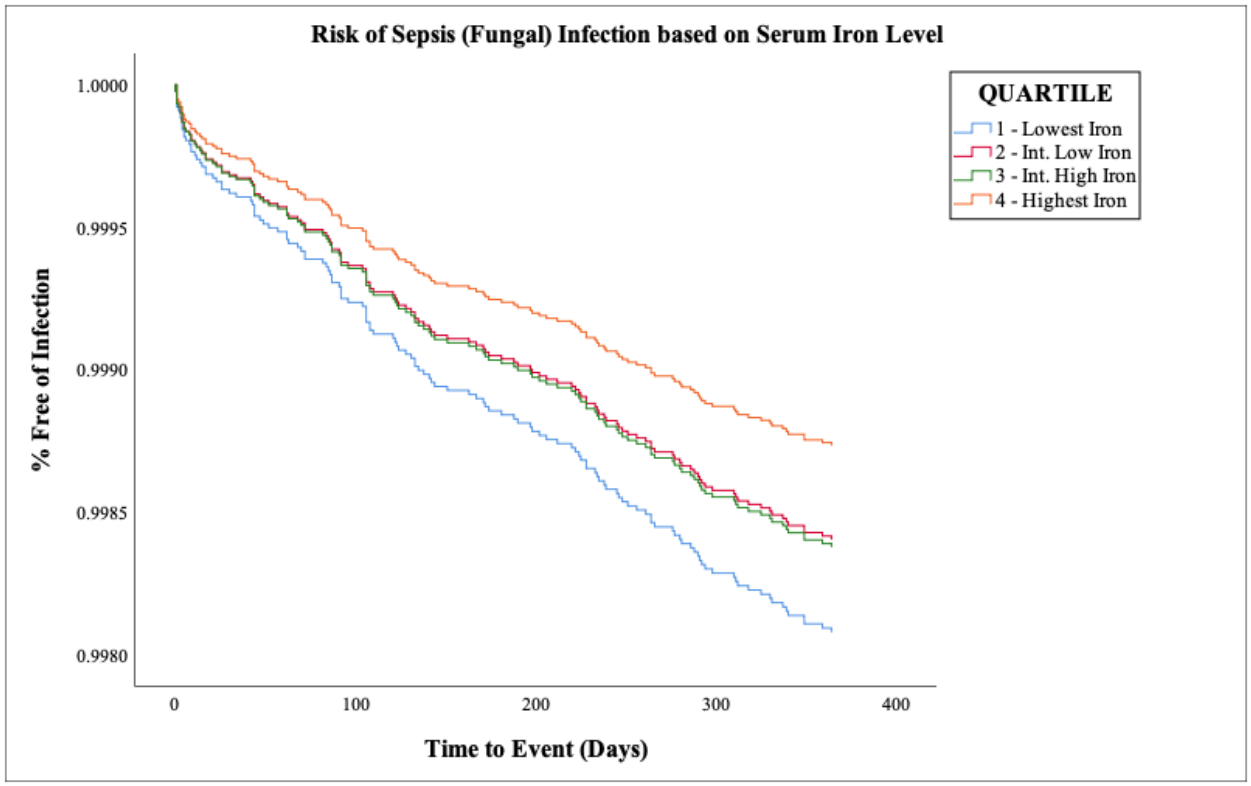


Table 4.9 Output displaying the contribution of covariates to the risk of sepsis (fungal) infections; α = 0.05

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	.003	.005	.331	1	.565	1.003	.994	1.012
Sex	.442	.176	6.321	1	.012	1.556	1.102	2.195
CRG Numeric	.319	.051	39.210	1	.000	1.376	1.245	1.520
Charlson Index	.206	.034	36.033	1	.000	1.228	1.149	1.314
Quartile			2.528	3	.470			
Quartile (2)	-.186	.222	.698	1	.403	.830	.537	1.284
Quartile (3)	-.169	.236	.514	1	.473	.844	.531	1.342
Quartile (4)	-.418	.268	2.442	1	.118	.658	.390	1.112

Figure 4.9 Graphical output examining the risk of strep throat infections based on serum iron level; *n* = 52,019

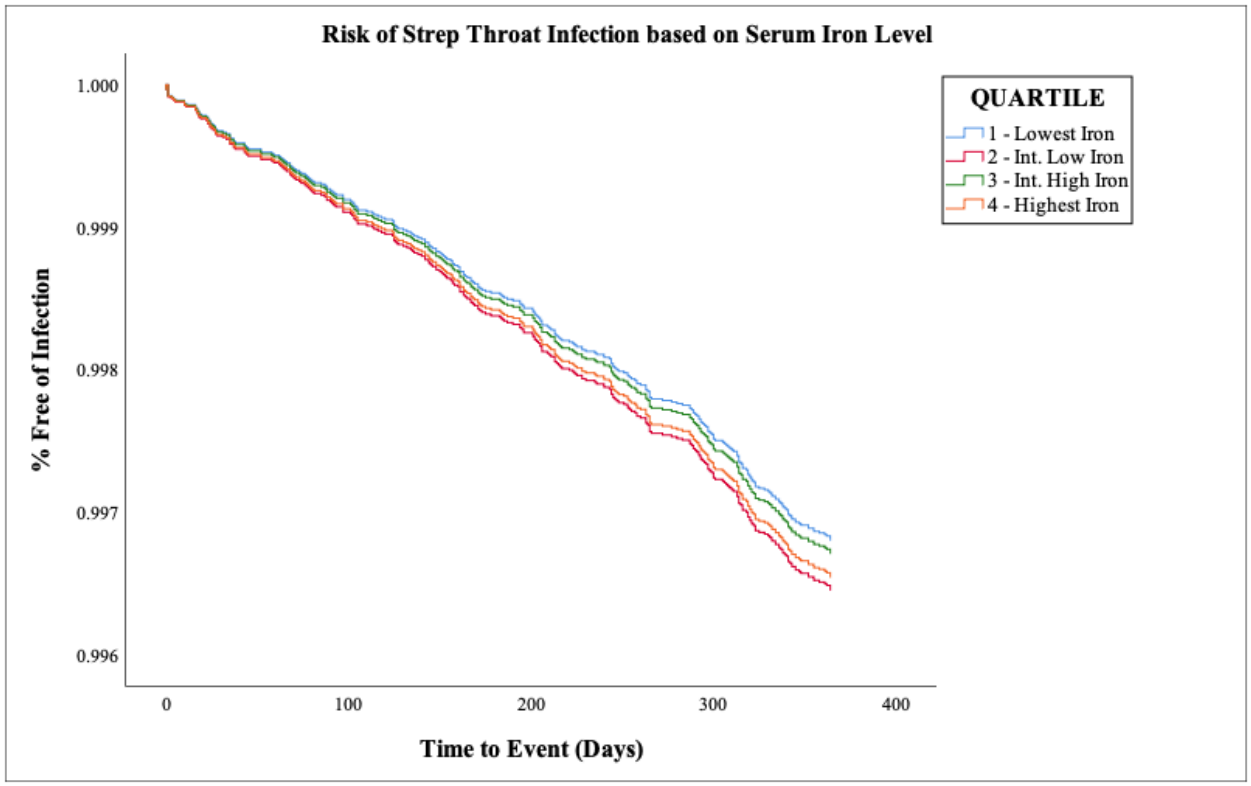


Table 4.10 Output displaying the contribution of covariates to the risk of strep throat infections; $\alpha = 0.05$

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	-.050	.003	208.440	1	.000	.951	.945	.958
Sex	-.284	.127	5.010	1	.025	.753	.587	.956
CRG Numeric	.013	.031	.177	1	.674	1.013	.953	1.077
Charlson Index	-.045	.102	.191	1	.662	.956	.783	1.168
Quartile			.501	3	.919			
Quartile (2)	.104	.161	.415	1	.519	1.110	.809	1.522
Quartile (3)	.029	.168	.029	1	.864	1.029	.741	1.430
Quartile (4)	.078	.168	.217	1	.641	1.081	.778	1.502

4.3.3 Cox Regressions by City Quadrant

The risk of overall infection within city quadrants, northwest, northeast, southwest, and southeast, was additionally examined. Serum iron level was found to be a significant predictor for infection in all quadrants of Calgary ($p < 0.001$; Tables 4.11 – 4.14). In the NW specifically (Figure 4.10), individuals in quartile 4 were 39.2% less likely to have an infection compared to those in quartile 1 (HR = .608, 95% CI .503 – .735). Furthermore, in the NW, older age, being a female, higher CRG, and higher CQCI were all significant risk factors for the development of infection ($p < 0.001$; Table 4.11).

In the NE, being in quartile 2 did not significantly contribute to the risk of infection ($p = 0.069$; Table 4.12). Individuals in quartile 4 were 26.9% less likely to have an infection compared to those in quartile 1 (HR = .731, 95% CI = .578 – .924) Younger age, being a female, higher CRG levels, and higher CQCI levels were all significant risk factors for the development of infection ($p < 0.001$; Figure 4.11).

Risk of infection was reduced for individuals in quartile 4 within SW Calgary by 39.5% as compared to those in quartile 1 (HR = .605, 95% CI .505 – .726; Table 4.13). Older age, being a female, higher CRG levels, and higher CQCI levels were all significant risk factors for the development of infection ($p < 0.001$). Figure 4.12 represents the visual survival output for the SW.

Individuals in quartile 4 in SE Calgary (Figure 4.13) were 48.6% less likely to develop an infection compared to those in quartile 1 (HR = .514, 95% CI .406 – .651; Table 4.14). Older age, being a female, higher CRG, and higher CQCI were all significant risk factors for the development of infection ($p < 0.001$).

Figure 4.10 Graphical output examining the risk of infection in the NW quadrant of Calgary based on serum iron level; *n* = 15,604

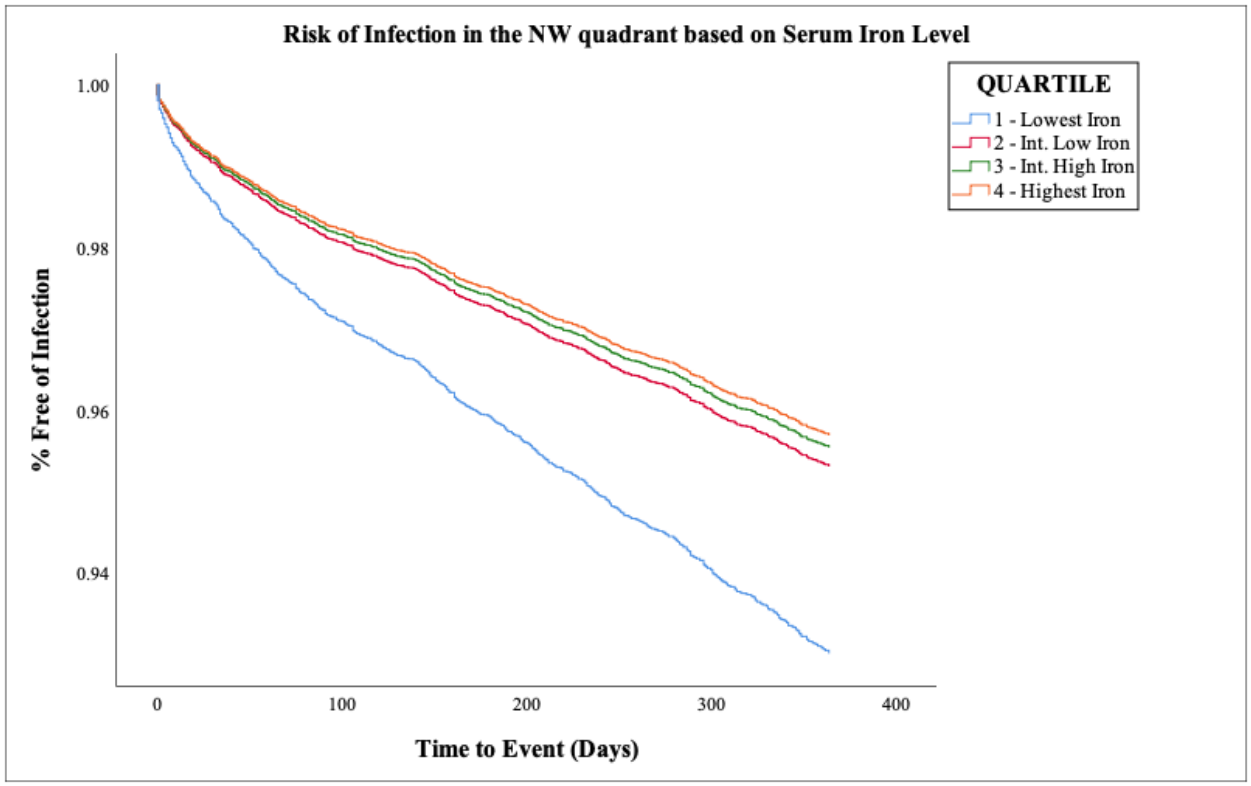


Table 4.11 Output displaying the contribution of covariates to the risk of infection overall in the NW; α = 0.05

Variables	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	.014	.002	58.862	1	.000	1.014	1.01	1.017
Sex	-.607	.072	71.594	1	.000	.545	.474	.627
CRG Numeric	.121	.018	43.397	1	.000	1.128	1.088	1.169
Charlson Index	.224	.014	246.433	1	.000	1.251	1.216	1.286
Quartile			45.74	3	.000			
Quartile (2)	-.411	.082	24.825	1	.000	.663	.564	.780
Quartile (3)	-.462	.090	26674	1	.000	.630	.528	.751
Quartile (4)	-.498	.097	26.419	1	.000	.608	.503	.735

Figure 4.11 Graphical output examining the risk of infection in the NE quadrant of Calgary based on serum iron level; *n* = 12,403

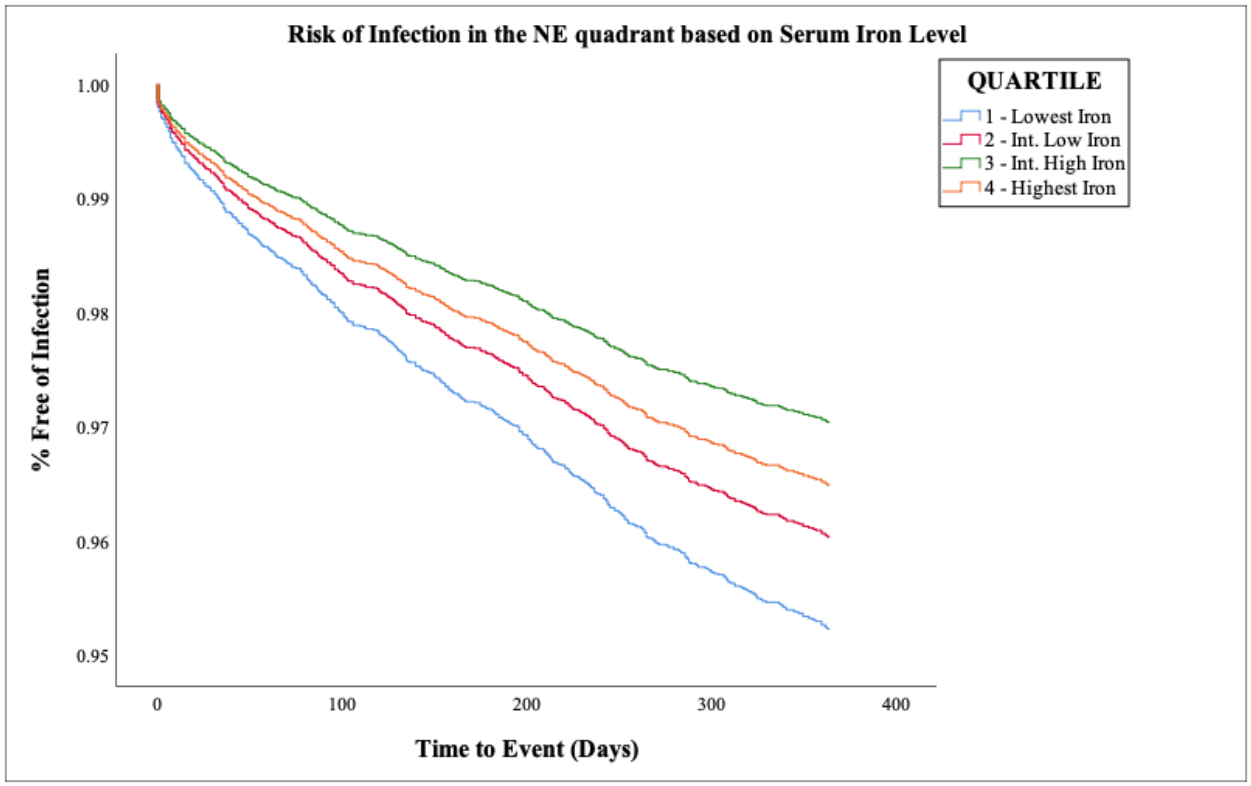


Table 4.12 Output displaying the contribution of covariates to the risk of infection overall in the NE; $\alpha = 0.05$

Variables	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	.009	.002	16.973	1	.000	1.010	1.005	1.014
Sex	-.748	.098	58.372	1	.000	.473	.391	.574
CRG Numeric	.131	.023	31.575	1	.000	1.140	1.089	1.193
Charlson Index	.242	.019	157.514	1	.000	1.274	1.227	1.323
Quartile			18.776	3	.000			
Quartile (2)	-.190	.104	3.317	1	.069	.827	.674	1.015
Quartile (3)	-.487	.119	16.903	1	.000	.614	.487	.775
Quartile (4)	-.314	.120	6.856	1	.009	.731	.578	.924

Figure 4.12 Graphical output examining the risk of infection in the SW quadrant of Calgary based on serum iron level; *n* = 15,737

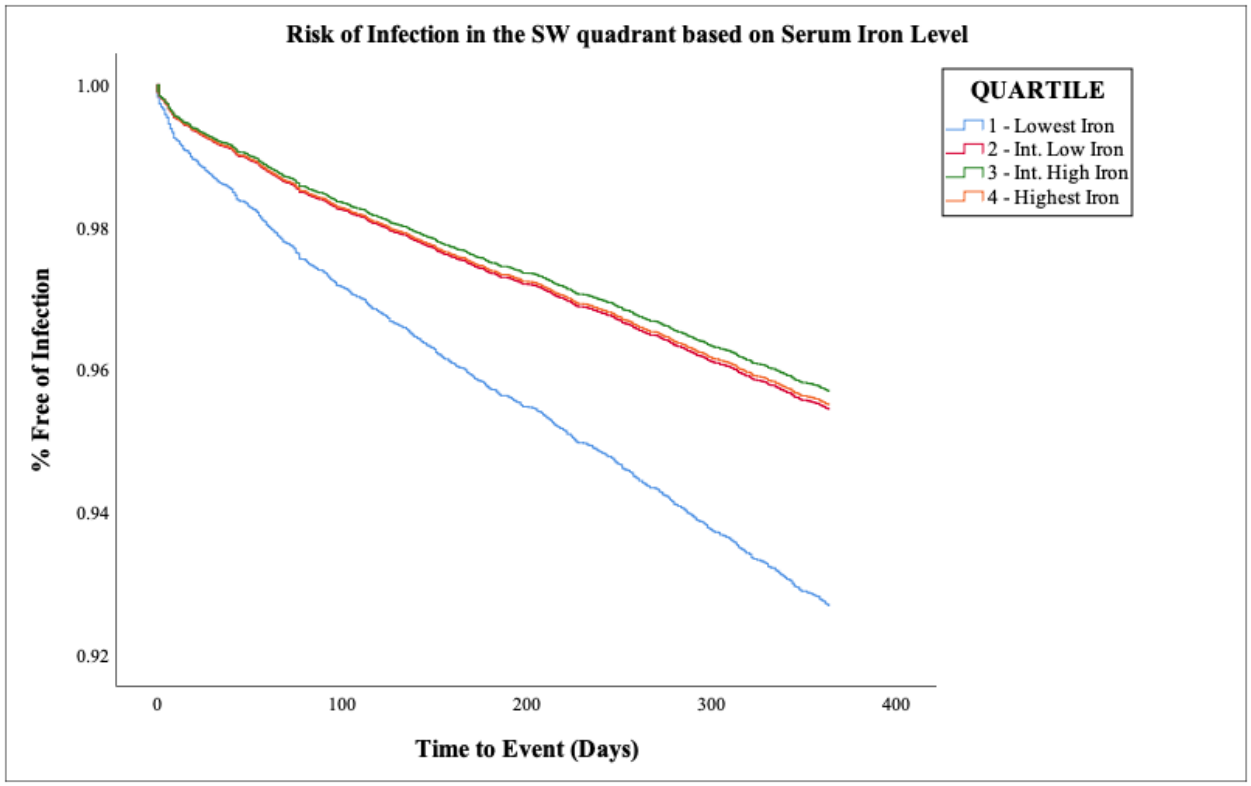


Table 4.13 Output displaying the contribution of covariates to the risk of infection overall in the SW; α = 0.05

Variables	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	.013	.002	54.903	1	.000	1.013	1.009	1.016
Sex	-.640	.069	86.71	1	.000	.527	.461	.603
CRG Numeric	.162	.019	76.447	1	.000	1.176	1.134	1.22
Charlson Index	.179	.013	197.106	1	.000	1.196	1.166	1.226
Quartile			62.155	3	.000			
Quartile (2)	-.488	.081	36.221	1	.000	.614	.524	.720
Quartile (3)	-.547	.089	37.796	1	.000	.579	.486	.689
Quartile (4)	-.502	.093	29.345	1	.000	.605	.505	.726

Figure 4.13 Graphical output examining the risk of infection in the SE quadrant of Calgary based on serum iron level; *n* = 11,416

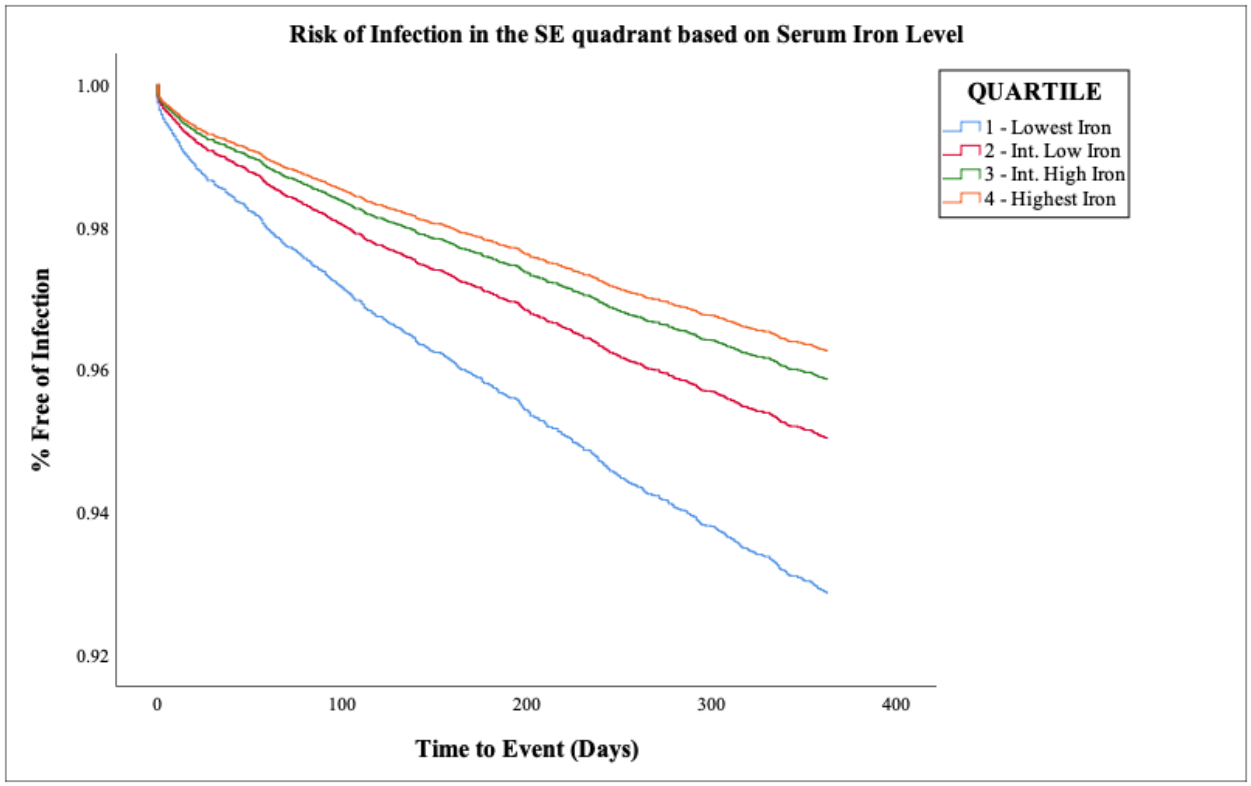


Table 4.14 Output displaying the contribution of covariates to the risk of infection overall in the SE; α = 0.05

Variables	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	.012	.002	34.059	1	.000	1.012	1.008	1.016
Sex	-.649	.082	62.342	1	.000	.522	.445	.614
CRG Numeric	.145	.021	46.11	1	.000	1.156	1.109	1.206
Charlson Index	.210	.014	218.991	1	.000	1.233	1.200	1.268
Quartile			47.865	3	.000			
Quartile (2)	-.374	.092	16.435	1	.000	.688	.574	.824
Quartile (3)	-.561	.106	28.102	1	.000	.571	.464	.702
Quartile (4)	-.665	.120	30.599	1	.000	.514	.406	.651

4.4 ArcGIS Maps of Mean Iron, Infection, and Sociodemographic Variables by CDA

The following figures are the ArcGIS Pro maps, representing the distribution of mean iron levels (Figure 4.14), infection count (Figure 4.15), median household income (Figure 4.16), education level (Figure 4.17), Indigenous status (Figure 4.18), and recent immigrant status (Figure 4.19). Notably, lower mean iron levels can be observed throughout the NE quadrant of Calgary. Clusters of the highest mean iron levels (18.37-24.00 µmol/L) can be observed throughout the city, although they are less prevalent in the NE. When infection count was broken down into CDAs, there were only two areas that had the highest infection count (between 16 and 22 infections). There was only one CDA in Calgary that reported a median income of over $100,000, indicated by the dark green colouring on the map, located in central Calgary (estimated to be around the downtown-core area of the city). CDAs with the highest percentage of post-secondary education (74% – 95%) clustered in the NW quadrant of the city. Regarding Indigenous status, there were three CDAs in the city that reported a population comprised of 35% to 52% Indigenous individuals (the highest levels reported in the NHS). There were several CDAs that reported a recent immigrant population of between 28% – 47% (indicated by the dark maroon colouring on Figure 4.19) however none of these CDAs were found in the NE.

Figure 4.14 Mean serum iron levels (µmol/L) by CDA

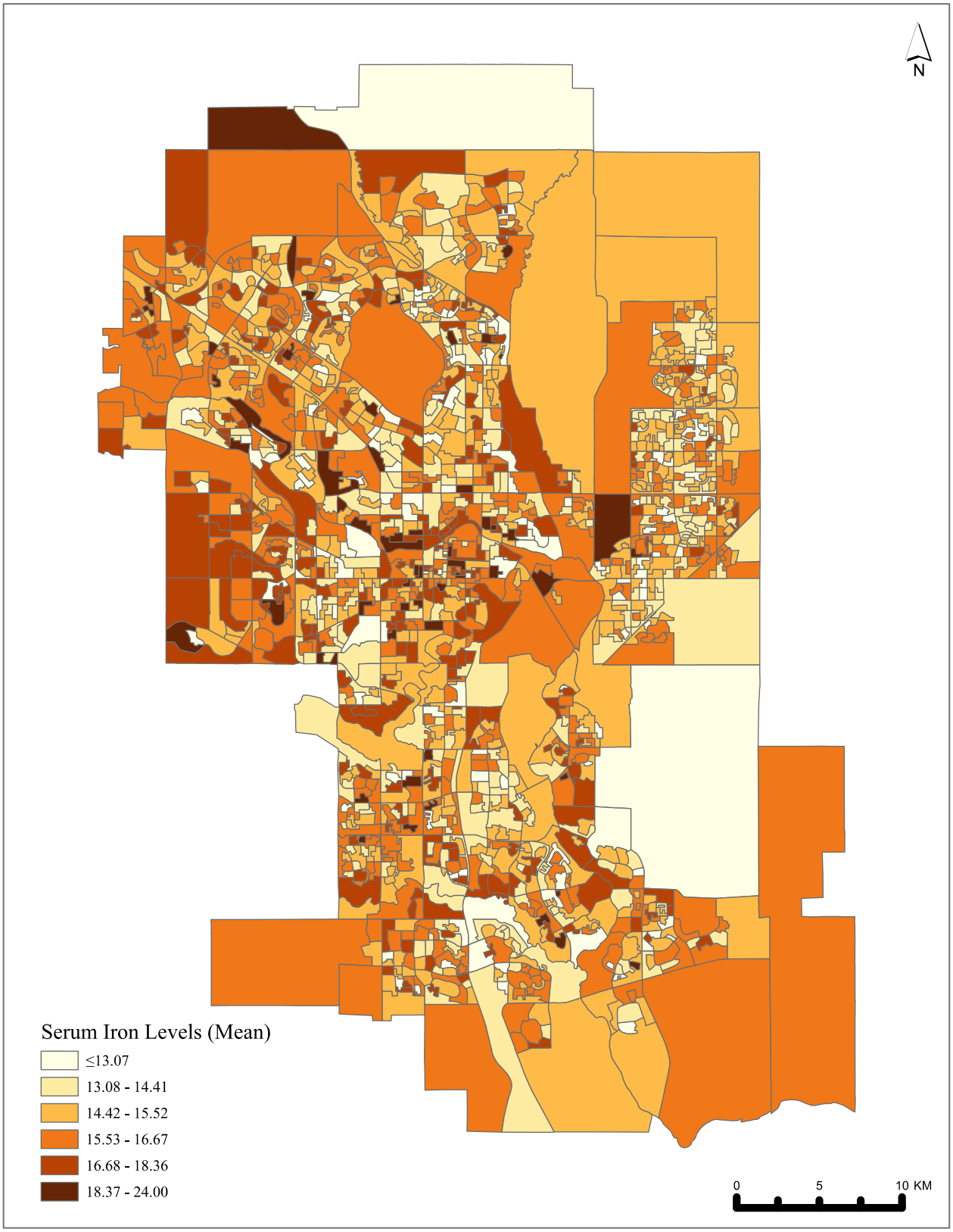


Figure 4.15 Infection counts by CDA

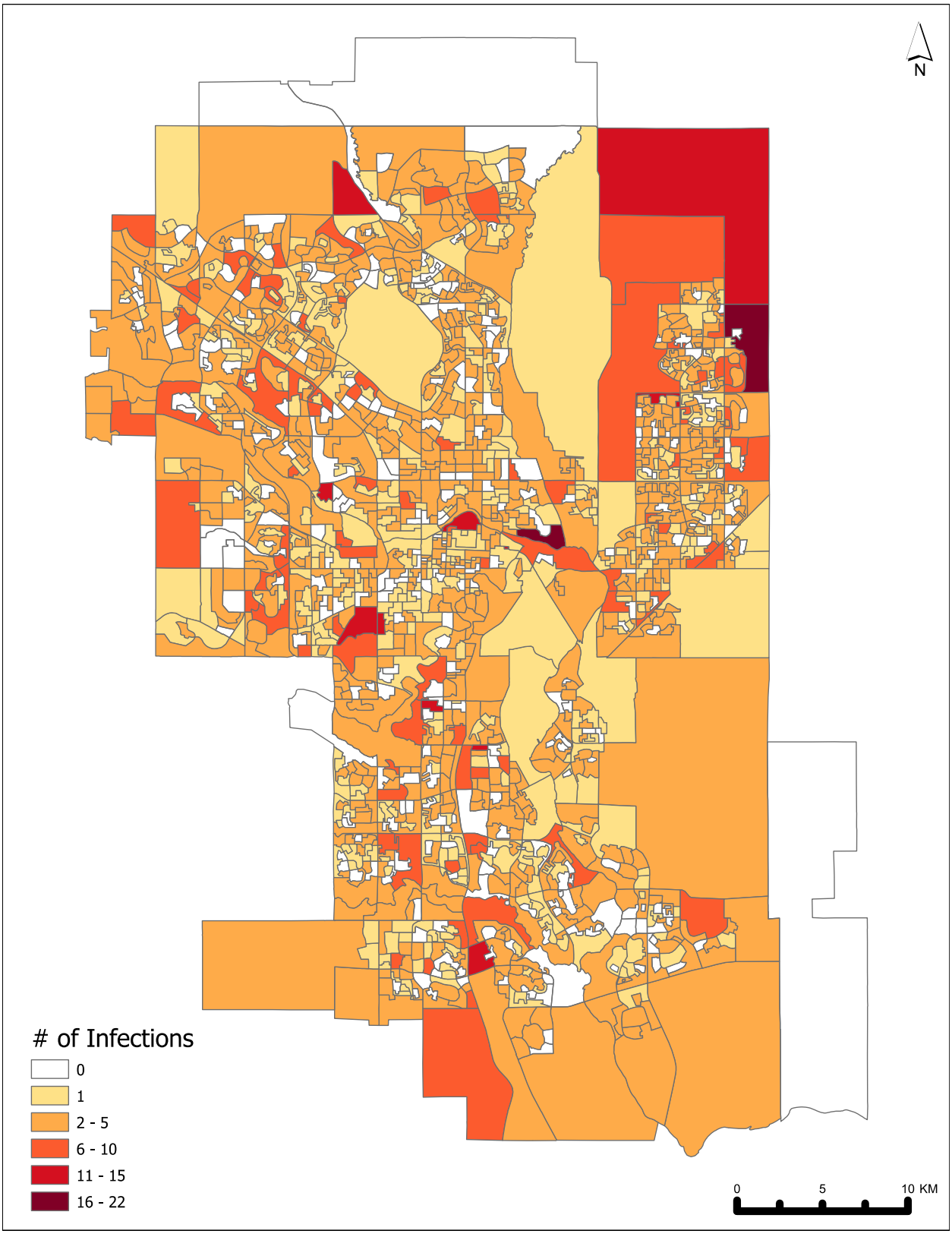


Figure 4.16 Median income by CDA

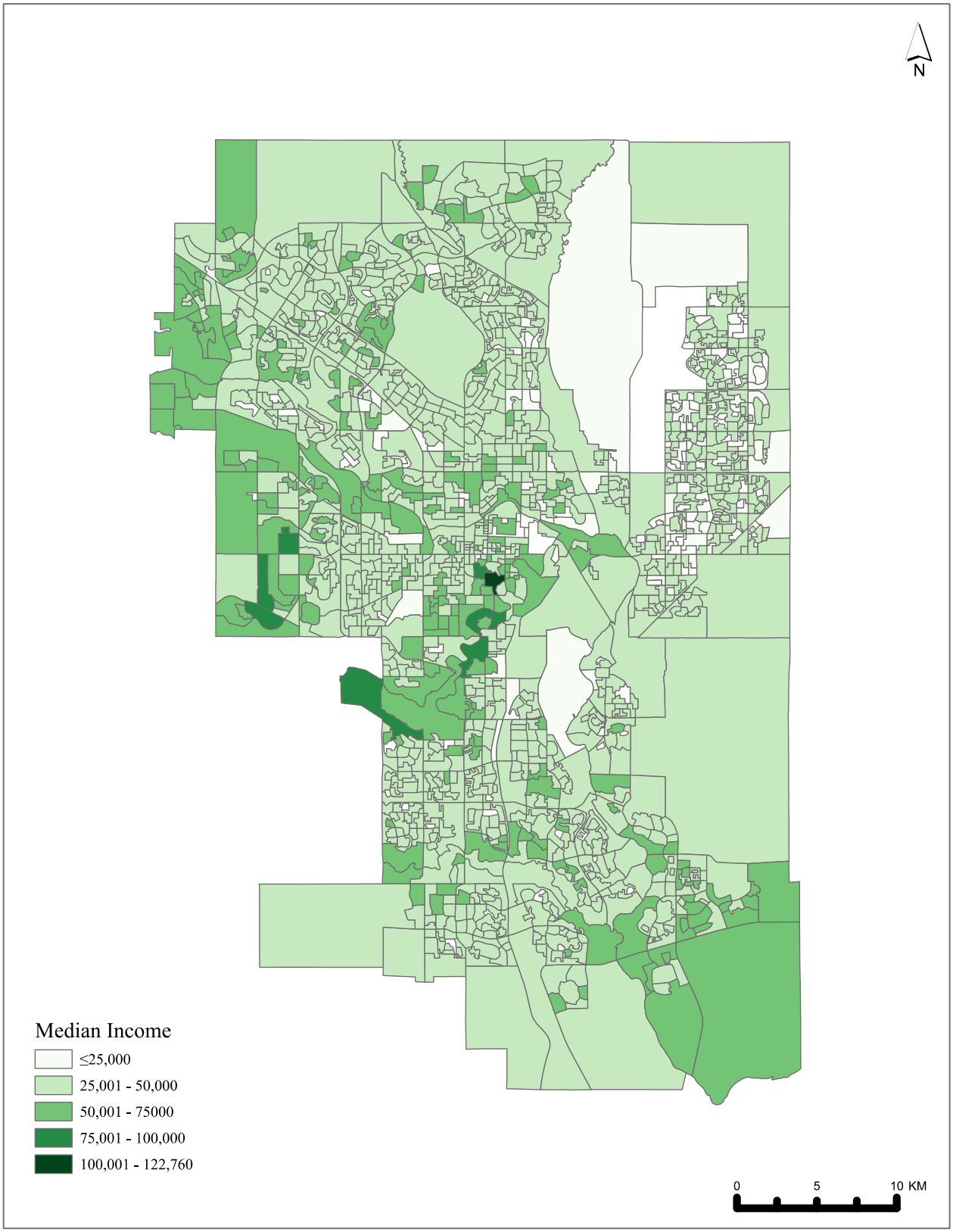


Figure 4.17 Percentage of individuals with at least some post-secondary education by CDA

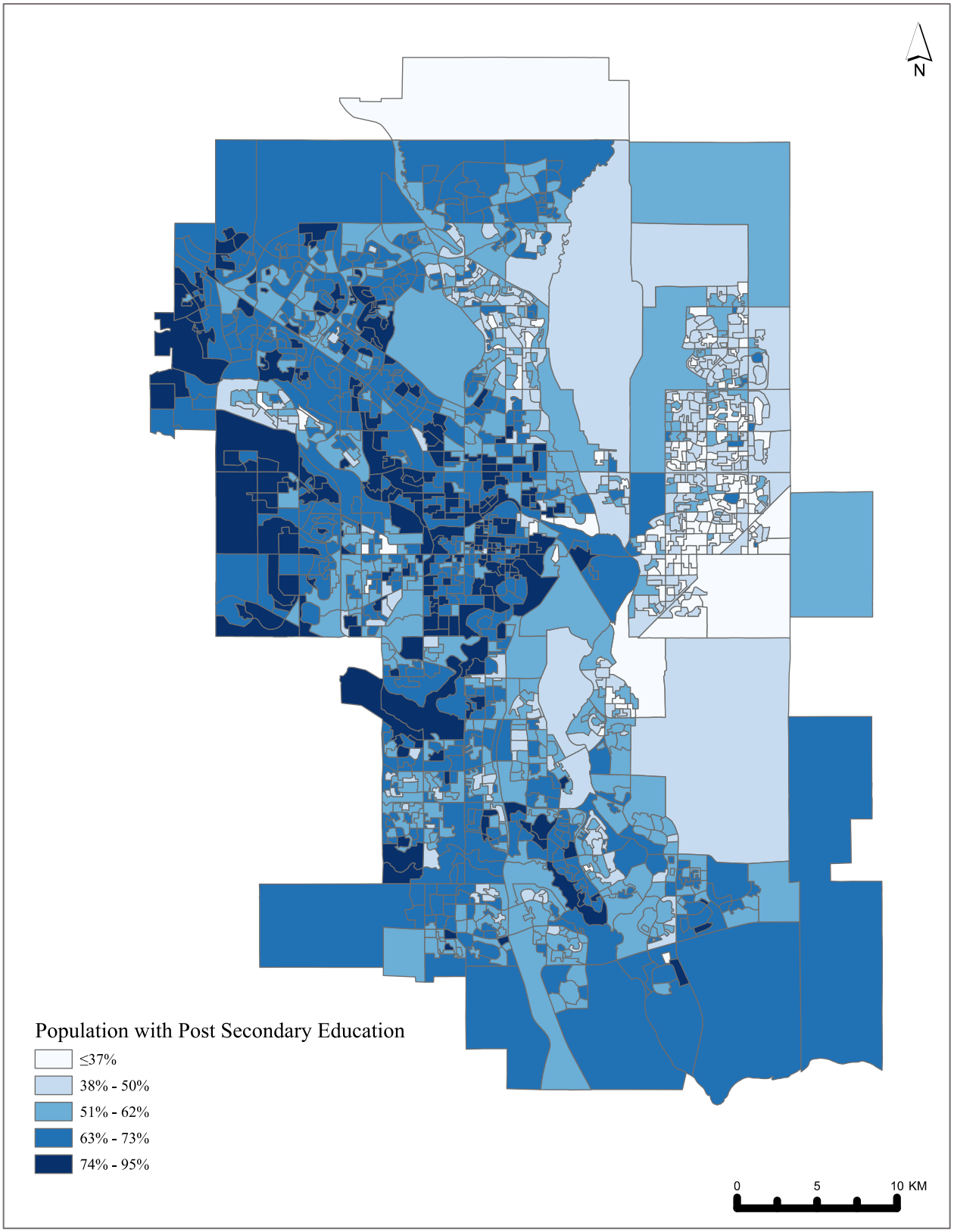


Figure 4.18 Percentage of individuals who self-identified as Indigenous/First Nations by CDA

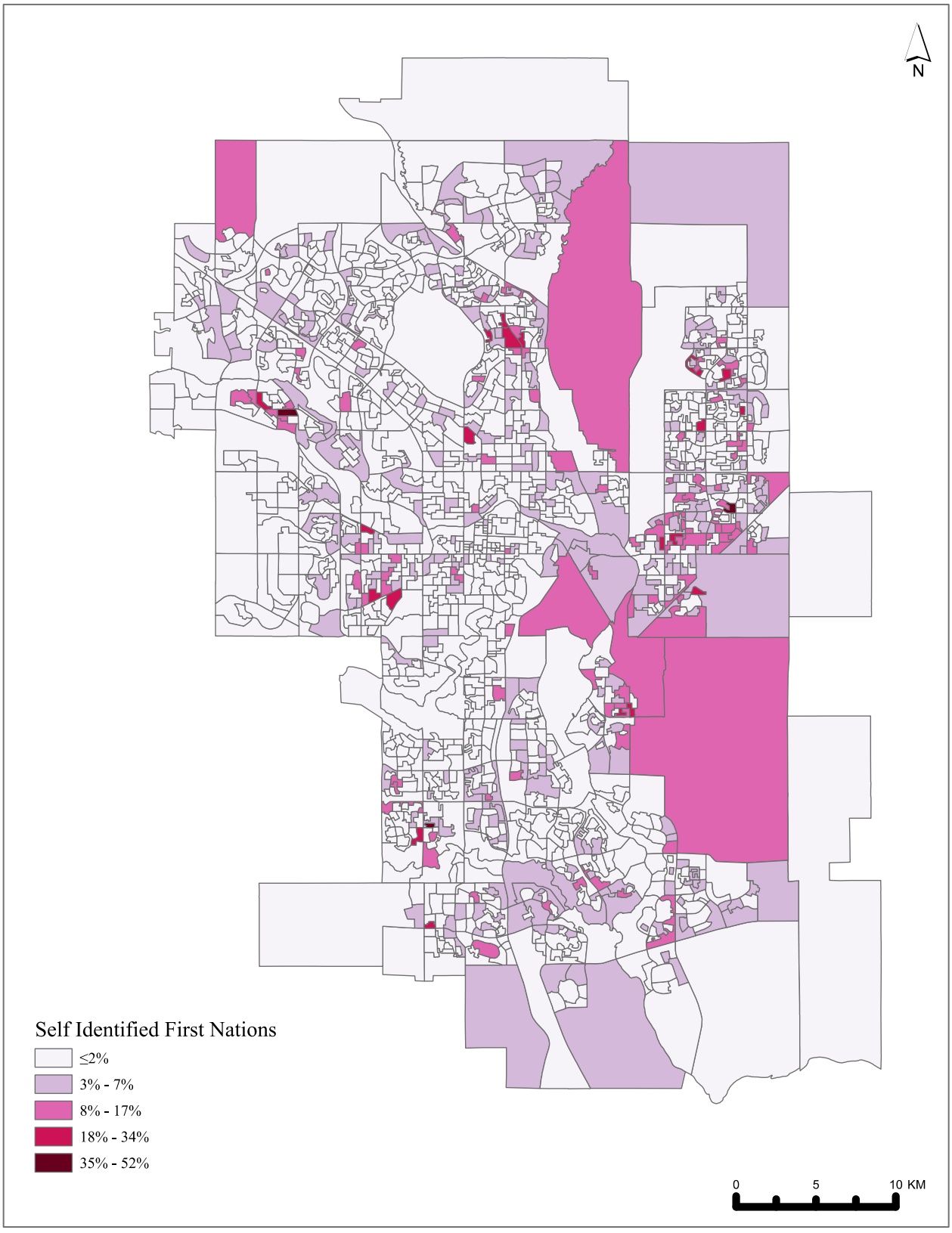
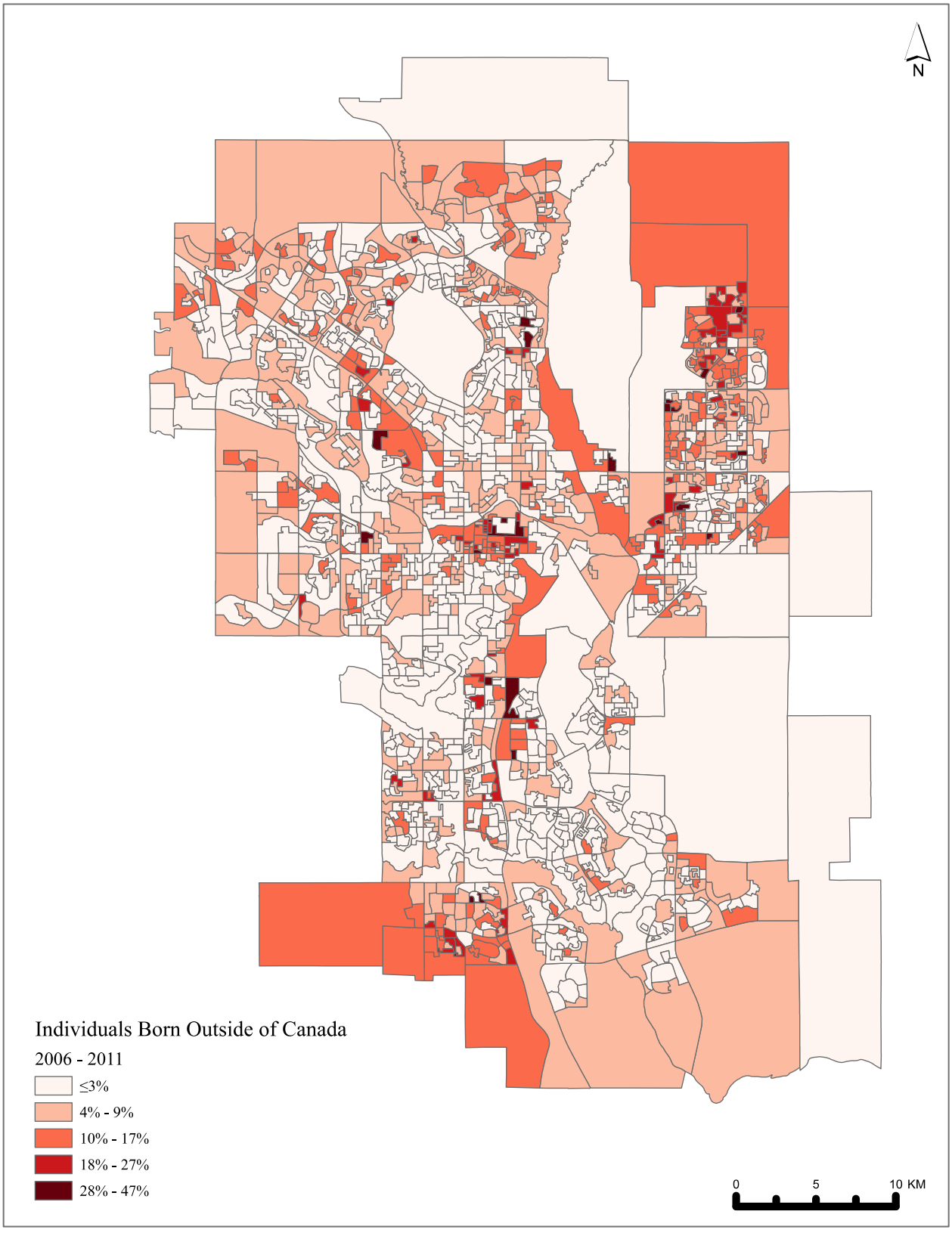


Figure 4.19 Percentage of recent immigrants (≤ 5 years) by CDA



4.5 Standard Multiple Regression Results of Infection and SDV, and Iron and SDV

4.5.1 Infection and SDV

The ANOVA test of the null hypothesis (i.e., all regression coefficients in the population equal 0) was not significant ($p = 0.296$), indicating that none of the sociodemographic variables were associated with infection.

4.5.2 Iron and SDV

Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, multicollinearity, and homoscedasticity. Mahalanobis' distances indicated 36 cases were outliers and they were subsequently removed. The model explained 5.8% of the variation seen in mean iron levels across the CDAs, F (4, 1543) = 23.71, $p < 0.001$. Median household income and achieved postsecondary education were statistically significant contributors to the model ($sr = .087$, $p < 0.001$; $sr = .132$, $p < 0.001$); that is, both higher median household income and more postsecondary education were associated with higher iron levels (Figures 4.20 & 4.21).

Table 4.15 Standard multiple regression analysis summary predicting mean iron level (µmol/L) with median income, recent immigrant status, Indigenous status, and postsecondary education

Predictor Variable	*B*	*SE B*	Beta	*sr*	R^2
					.058
Constant	13.48	.205			
Median Household Income ($)	.000	.000	.112***	.087	
Recent Immigrant Status	.004	.007	.016	.015	
Indigenous Status	.012	.013	.023	.023	
Post-Secondary Education	.019	.004	.165***	.132	

Note. *sr* = semi-partial correlation coefficient
***$p < .001$

Figure 4.20 Scatterplot of median household income by mean iron level

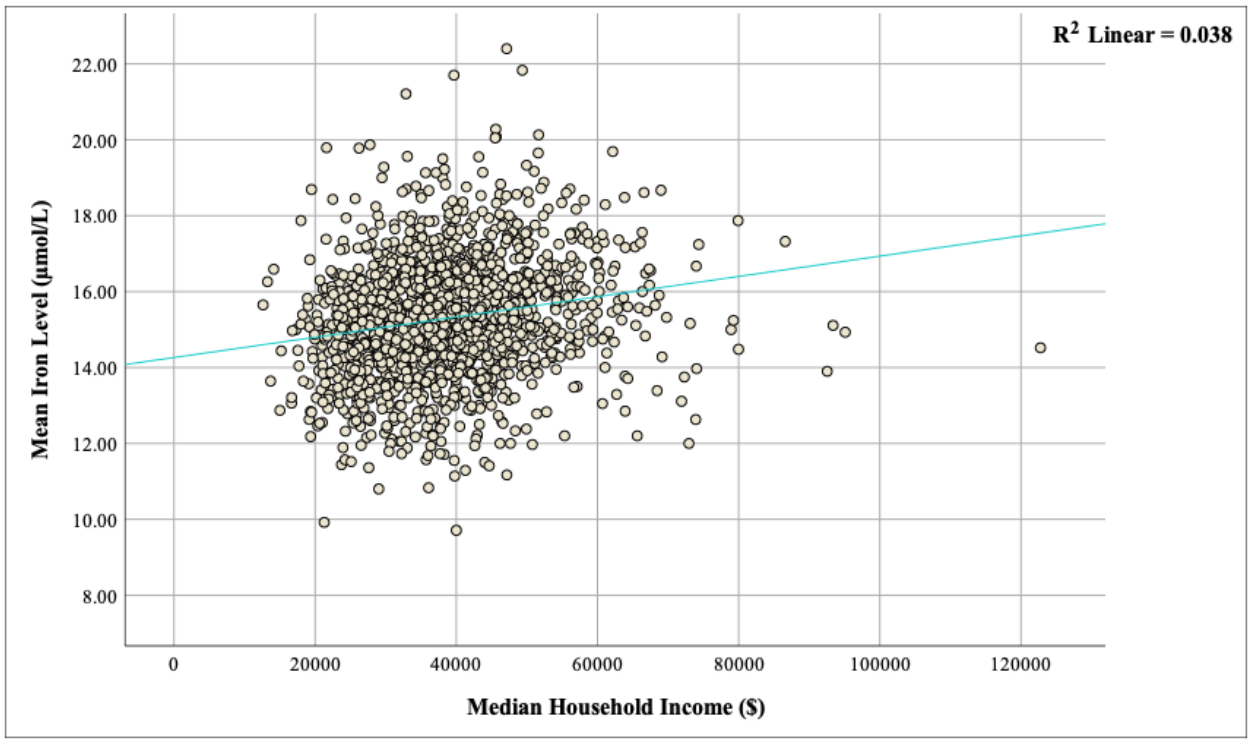
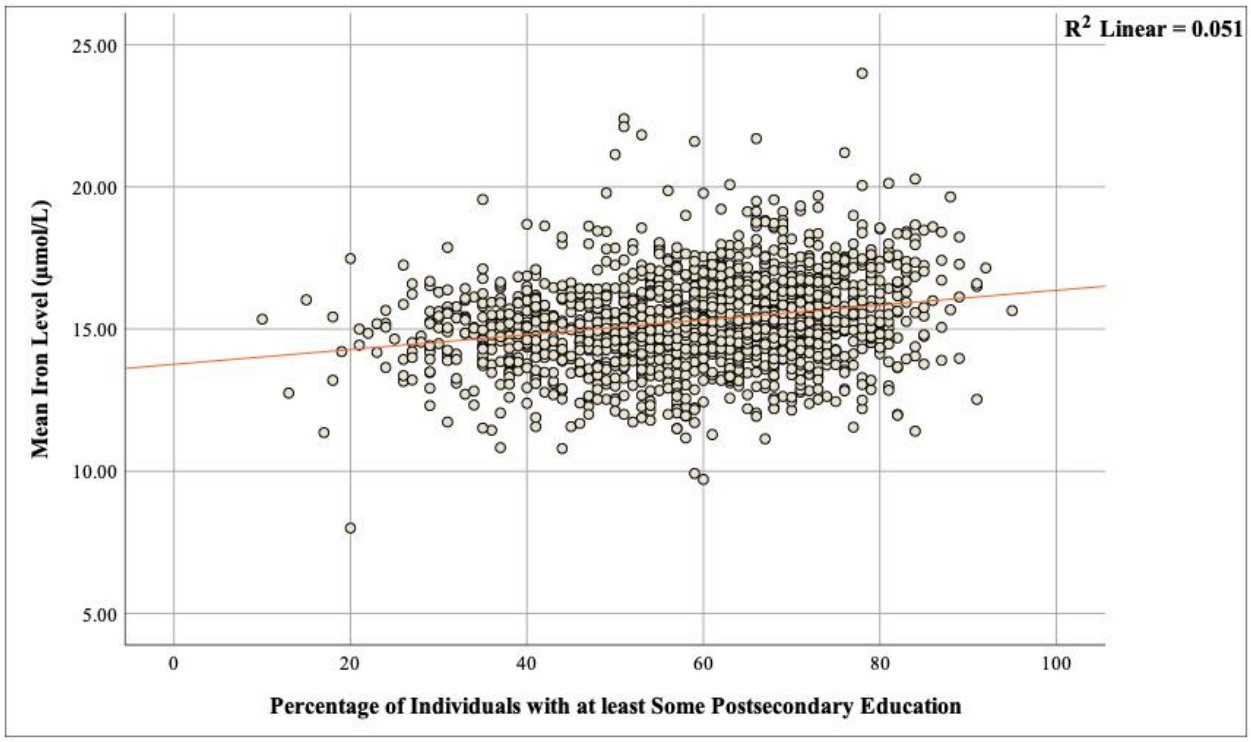


Figure 4.21 Scatterplot of postsecondary education by mean iron level



Chapter Five: Discussion and Conclusion

5.1 Review of Research Questions and Hypotheses

Globally, iron deficiency (ID) and iron deficiency anemia (IDA) are public health crises that have captured the attention of health professionals for decades. In 1992, for example, the WHO urged member states to establish a health and nutrition monitoring system that included ID disorders (WHO, 2001). By 1993, 90 member states had engaged in preventative measures against ID. Unfortunately, these measures were "neither systematically implemented nor well monitored or evaluated" (WHO, 2001: p. 1). Perhaps not surprisingly, ID and IDA remain the most common micronutrient disorders in the world (Pasricha & Drakesmith, 2016; Bailey et al., 2015) with potentially tragic consequences.

While an individual's normal iron level depends on their sex, age, and the biomarker of iron chosen, the WHO (2001) recommends hemoglobin levels of $\geq$110 g/dL for pregnant women, $\geq$120 g/dL for non-pregnant women, and $\geq$130 g/dL for men for those aged 20-49, to maintain physiological homeostasis and proper immune function. Hence, ID and IDA have been found to have pernicious health implications. The role of iron in immune function alone might lead one to conclude that individuals with lower iron levels will have more infections than those with higher iron levels. That said, given the iron requirements of some pathogens, ID and IDA might deprive these pathogens of an essential nutrient and confer benefits to their hosts (Denic & Agarwal, 2007; Flakenberg, 2016; Pietrangelo, 2015; Schrimshaw & SanGiovanni, 1997). Indeed, iron sequestration is a phenotypically plastic adaptation to infectious disease. However, few studies exist examining the relationship between host serum iron levels and subsequent incidents of infection in WEIRD countries (Cardenas et al., 2011; Fagan et al., 2009). In fact, the majority of research on the link between iron and infection has focused on low-to-middle-income countries with levels of infectious disease endemicity that exceed those seen in WEIRD countries. Further, the literature is largely silent on sociodemographic risk factors for ID and IDA in WEIRD countries (Bayoumi et al., 2020; Cooper et al., 2012). As decades of research have documented that variation in health outcomes must be understood in light of the sociodemographics, this gap is surprising (e.g., Little et al., 2021; Kumar, 2021; Bauer, 2014; Feagin & Bennefield, 2014; Eckersley, 1993). To address these gaps in the literature, this study of a sample of Canadians sought to answer two research questions:

1) Does baseline serum iron predict infection up to one year out?
2) Is sociodemographic context associated with (1) mean iron level and (2) infection across Calgary?

Specifically, this study was designed to evaluate two hypotheses:

1) H_{A1} – Low baseline serum predicts a lower risk of infection up to one year out.
2) H_{A2} – Sociodemographic variables including higher median income, more postsecondary education, non-immigrant status, and non-Indigenous status, will be associated with higher serum iron levels.

This research, the first large-scale, population-based, retrospective study examining the association between infectious disease and host iron levels in Canada, should contribute to the understanding of the costs and benefits of ID and IDA. Moreover, the declining effectiveness of antibiotics and the rapid emergence of antibiotic resistant infections suggests that it is expedient to explore all possible interventions for infections. If, for example, ID and/or mild to moderate IDA do provide an adaptive defense against pathogens, then simple nutritional modifications, such as low iron diets or the use of iron chelators, may help in the treatment and prevention of infectious diseases.

5.2 Summary of Key Findings

In short, baseline serum iron does predict infection up to one year out, but not as hypothesized: low serum iron is predictive of higher rates of infection. Hence, this study fails to support H_{A1}. As for the relationship between SDVs and iron and infection, the results varied. None of the SDVs were linked to infection, while higher median household income and having post-secondary education were associated with higher iron levels. Hence, this study does not enable us to completely reject H_{A2}.

Of the 55,437 individuals residing in Calgary who had received a blood test and subsequent microbiology test between 2010-2011, individuals in the highest iron level quartile were 39.2% less likely to develop an infection compared to those in the lowest iron level quartile. In an exploration of the association between baseline iron levels and infection by quadrants of Calgary,

those in the highest iron quartile in the NW, NE, SW, and SE were 39.2%, 26.9%, 39.5%, and 48.6%, respectively, less likely to develop an infection compared to those in the lowest iron level quartile in their quadrant of Calgary. Other risk factors for higher levels of infection included older age (except for residents of the NE, where younger age was a risk factor for infection), being a female, and higher CRG/CQCI levels. When examining the risk of infection by quartile for each type of infection separately, a lower iron level was significantly associated with higher risk of sepsis (blood) and urinary tract infections but was not associated with sepsis (fungal) or Group A streptococcal infections. Older age, being a female, and higher CRG and CQCI levels were risk factors for the development of UTIs. Conversely, being a male and higher CRG and CQCI levels were risk factors for the development of sepsis (blood) and sepsis (fungal) infections. Age was not a risk factor for either sepsis infection. Being a female and younger age increased an individual's risk of contracting a strep throat infection.

Multiple regression analysis did not find a link between sociodemographic variables and infection. In terms of SDVs and iron, 5.8% of the variation observed in mean iron across Calgary's CDAs could be explained by the model as a whole. Both higher median household income and more postsecondary education were associated with higher iron levels, although postsecondary education had a greater effect on the variation of iron levels as compared to median household income ($sr = .132$, $p < 0.001$).

5.3 Findings in the Context of Literature

As discussed above, iron is both an essential nutrient for humans and for most pathogens. Humans acquire iron dietarily, where it is then absorbed and processed inside the small intestine and directed to various storage sites throughout the body. The lack of a gastrointestinal tract in pathogenic organisms, however, means that to maintain the iron levels needed for proliferation, pathogens have adapted mechanisms to steal iron from their hosts (e.g., siderophores, hemophores – Chapter Two, section 2.3.3). As such, it is in the interest of hosts to reduce iron levels in an effort to reduce pathogenicity (Hennigar & McClung, 2016; Weinberg, 1975). This evolutionary arms race between hosts and pathogens (Chapter Two, section 2.2.3) has led some to theorize that iron deficiency and iron deficiency anemia are adaptations to infectious disease (e.g., Goheen et al., 2016; Hunter et al., 2013; Wander et al., 2009). Yet, despite the potential

benefits of low iron in the face of infection, host immune systems rely heavily on the nutrient for things like effective inflammatory responses and the growth and maintenance of B and T lymphocytes (Chapter Two, section 2.3.5). So, how might the relationship between infection and iron levels be understood? Clearly, all adaptations entail trade-offs. Moreover, adaptations can best be understood in the environment in which they were selected.

Given that the present study has been the first of its kind in a WEIRD country, it is difficult to situate the results directly alongside other studies on the association between iron levels and infection, the majority of which have been situated in low-middle income countries with high levels of infectious disease endemicity. Hence, the findings will be viewed considering the broader body of literature regarding iron levels and infection.

5.3.1 H_{A1}: Low baseline serum predicts a lower risk of infection up to one year out.

Per the first hypotheses, and as noted in Chapter Two, it was expected that individuals with lower levels of iron (Q1 and Q2) to be better protected against the risk of infectious disease. The reverse in fact was true: the risk of infection was greater for those with lower levels of iron in seven of the nine cox regressions conducted[ggg]. This finding was not entirely unsurprising, given that iron is a critical component of a properly functioning immune system and low levels of iron have proven to be detrimental for the host (Cherayil, 2010; Srikantia et al., 1976). However, these results are inconsistent with research such as Wander et al.'s (2009), in which residents of the Marasabit District in northern Kenya with normal levels of iron had increased odds of infection and clinical iron deficiency was deemed potentially protective. Similarly, Nyakeriga et al.'s (2004) study in the Ngerenya region of the Kenyan coast concluded that iron deficiency was associated with a small but significant degree of protection from episodes of clinical malaria. Examining the risk of malaria in pregnant women in the Philippines, Senga et al.'s (2011) results were also dissimilar to the current study in that iron deplete mothers were found to be better protected against instances of placental malaria. Also inconsistent with the present study's results, Wander et al.'s (2017) exploration of the associations between iron status and both pre-existing and new infections in Kilimanjaro, Tanzania, finds a protective effect of mild-to-moderate iron deficiency against respiratory infections among the children sampled.

[ggg] Serum iron level was not a significant risk factor for sepsis (fungal) and strep throat infections.

Why might the current findings be inconsistent with those noted above? There are three differences between the present study and those of Wander et al. (2009, 2017) and Nyakeriga et al. (2004) that may help to answer this question.

First, perhaps most importantly, different measures of iron and infection were chosen. The present study examined serum iron levels and identified infection via a positive microbiology test; in contrast, other authors primarily utilized transferrin receptors (TfR) and C-reactive protein (CRP) as ways to measure iron and infection (Wander et al., 2017[hhh]; Senga et al., 2011; Wander et al., 2009; Nyakeriga et al., 2004). The choice of biomarker may be a significant reason why a protective effect of low iron was not observed. TfR is considered a more robust biomarker of iron status as it does not change in the presence of inflammation (Wander et al., 2009; Harthoorn-Lasthuzien et al., 2000). Inflammation is known to affect the interpretation of iron status indicators, especially those such as serum iron or serum ferritin (Pfieffer & Looker, 2017), and because inflammation often accompanies infection[iii], the low iron levels recorded in the current study may be an artifact of infection and/or inflammation as opposed to dietary intake. Additionally, as stated by Pfieffer and Looker (2017), the intra-individual variation for serum iron (~30%) is much higher than it is for serum ferritin (~10-25%) or even TfR (~10%), suggesting that researchers should take more than one sample of serum ferritin if it is to be used in a clinical evaluation. Only the first serum iron result was utilized in this research and thus it could have been an inaccurate reflection of the individual's serum iron levels. It should be noted, however, that there is also no single, widely accepted cut-off value for TfR that is used to identify iron deficiency, and therefore researchers such as Wander et al. (2009) selected cut-offs based on TfR's sensitivity to ZPP:H, as mentioned in section 2.3.6.

Second, the studies of Wander et al. (2009, 2017), Nyakeriga et al. (2004), and Senga et al. (2011) were conducted in environments that differ in a notable way from that of Calgary in 2011. Senga and colleagues' (2011) work in the Philippines, described in section 2.3.6, Wander et al. (2017) in Tanzania, and Nyakeriga et al. (2004) and Wander et al. (2009) in Kenya, were conducted in regions with high rates of endemic infection where low iron may be adaptive. This

[hhh] It is relevant to note that Wander et al. (2017) further incorporated physician-diagnosed infections as well as utilized a_1-glycoprotein to record infections and inflammation, alongside CRP.
[iii] Infection almost always causes inflammation, but inflammation can also be present in the absence of infection.

could also be said of Sub-Saharan Africa in general, where diseases such as acute respiratory infections, HIV/AIDS, diarrhea, malaria, and tuberculosis have been listed as the continent's top five infectious causes of mortality (Boutayeb, 2010). Canada, comparatively, does not suffer from the same rates of infectious disease endemicity and the types of infections experienced by Canadians are often different. For example, out of the 284,082 total deaths across Canada in 2019, 4,323 (1.5%) were deaths caused by infectious diseases, and of these infections, none were categorized as malaria (Statistics Canada, 2020). On the other hand, six countries from Sub-Saharan Africa accounted for approximately half of all malaria deaths worldwide in 2019 alone: Nigeria (23%), the Democratic Republic of the Congo (11%), United Republic of Tanzania (5%), Burkina Faso (4%), Mozambique (4%), and Niger (4%) (WHO, 2021)[jjj]. So, while low iron may be an adaptation in areas with high infectious disease endemicity, it appears to become a liability in an area such as Canada where overall infectious disease rates are low. It is therefore imperative to consider the context of a study as an adaptation cannot be considered outside of the environment in which it was selected for, and context can greatly influence the perceived adaptiveness of a trait and any associated trade-offs (Chapter Two, section 2.1.3).

Third, the current study differs from those of Wander et al. (2017, 2009), Senga et al. (2011), and Nyakeriga et al. (2004) in terms of the population sampled. Wander et al.'s (2017, 2009) samples consisted solely of children (5-10 years and 2-7 years, respectively) and the cohort in Nyakeriga et al. (2004) consisted of children recruited at birth until they were eight years old. In Senga et al.'s (2011) study, the cohort consisted of exclusively perinatal women (no ages provided). In contrast, the present study encompassed a range of individuals from birth to 103 years old and the mean age of those in the current sample with infections was 57.22 $\pm$24.24 years. This may well matter as increasing age has been linked to an increased risk of infection and potentially can affect hemoglobin levels (findings on the relationship between age and hemoglobin are equivocal – see Chapter Three, section 3.2.1), therefore the broader age range utilized by this study may have impacted the results. Furthermore, the majority of the current sample was comprised of females (63%), whereas studies such as Wander et al. (2009) were more equally representative, with 52% males and 48% females. It is interesting that females were found to be more at-risk for infection in the present study, while Wander et al. (2017) concluded that sex did not affect the

[jjj] The WHO (2021) estimates that 409,000 individuals died of malaria in 2019.

risk of infection at all. It is possible that because the present study was comprised mainly of females (63%) and the infected subsample was similar (73%), sex was found to be a significant risk factor because of the overrepresentation of females in this research. So, the inconsistencies between the current research and that of Wander et al. (2017, 2009), Senga et al. (2011), and Nyakeringa et al. (2004) may be explained by the different biomarkers used, levels of infectious disease endemicity, and research samples.

5.3.2 H_{A2}: Higher median household income, higher levels of postsecondary education, non-immigrant, and non-Indigenous status will be associated with higher serum iron levels.

Per the second hypothesis, as noted in Chapter Three, it was expected that higher median household income, higher levels of postsecondary education, non-immigrant, and non-Indigenous statuses associated with higher mean serum iron levels across Calgary (Chapter Three, section 3.3.1). The non-significant association between the SDVs and infection was very unexpected. Previous studies have emphasized the importance of SDVs in explaining health disparities (Mattsson et al., 2017; Irwin et al., 2006; Marmot, 2015) therefore to have found no relationship is unusual. While the regression model did not find a significant association between infection and the sociodemographic variables considered, this may be a function of aggregating the individual samples to a population estimate, thereby masking the true variation in the sample. This was the best that could be done given the data provided and the Covid-19 pandemic, which made it far less feasible to conduct person-to-person research in Calgary.

Immigrant status and Indigenous status did not affect serum iron level. These results are inconsistent with previous studies that have reported elevated levels of ID and IDA in Canadian Indigenous populations, especially in women and children (Christofides et al., 2005a; Willows et al., 2000; Hodgins et al., 1998). However, more current information regarding iron status in Canadian Indigenous communities is not available, therefore these differences may be a factor of outdated research. A non-significant result for immigrant status was similarly unanticipated as recent immigrants to Canada are known to suffer from conditions of iron deficiency (Pottie et al., 2011). Recent immigrants comprised over a quarter of Calgary's population in 2011 (Statistics Canada, 2013) and for this variable to have no influence on average serum iron level is perplexing. Conversely, finding a significant association between average serum iron level and

median household income was anticipated. It was interesting that postsecondary education had more of an influence on serum iron compared to median household income, although the overall contributions by both variables to the model were minor (Table 4.5, Figures 4.20 & 4.21). As noted above, these results are compromised by the fact that sociodemographic data were not available for individuals in this study. Having to aggregate and compare average values to census-level data may have masked the effects of individual variation. For example, median household income is significantly associated with mean iron level by region, but conclusions cannot be drawn regarding median household income's relationship to serum iron on a case-by-case basis. Therefore, while regions with a higher median household income, or higher postsecondary education percentage may be associated with higher mean serum iron, this may not hold true for individual cases within the city.

This section aimed to situate the present study in the context of existing literature and describe the differences in study design that may explain inconsistent findings. The following section will detail possible interpretations of the current findings and what these results might mean.

5.4 Interpretation of Results

The findings presented in Chapter Four indicate that low iron appears to be a risk factor for the development of infection, and therefore H_{A1} is not supported. The non-significant result found with sepsis (fungal) is unexpected as fungal infections directly affect the bloodstream and have been noted to increase an individual's transferrin saturation levels and ferritin concentrations, in addition to lowering serum transferrin and increasing serum iron concentrations (Iglesias-Osma et al., 1995). Conversely, it is understandable that the strep throat infections also yielded a non-significant result as streptococcus infection typically does not interact directly with the blood. Strep throat can, however, progress to streptococcal toxic shock syndrome (Chapter Three, section 3.2.1) if the bacteria are able to enter the bloodstream. UTIs are similar to strep throat in that they must progress to a more severe form (known as urosepsis) if they are to directly affect the blood, which is why it is curious that serum iron level was a significant risk factor for a urinary tract infection. This may simply be a product of how many individuals, both male and female, tested positive for a UTI (77% of all infected individuals), whereas the sepsis (fungal) sample may have been too small to capture a relationship (4.4% of all infected cases).

Evolutionary considerations, as noted above in the differences in research context, particularly variation in infectious disease endemicity, may be particularly important in understanding why the current study failed to find an association between iron and infection. Darwin (1859) emphasized that adaptations must be considered in light of the environment. As well, an adaptation to one physiological domain may compromise another physiological domain (Mazess, 1975; Chapter Two, section 2.1.3). An example of this is manifest in genes that code for higher arterial hemoglobin levels among the Aymara, a population indigenous to the high Andes (Decker et al. 1998). This trait increases the Aymara's ability to transport oxygen in a hypoxic environment but requires their heart to work harder to move more viscous blood – a benefit at a high altitude but a potential liability at sea level. The relative importance of the environment and adaptive trade-offs is also manifest in the balanced polymorphism, sickle-cell anemia. As discussed in Chapter Two section 2.2.4, individuals who are heterozygous for the sickle-cell allele (HbAS) fair better in malaria-endemic environments than those without the allele (Allison, 1954). The downside, however, is that being heterozygous renders an individual's red blood cells less stable and predisposes them to a sickling event in the case of oxygen stress (Mitchell, 2018; Nelson et al., 2016; Austin et al., 2007). This phenomenon is not uncommon in young African Americans who compete as college athletes; in 1974, a 19-year-old football player at the University of Colorado, Boulder, collapsed during conditioning and later died because of sickle cell complications (Mitchell, 2018). Additionally, should two HbAS individuals have a child together, there is a 25% chance that their child will have sickle-cell anemia (HbSS) and will experience early death without extensive medical intervention.

In the case of iron sequestration in the face of infectious disease, it is believed that the environment in which it originated emerged because soluble ferrous iron oxidized into insoluble ferric iron (Chapter Two, section 2.3.3) some 2.4 billion years ago, thereby leading to the battle of iron piracy between pathogens and hosts[kkk]. In fact, one of the earliest cases of this battle was discovered by Barber and Elde (2014), who found evidence of rapid primate transferrin evolution that was driven by interactions with the bacterial transferrin-binding protein (TbpA) in Old World and New World monkey species[lll]. As humans evolved and the agricultural revolution

[kkk] This is referred to as the Great Oxygenation Event, in which cyanobacteria began to accumulate in the earth's atmosphere, producing oxygen and causing the oxidization of ferrous iron into ferric iron.
[lll] TbpA is used by bacteria to bind to transferrin and steal iron from their hosts.

took place, researchers like Denic & Agarwal (p. 611, 2007) posited that the combined effects of a dietary shift towards low-iron foods and an increased population density led to a 'pandemic of iron deficiency', which in turn was followed by the emergence of new infectious diseases (Chapter Two, section 2.2.2). Iron deficiency, they argue, was likely protective against diseases like malaria or tuberculosis and was therefore selected for in these early human populations (Denic & Agarwal, 2007). Similarly, this may also be the case when considering the roles and influence of hepcidin on a host's iron levels. As discussed in Chapter Two, section 2.3.1, hepcidin is found as a single molecule in humans yet it maintains two functions: acting as an antimicrobial peptide in the innate immune response, and as an iron limiter in the acute phase protein response. As to why these two roles exist inside one molecule, it may be because both had a function in fighting infection; that is, hepcidin could have evolved into this dual-pronged peptide due to pressures placed by high infectious disease loads. In other words, hepcidin's duality may have been adaptive in previous conditions with endemic infectious disease because it lowers the available iron in a host *and* destroys infectious molecules. However, while low iron and hepcidin may have been adaptive in these early agricultural societies that suffered from endemic infectious diseases, and while they may continue to provide mild-to-moderate protection in modern, agricultural-based societies such as Tanzania (e.g., Wander et al., 2017), these adaptations might not be relevant in a high-income country like Canada where factors such as advanced and accessible biomedical services are readily available to treat and prevent disease. As such, it is extremely important to consider context when attempting to understand an adaptation, as we have seen the trade-offs evident with low iron (namely, increased risk of infection) in an environment without high disease endemicity such as Canada (Darwin, 1859).

As regards the second hypothesis, when sociodemographic variables are controlled for, this study still finds that iron remains the key player in the risk for infection. The multiple regression model suggested that median household income and postsecondary education do have an impact on iron level, although the effects are minimal. These findings are likely compromised by the fact that markers of SES, postsecondary education, immigrant status, and Indigenous status are not available at the individual level for the cases in this study. As a result, generalized conclusions had to be drawn regarding SDVs based on postal codes and census dissemination areas.

5.5 Limitations and Future Directions

Potential limitations of the present study include the methodology utilized to analyze the relationship between iron and infection and sociodemographic variables and the selection of biomarkers used to analyze iron and infection. The average serum iron level and percentage of infections per CDA were compared to population-level data; that is, individual data points were available for iron level and infection, but these could not be matched up to individual measures of median household income, postsecondary education, immigrant status, or Indigenous status. As such, some of the more nuanced relationships between iron and infection and the chosen SDVs are likely being clouded by the proxy measure of SES. For example, the multiple regression analysis between iron and the selected SDVs indicated that areas of Calgary with a higher median income were less likely to experience an infection, yet this is likely not the case for every individual residing in these areas. In regions like Nose Hill, whose median household income was recorded as $101,191 in 2011, there was still a gradient of income present, and individuals with an income lower than the median value could very well have experienced infection despite the higher regional reflection of socioeconomic status. More detailed sociodemographic data are required to accurately explore the relationships between iron, infection, and SDVs in Calgary. Unfortunately, such work will have to wait until it is again safe to be meeting in-person with study participants.

As regards the biomarkers chosen to assess iron level, serum iron is not as robust as the transferrin receptor (TfR) in the face of inflammation, and although any individual who tested positive for an infection within 30 days of their blood test was removed from the study (Chapter Three, section 3.1.4), inflammation can exist without infection. The serum iron results that were utilized, therefore, may have been an inaccurate measurement of an individual's iron levels. As stated by Wander et al. (2009: p. 3) "no single biomarker is sufficient to characterize an individual's iron status as each indicator is vulnerable to one or more sources of misclassification", therefore another limitation of this study was only selecting one measure of iron to analyze against infection. Moreover, while a positive microbiology test is a reliable indication of infection, the present study could have been strengthened by analyzing measures of C-reactive protein (CRP) because it rises in response to inflammation. This measure would have helped identify and potentially exclude additional individuals who had not tested positive for an

infection but still had had elevated levels of CRP, especially since serum iron was the only measure for iron status.

Future research regarding the association between iron levels and infection should examine the differences in infection by sex. For example, females were vastly overrepresented in the infected subsample (72.5%), yet males were found to be more at risk for both types of sepsis infections (fungal and blood). These results are consistent with Navaneelan's (2016) report on sepsis in Canada, which states that deaths involving sepsis were significantly higher among males than females in 2011. Research by Esper et al. (2006) further aligns with these results, suggesting that hormonal differences between the sexes may be a partial reason for the differences in sepsis patients. It would be beneficial to examine the frequency of sepsis amongst Calgarians and establish how, if at all, males and females differ in their susceptibility and explore why, given that sepsis can have adverse effects if left untreated. It would be also worthwhile to explore why females in general comprised significantly more of the sample demographic. Perhaps there are sociocultural explanations for this difference that have not yet been explored in Calgary, which could then be used to evaluate the existing medical systems and how they could be better adapted to the population's needs.

Additionally, future studies should include individual assessments of median household income, postsecondary education, recent immigrant status, and Indigenous status, in order to better evaluate how these variables might interact with or influence one another. For example, when examining infection by city quadrant, it was posited that the higher immigrant population and lower median income in the NE (Chapter Three, section 3.3.1 and Table 3.2) would translate into the highest number of infections. It was therefore unexpected to see the lowest percentage of infections in the NE (17.65%), while simultaneously displaying the lowest average serum iron levels (14.89 $\pm$ 7.11 µmol/L). Further, the NE had the lowest average age of the quadrants (40.26 $\pm$ 20.77 years) yet being younger was a significant risk factor for infection as opposed to being older. The NE thus presents results that are contrary to research suggesting older individuals are at a greater risk for infection (Bender, 2003). In contrast, the SW had the highest average age at 49.32 $\pm$21.66 years and the highest percentage of infections (30.92%). The number of infections were surprising when considering social determinants of health: the SW is a predominantly

wealthy quadrant with a median household income of $88,320 (Figure 3.1B, Table 3.2). As the first determinant of health in Canada (Government of Canada, 2016) and the chosen marker for socioeconomic status, it was hypothesized that areas with higher median household income would have higher average serum iron levels compared to areas with lower median incomes. While the iron levels were higher, they were accompanied by a greater number of infections. Older average ages may be a factor in why elevated levels of infection were observed in the western (and wealthier) side of the city; the older an individual was, their risk of infection significantly increased, although the ANOVA test comparing average ages across all four quadrants returned a small effect size for significance (0.02; section 4.2). These findings emphasize the importance of community-specific research and careful ethnography to truly uncover and highlight the differences between infection rates across the city of Calgary.

5.6 Significance and Conclusion

ID remains one of the top global micronutrient deficiencies, affecting approximately 25% of the population (WHO, 2021). Significantly higher rates of ID and IDA are found in women of reproductive/postpartum ages, children, and the elderly, both in low-middle income and high-income countries, such as Canada (Goonewardene et al., 2011; Shavelle et al., 2012; Abbaspour et al., 2014). Further, ID has a complex etiology, with risk factors ranging from genetic (Finberg et al., 2008; Fairweather-Tait et al., 2013), to blood loss, dietary inadequacy, iron malabsorption, rapid growth of parasitic infections (Kumar & Coltran, 2014), poverty, Indigenous status (in Canada) (Christofides et al., 2005a; Willows et al., 2000; Hodgins et al., 1998), immigrant status (Pottie et al., 2011; Hayes et al., 1998; Bindra & Gibson, 1986), and viral, bacterial, or fungal infectious diseases (Figure 1.1). Left untreated, ID and IDA can lead to devastating health consequences due to iron's role in the immune system and growth and development (e.g., Black et al., 2011; Georgieff et al., 2011; CDC, 2010). That said, the origin and subsequent pervasiveness of ID and IDA may be understood as an adaptation to pathogenic iron piracy, thereby reducing pathogen virulence and susceptibility to infectious diseases (Denic & Agarwal, 2007; Falkenberg, 2016; Pietrangelo, 2015; Scrimshaw & SanGiovanni, 1997). Analyses of the association between iron and infection, specifically low iron/iron deficiency, and a host of sociodemographic variables that could affect the relationship between the two may help inform

global health priorities and the strategies being used to mitigate the effects of ID and IDA in vulnerable populations.

The present study examined the relationship between serum iron and four infectious diseases to determine if low levels of serum iron would be protective against infection. The associations between sociodemographic variables and iron and infection were also examined to see if there were other factors influencing the primary relationship between iron and infection. This study contributes to the literature as previous research on iron and infection has primarily been conducted in low-middle income countries with high rates of infectious disease endemicity. To the author's knowledge, there have been very few studies on iron and infection in a WEIRD country such as Canada where infectious disease rates are low. Low serum iron was found to not be protective against infectious diseases as predicted but was in fact a risk factor for increased infections. Higher median income and more postsecondary education can explain a small amount of the variation observed in mean serum iron levels, but the correlation coefficients are small indicating a minimal effect. There were no significant relationships between sociodemographic variables and infection. The results of the study were inconsistent with the literature, likely due to three reasons: (1) the difference in biomarkers used to measure iron and infection, (2) the difference in study context, and (3) the difference in sample demographics. These findings indicate that low serum iron levels in Calgary are detrimental to an individual's risk of an infection as opposed to adaptive, as seen in areas such as Africa or southeast Asia. Furthermore, these findings suggest that a closer examination of sociodemographic variables such as income, education level, and immigrant and Indigenous status at an individual level may reveal more nuanced effects on iron level and susceptibility to infection in Calgary.

References

3M Health Information Systems. (2016). *3M clinical risk groups: Measuring risk, managing care*. https://multimedia.3m.com/mws/media/765833O/3m-crgs-measuring-risk-managing-care-white-paper.pdf

Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014). Review on iron and its importance for human health. *Journal of Research in Medical Sciences*, 12.

Adolph, E. F. (1961) Early concepts of physiological regulations. Physiol. Rev. 41:737–770. doi: 10.1152/physrev.1961.41.4.737

Ahmmad, Z., Wen, M., & Li, K. (2021). Self-rated Health Disparities Among Asian Americans: Mediating Roles of Education Level and Household Income. *Journal of Immigrant and Minority Health*, *23*(3), 583–590. https://doi.org/10.1007/s10903-020-01051-0

Alberta Health Services. (2014). Data integration, measurement & reporting: Clinical risk groups. Edmonton, Alberta.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (n.d.). *Innate Immunity*. NCBI. https://www.ncbi.nlm.nih.gov/books/NBK26846/

Allison, A. C. (1954). Protection afforded by sickle-cell trait against subtertian malarial infection. British Medical Journal *48*(57), 290–294

Angeles, I. T., Schultink, W. J., Matulessi, P., Gross, R., & Sastroamidjojo, S. (1993). Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. *The American Journal of Clinical Nutrition*, *58*(3), 339–342. https://doi.org/10.1093/ajcn/58.3.339

Annibale, B. (2003). Concomitant alterations in intragastric pH and ascorbic acid concentration in patients with Helicobacter pylori gastritis and associated iron deficiency anaemia. *Gut*, *52*(4), 496–501. https://doi.org/10.1136/gut.52.4.496

Armando Hasundungan, (2014, August 25). *Iron Physiology* [Video]. Youtube. https://www.youtube.com/watch?v=ahCy97FVUpM&list=PLYE4ac67Y66sgKe7Ewd81nEETjNPvCdcL&index=3

Armelagos, G. J., Brown, P. J., & Turner, B. (2005). Evolutionary, historical and political economic perspectives on health and disease. *Social Science & Medicine*, *61*(4), 755–765. https://doi.org/10.1016/j.socscimed.2004.08.066

Austin, H., Key, N. S., Benson, J. M., Lally, C., Dowling, N. F., Whitsett, C., & Hooper, W. C. (2007). Sickle cell trait and the risk of venous thromboembolism among blacks. *Blood*, *110*(3), 908–912. https://doi.org/10.1182/blood-2006-11-057604

Balarajan, Y., Ramakrishnan, U., Özaltin, E., Shankar, A. H., & Subramanian, S. (2011). Anaemia in low-income and middle-income countries. *The Lancet*, *378*(9809), 2123–2135. https://doi.org/10.1016/S0140-6736(10)62304-5

Bailey, R. L., West Jr., K. P., & Black, R. E. (2015). The Epidemiology of Global Micronutrient Deficiencies. *Annals of Nutrition and Metabolism*, *66*(Suppl. 2), 22–33. https://doi.org/10.1159/000371618

Barber, M. F., & Elde, N. C. (2014). Escape from bacterial iron piracy through rapid evolution of transferrin. *Science*, *346*(6215), 1362–1366. https://doi.org/10.1126/science.1259329

Barton, J. C., Wiener, H. H., Acton, R. T., Adams, P. C., Eckfeldt, J. H., Gordeuk, V. R., Harris, E. L., McLaren, C. E., Harrison, H., McLaren, G. D., & Reboussin, D. M. (2020). Prevalence of iron deficiency in 62,685 women of seven race/ethnicity groups: The HEIRS Study. *PLOS ONE*, *15*(4), e0232125. https://doi.org/10.1371/journal.pone.0232125

Basta, S. S., Soekirman, Karyadi, D., & Scrimshaw, N. S. (1979). Iron deficiency anemia and the productivity of adult males in Indonesia. *The American Journal of Clinical Nutrition*, *32*(4), 916–925. https://doi.org/10.1093/ajcn/32.4.916

Bauer, G. R. (2014). Incorporating intersectionality theory into population health research methodology: Challenges and the potential to advance health equity. *Social Science & Medicine*, *110*, 10–17. https://doi.org/10.1016/j.socscimed.2014.03.022

Bayoumi, I., Parkin, P. C., Birken, C. S., Maguire, J. L., Borkhoff, C. M., & for the TARGet Kids! Collaboration. (2020). Association of Family Income and Risk of Food Insecurity With Iron Status in Young Children. *JAMA Network Open*, *3*(7), e208603. https://doi.org/10.1001/jamanetworkopen.2020.8603

Bender, B. S. (2003). Infectious disease risk in the elderly. *Immunology and Allergy Clinics of North America*, *23*(1), 57–64. https://doi.org/10.1016/S0889-8561(02)00078-4

Bennett, J. E., Dolin, R., Blaser, M., Sobel, J. D., & Brown, P. (2020). Urinary Tract Infections. In *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases* (9th ed., pp. 962–989). Elsevier.

Bernard, C. (1927) An Introduction to the Study of Experimental Medicine (originally published in 1865; first English translation by Henry Copley Greene, published by Macmillan & Co., Ltd., 1927)
Bewick, V., Cheek, L., & Ball, J. (2004). Statistics review 12: Survival analysis. *Critical Care*, *8*(5), 389. https://doi.org/10.1186/cc2955

Bindra, G. S., & Gibson, R. S. (1986). Iron status of predominantly lacto-ovo vegetarian East Indian immigrants to Canada: A model approach. *The American Journal of Clinical Nutrition*, *44*(5), 643–652. https://doi.org/10.1093/ajcn/44.5.643

Black, M. M., Quigg, A. M., Hurley, K. M., & Pepper, M. R. (2011). Iron deficiency and iron-deficiency anemia in the first two years of life: Strategies to prevent loss of developmental potential: Nutrition Reviews©, Vol. 66, No. S1. *Nutrition Reviews*, *69*, S64–S70. https://doi.org/10.1111/j.1753-4887.2011.00435.x

Boutayeb, A. (2010). The Impact of Infectious Diseases on the Development of Africa. In V. R. Preedy & R. R. Watson (Eds.), *Handbook of Disease Burdens and Quality of Life Measures* (pp. 1171–1188). Springer. https://doi.org/10.1007/978-0-387-78665-0_66

Bullen, J. J., Rogers, H. J., Spalding, P. B., & Ward, C. G. (2006). Natural resistance, iron and infection: A challenge for clinical medicine. *Journal of Medical Microbiology*, *55*(3), 251–258. https://doi.org/10.1099/jmm.0.46386-0

Cagliani, R., & Sironi, M. (2013). Pathogen-Driven Selection in the Human Genome. *International Journal of Evolutionary Biology*, *2013*, 1–6. https://doi.org/10.1155/2013/204240

Canadian Population Health Initiative. (2008). Reducing gaps in health: a focus on socioeconomic status in urban Canada. Ottawa (ON): CIHI; 2008.

Cardenas, V. M., Prieto-Jimenez, C. A., Mulla, Z. D., Rivera, J. O., Dominguez, D. C., Graham, D. Y., & Ortiz, M. (2011). Helicobacter pylori Eradication and Change in Markers of Iron Stores Among Non–iron-deficient Children in El Paso, Texas: An Etiologic Intervention Study. *Journal of Pediatric Gastroenterology & Nutrition*, *52*(3), 326–332. https://doi.org/10.1097/MPG.0b013e3182054123

Carroll, L. (1990). *Through the looking glass* (K. Baker, Trans.). New York, New York: Berkley Publishing Group and First Publishing, Inc.; First edition.

Casals, F., Sikora, M., Laayouni, H., Montanucci, L., Muntasell, A., Lazarus, R., Calafell, F., Awadalla, P., Netea, M. G., & Bertranpetit, J. (2011). Genetic adaptation of the antibacterial human innate immunity network. *BMC Evolutionary Biology*, *11*(1), 202. https://doi.org/10.1186/1471-2148-11-202

Cassat, J. E., & Skaar, E. P. (2013). Iron in Infection and Immunity. *Cell Host & Microbe*, *13*(5), 509–519. https://doi.org/10.1016/j.chom.2013.04.010

Centers for Disease Control and Prevention. (1998, April 3). *Recommendations to Prevent and Control Iron Deficiency in the United States.* Recommendations and Reports. https://www.cdc.gov/mmwr/preview/mmwrhtml/00051880.htm

Centers for Disease Control and Prevention. Breastfeeding Report Card, United States: Outcome Indicators (Publication, from Centers for Disease Control and Prevention, National Immunization Survey, 2010. http://www.cdc. gov/breastfeeding/data/index.htm [Last accessed on 22 April 2020].

Centers for Disease Control and Prevention. (2018). Pharyngitis. National Center for Immunization and Respiratory Diseases, Division of Bacterial Diseases.

Centers for Disease Control and Prevention (2018). Sepsis. U.S. Centers for Disease Control and Prevention: National Center for Emerging and Zoonotic Infectious Diseases.

Charles, D. H. M., Ness, A. R., Campbell, D., Smith, G. D., Whitley, E., & Hall, M. H. (2005). Folic acid supplements in pregnancy and birth outcome: Re-analysis of a large randomised controlled trial and update of Cochrane review. *Paediatric and Perinatal Epidemiology*, *19*(2), 112–124.

Cherayil, B. J. (2010). Iron and Immunity: Immunological Consequences of Iron Deficiency and Overload. *Archivum Immunologiae et Therapiae Experimentalis*, *58*(6), 407–415. https://doi.org/10.1007/s00005-010-0095-9

Chlosta, S., Fishman, D. S., Harrington, L., Johnson, E. E., Knutson, M. D., Wessling-Resnick, M., & Cherayil, B. J. (2006). The iron efflux protein ferroportin regulates the intracellular growth of Salmonella enterica. *Infection and Immunity*, *74*(5), 3065–3067. https://doi.org/10.1128/IAI.74.5.3065-3067.2006

Choe, Y. H., Kim, S. K., Son, B. K., Lee, D. H., Hong, Y. C., & Pai, S. H. (1999). Randomized Placebo-Controlled Trial of Helicobacter pylori Eradication for Iron-Deficiency Anemia in Preadolescent Children and Adolescents. *Helicobacter (Cambridge, Mass.)*, *4*(2), 135–139.

Choe, Y., Lee, J., & Kim, S. (2000). Effect of Helicobacter pylori eradication on sideropenic refractory anaemia in adolescent girls with Helicobacter pylori infection. *Acta Pædiatrica*, *89*(2), 154–157.

Christofides, A., Schauer, C., & Zlotkin, S. H. (2005a). Iron Deficiency and Anemia Prevalence and Associated Etiologic Risk Factors in First Nations and Inuit Communities in Northern Ontario and Nunavut. *Canadian Journal of Public Health*, *96*(4), 304–307. https://doi.org/10.1007/BF03405171

Christofides, A., Schauer, C., & Zlotkin, S. H. (2005b). Iron deficiency anemia among children: Addressing a global public health problem within a Canadian context. *Paediatrics & Child Health*, *10*(10), 597–601. https://doi.org/10.1093/pch/10.10.597

City of Calgary [@cityofcalgary] (2017, April 20). *#greencart will be rolled out by quadrant. Once everyone in a quadrant has their carts, collection will begin as follows.* [Image attached] [Tweet] Twitter. *https://twitter.com/cityofcalgary/status/855091352991711233?lang=ca*

Collins, J. F., Wessling-Resnick, M., & Knutson, M. D. (2008). Hepcidin Regulation of Iron Transport. *The Journal of Nutrition*, *138*(11), 2284–2288. https://doi.org/10.3945/jn.108.096347

Cooper, M., Greene-Finestone, L., Lowell, H., Levesque, J., & Robinson, S. (2012). Iron sufficiency of Canadians. *Health Reports*, *23*(82), 10.

Cooper, S. J. (2008). From Claude Bernard to Walter Cannon. Emergence of the concept of homeostasis. *Appetite*, *51*(3), 419–427. https://doi.org/10.1016/j.appet.2008.06.005

Darin-Mattsson, A., Fors, S., & Kåreholt, I. (2017). Different indicators of socioeconomic status and their relative importance as determinants of health in old age. *International Journal for Equity in Health*, *16*(1), 173. https://doi.org/10.1186/s12939-017-0670-3

Darwin, C. (1859). *On the origin of species*. Lerner Publishing Group.

de Koning, L., Henne, D., Woods, P., Hemmelgarn, B. R., & Naugler, C. (2014). Sociodemographic correlates of 25-hydroxyvitamin D test utilization in Calgary, Alberta. *BMC Health Services Research*, *14*(1), 339. https://doi.org/10.1186/1472-6963-14-339

Decker, M., Vargas, E., Villena, M., Soria, R., Alarcon, A., & Gonzales, C. (1998). Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. *American Journal of Physical Anthropology*, *106*, 385–400.

Degroot, V., Beckerman, H., Lankhorst, G., & Bouter, L. (2003). How to measure comorbiditya critical review of available methods. *Journal of Clinical Epidemiology*, *56*(3), 221–229. https://doi.org/10.1016/S0895-4356(02)00585-1

Denic, S., & Agarwal, M. M. (2007). Nutritional iron deficiency: An evolutionary perspective. *Nutrition*, *23*(7–8), 603–614. https://doi.org/10.1016/j.nut.2007.05.002

Devi, U., Rao, C. M., Srivastava, V. K., Rath, P. K., & Das, B. S. (2003). Effect of iron supplementation on mild to moderate anaemia in pulmonary tuberculosis. *British Journal of Nutrition*, *90*(3), 541–550. https://doi.org/10.1079/BJN2003936

Dijkhuizen, M. A., Wieringa, F. T., West, C. E., Martuti, S., & Muhilal. (2001). Effects of Iron and Zinc Supplementation in Indonesian Infants on Micronutrient Status and Growth. *The Journal of Nutrition*, *131*(11), 2860–2865. https://doi.org/10.1093/jn/131.11.2860

Eckersley, R. (1993). Failing a generation: The impact of culture on the health and well-being of youth. *Journal of Paediatrics and Child Health*, *29*(s1), S16–S19.

Esper, A., M. (2006). DISPARITIES IN SEPSIS: *Shock*, *25*(Supplement 1), 4. https://doi.org/10.1097/00024382-200606001-00011

Fagan, R. P., Dunaway, C. E., Bruden, D. L., Parkinson, A. J., & Gessner, B. D. (2009). Controlled, Household-Randomized, Open-Label Trial of the Effect of Treatment of Helicobacter pylori Infection on Iron Deficiency among Children in Rural Alaska: Results at 40 Months. *The Journal of Infectious Diseases*, *199*(5), 652–660.

Fairweather-Tait, S. J., Guile, G. R., Valdes, A. M., Wawer, A. A., Hurst, R., Skinner, J., & Macgregor, A. J. (2013). The Contribution of Diet and Genotype to Iron Status in Women: A Classical Twin Study. *PLoS ONE*, *8*(12), e83047. https://doi.org/10.1371/journal.pone.0083047

Fairweather-Tait, S. J., Wawer, A. A., Gillings, R., Jennings, A., & Myint, P. K. (2014). Iron status in the elderly. *Mechanisms of Ageing and Development*, *136–137*, 22–28. https://doi.org/10.1016/j.mad.2013.11.005

Falkenberg, R. I. (2016). Review: An Evolutionary Perspective on Iron Deficiency. Human Body, 1:24-28.

Farmer, P. (1996). Social Inequalities and Emerging Infectious Diseases. *Emerging Infectious Diseases*, *2*(4), 259–269. https://doi.org/10.3201/eid0204.960402

Feagin, J., & Bennefield, Z. (2014). Systemic racism and U.S. health care. *Social Science & Medicine*, *103*, 7–14. https://doi.org/10.1016/j.socscimed.2013.09.006

Finberg, K. E., Heeney, M. M., Campagna, D. R., Aydınok, Y., Pearson, H. A., Hartman, K. R., Mayo, M. M., Samuel, S. M., Strouse, J. J., Markianos, K., Andrews, N. C., & Fleming, M. D. (2008). Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). *Nature Genetics*, *40*(5), 569–571. https://doi.org/10.1038/ng.130

Foy, M. R., Kim, J. J., Shors, T. J., & Thompson, R. F. (2006). Neurobiological Foundations of Stress. In *Nutrients, Stress, and Medical Disorders*. Humana Press.

Franco, A. V. M. (2005). Recurrent urinary tract infections. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *19*(6), 861–873. https://doi.org/10.1016/j.bpobgyn.2005.08.003

Fumagalli, M., Sironi, M., Pozzoli, U., Ferrer-Admettla, A., Pattini, L., & Nielsen, R. (2011). Signatures of Environmental Genetic Adaptation Pinpoint Pathogens as the Main Selective Pressure Through Human Evolution. *PLoS Genetics*, *7*(11).

Fusco, G., & Minelli, A. (2010). Phenotypic plasticity in development and evolution: Facts and concepts. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1540), 547–556. https://doi.org/10.1098/rstb.2009.0267

Gangaidzo, I. T., Moyo, V. M., Mvundura, E., Aggrey, G., Murphree, N. L., Khumalo, H., Saungweme, T., Kasvosve, I., Gomo, Z. A. R., Rouault, T., Boelaert, J. R., & Gordeuk, V. R. (2001). Association of Pulmonary Tuberculosis with Increased Dietary Iron. *The Journal of Infectious Diseases*, *184*(7), 936–939. https://doi.org/10.1086/323203

Gaynes, R. (2017). The Discovery of Penicillin—New Insights After More Than 75 Years of Clinical Use. *Emerging Infectious Diseases*, *23*(5), 849–853. https://doi.org/10.3201/eid2305.161556

Georgieff, M. K. (2011). Long-term brain and behavioral consequences of early iron deficiency: Nutrition Reviews©, Vol. 69, No. s1. *Nutrition Reviews*, *69*, S43–S48. https://doi.org/10.1111/j.1753-4887.2011.00432.x

Georgiev, A. V., Kuzawa, C. W., & McDade, T. W. (2016). Early developmental exposures shape trade-offs between acquired and innate immunity in humans. *Evolution, Medicine, and Public Health*, *2016*(1), 256–269. https://doi.org/10.1093/emph/eow022

Gera, T. (2002). Effect of iron supplementation on incidence of infectious illness in children: Systematic review. *BMJ*, *325*(7373), 1142–1142. https://doi.org/10.1136/bmj.325.7373.1142

Geyer, S. (2006). Education, income, and occupational class cannot be used interchangeably in social epidemiology. Empirical evidence against a common practice. *Journal of Epidemiology & Community Health*, *60*(9), 804–810. https://doi.org/10.1136/jech.2005.041319

Goheen, M. M., Wegmüller, R., Bah, A., Darboe, B., Danso, E., Affara, M., Gardner, D., Patel, J. C., Prentice, A. M., & Cerami, C. (2016). Anemia Offers Stronger Protection Than Sickle Cell Trait Against the Erythrocytic Stage of Falciparum Malaria and This Protection Is Reversed by Iron Supplementation. *EBioMedicine*, *14*, 123–130. https://doi.org/10.1016/j.ebiom.2016.11.011

Goonewardene, M., Shehata, M., & Hamad, A. (2012). Anaemia in pregnancy. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *26*(1), 3–24. https://doi.org/10.1016/j.bpobgyn.2011.10.010

Gould, S. J., & Lewontin, R. C. (1979). The Evolution of Adaptation by Natural Selection. *Proceedings of the Royal Society of London*, *205*(1161), 581–598.

Grenier, D., Huot, M.-P., Mayrand, D., & Dentaire, M. (2000). *Iron-Chelating Activity of Tetracyclines and Its Impact on the Susceptibility of Actinobacillus actinomycetemcomitans to These Antibiotics* (Vol. 44, Issue 3, pp. 763–766). http://aac.asm.org/

Government of Canada. (2020, October 7). *Social determinants of health and health inequalities.* Canada. https://www.canada.ca/en/public-health/services/health-promotion/population-health/what-determines-health.html

Gräsbeck, R., Kouvonen, I., Lundberg, M., & Tenhunen, R. (1979). An intestinal receptor for heme. *Scandinavian Journal of Haematology*, 23(1), 5-9.

Grenier, D., Huot, M.P., & Mayrand, D. (2000). Iron-chelating activity of tetracyclines and its impact on the susceptibility of Actinobacillus actinomycetemcomitans to these antibiotics. *Antimicrobial Agents and Chemotherapy*, 44(3): 763-766.

Haas, J. D., & Brownlie, T. (2001). Iron Deficiency and Reduced Work Capacity: A Critical Review of the Research to Determine a Causal Relationship. *The Journal of Nutrition*, *131*(2), 676S-690S. https://doi.org/10.1093/jn/131.2.676S

Hadley, C., & DeCaro, J. A. (2015). Does moderate iron deficiency protect against childhood illness? A test of the optimal iron hypothesis in Tanzania: IRON AND INFECTION. *American Journal of Physical Anthropology*, *157*(4), 675–679. https://doi.org/10.1002/ajpa.22756

Hagan, A. (n.d.). Siderophores: Bacterial iron scavengers. Retrieved from https://www.asm.org/Articles/2017/May/siderophores-bacterial-iron-scavengers

Hager, D. N., Krishnan, J. A., Hayden, D. L., Brower, R. G., & Network, A. C. T. (2005). Tidal Volume Reduction in Patients with Acute Lung Injury When Plateau Pressures Are Not High. *American Journal of Respiratory and Critical Care Medicine*, *172*(10), 1241–1245.

Haldane, J. B. S. (1949). THE ASSOCIATION OF CHARACTERS AS A RESULT OF INBREEDING AND LINKAGE. *Annals of Eugenics*, *15*(1), 15–23. https://doi.org/10.1111/j.1469-1809.1949.tb02418.x

Hall, W. H., Ramachandran, R., Narayan, S., Jani, A. B., & Vijayakumar, S. (2004). An electronic application for rapidly calculating Charlson comorbidity score. *BMC Cancer*, *4*(1), 94. https://doi.org/10.1186/1471-2407-4-94

Harju, E. (1988). Empty iron stores as a significant risk factor in abdominal surgery. *Journal of Parenteral and Enteral Nutrition*, *12*(3), 282–285. https://doi.org/10.1177/0148607188012003282

Harper, K., & Armelagos, G. (2010). The Changing Disease-Scape in the Third Epidemiological Transition. *International Journal of Environmental Research and Public Health*, *7*(2), 675–697. https://doi.org/10.3390/ijerph7020675

Harris, J. B., LaRocque, R. C., Qadri, F., Ryan, E. T., & Calderwood, S. B. (2012). Cholera. *The Lancet*, *379*(9835), 2466–2476. https://doi.org/10.1016/S0140-6736(12)60436-X

Harthoorn-Lasthuizen, E. J., van't Sant, P., Lindemans, J., & Langenhuijsen, M. M. A. C. (2000). Serum Transferrin Receptor and Erythrocyte Zinc Protoporphyrin in Patients with Anemia. *Clinical Chemistry*, *46*(5), 719–722. https://doi.org/doi.org/10.1093/clinchem/46.5.719

Hayes, E. B., Talbot, S. B., Matheson, E. S., Pressler, H. M., Hanna, A. B., & McCarthy, C. A. (1998). Health Status of Pediatric Refugees in Portland, Me. *Archives of Pediatrics & Adolescent Medicine*, *152*(6). https://doi.org/10.1001/archpedi.152.6.564

Health Quality Ontario. (2016). *Income and health: Opportunities to achieve health equity in Ontario.* https://www.deslibris.ca/ID/10050861

Hennigar, S. R., & McClung, J. P. (2016). Nutritional Immunity: Starving Pathogens of Trace Minerals. *American Journal of Lifestyle Medicine*, *10*(3), 170–173. https://doi.org/10.1177/1559827616629117

Henrich, J., Heine, S. J., & Norenzayan, A. (2010). The weirdest people in the world? *Behavioral and Brain Sciences*, *33*(2–3), 61–83. https://doi.org/10.1017/S0140525X0999152X

Hershko, C., Hoffbrand, A. V., Keret, D., Souroujon, M., Maschler, I., Monselise, Y., & Lahad, A. (2005). Role of autoimmune gastritis, Helicobacter pylori and celiac disease in refractory or unexplained iron deficiency anemia. *Haematologica*, *90*(5), 585–595.

Hershko, C., Ronson, A., Souroujon, M., Maschler, I., Heyd, J., & Patz, J. (2006). Variable hematologic presentation of autoimmune gastritis: Age-related progression from iron deficiency to cobalamin depletion. *Blood*, *107*(4), 1673–1679. https://doi.org/10.1182/blood-2005-09-3534

Hindré, T., Brüggemann, H., Buchrieser, C., & Héchard, Y. (2008). Transcriptional profiling of Legionella pneumophila biofilm cells and the influence of iron on biofilm formation. *Microbiology*, *154*(1), 30–41. https://doi.org/10.1099/mic.0.2007/008698-0

Hodgins, S., Dewailly, E., Chatwood, S., Bruneau, S., & Bernier, F. (1998). Iron-deficiency anemia in Nunavik: pregnancy and infancy. *International journal of circumpolar health*, *57 Suppl 1*, 135–140.

Horton, S., & Ross, J. (2003). The economics of iron deficiency. *Food Policy*, *28*(1), 51–75. https://doi.org/10.1016/S0306-9192(02)00070-2

Huber, B (1939) Siebrohrensystem unserer Baume und seine jahreszeitlichen Veranderungen. Jahrbücher für Wissenschaftliche Botanik 88:176–242.

Humphries, K. H., & van Doorslaer, E. (2000). Income-related health inequality in Canada. *Social Science & Medicine*, *50*(5), 663–671. https://doi.org/10.1016/S0277-9536(99)00319-6

Hunter, R. C., Asfour, F., Dingemans, J., Osuna, B. L., Samad, T., Malfroot, A., Cornelis, P., & Newman, D. K. (2013). Ferrous Iron Is a Significant Component of Bioavailable Iron in Cystic Fibrosis Airways. *MBio*, *4*(4). https://doi.org/10.1128/mBio.00557-13

Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. *The American Journal of Clinical Nutrition*, *91*(5), 1461S-1467S. https://doi.org/10.3945/ajcn.2010.28674F

Hussain Biology. (2018, September 10). *Iron Metabolism* [Video]. Youtube. https://www.youtube.com/watch?v=riUHZn0xWRA&list=PLYE4ac67Y66sgKe7Ewd81nEETjNPvCdcL&index=1

Iglesias-Osma, C., Gonzalez-Villaron, L., San Miguel, J. F., Caballero, M. D., Vasquez, L., & de Castro, S. (1995). Iron metabolism and fungal infections in patients with haematological malignancies. *Journal of Clinical Pathology*, *48*(3), 223–225. https://doi.org/doi.org/10.1136/jcp.48.3.223

Inhorn, M., & Brown, P. (1990). The Anthropology of Infectious Disease. *Annual Review of Anthropology, 19*, 89-117. Retrieved December 14, 2020, from http://www.jstor.org/stable/2155960

Irwin, A., Valentine, N., Brown, C., Loewenson, R., Solar, O., Brown, H., Koller, T., & Vega, J. (2006). The Commission on Social Determinants of Health: Tackling the Social Roots of Health Inequities. *PLoS Medicine*, *3*(6), e106. https://doi.org/10.1371/journal.pmed.0030106

Jablonski, N. G., & Chaplin, G. (2010). Human skin pigmentation as an adaptation to UV radiation. *Proceedings of the National Academy of Sciences*, *107*(Supplement_2), 8962–8968. https://doi.org/10.1073/pnas.0914628107

Johnson-Wimbley, T. D., & Graham, D. Y. (2011). Diagnosis and management of iron deficiency anemia in the 21st century. *Therapeutic Advances in Gastroenterology*, *4*(3), 177–184. https://doi.org/10.1177/1756283X11398736

Jonker, F. A. M., Calis, J. C. J., van Hensbroek, M. B., Phiri, K., Geskus, R. B., Brabin, B. J., & Leenstra, T. (2012). Iron Status Predicts Malaria Risk in Malawian Preschool Children. *PLoS ONE*, *7*(8), e42670. https://doi.org/10.1371/journal.pone.0042670

Joynson, D., Walker, D., Jacobs, A., & Dolby, A. (1972). Defect of cell-mediated immunity in patients with iron-deficiency anaemia. Lancet (London, England), 2(7786), 1058-1059.

Kortman, G. A. M., Boleij, A., Swinkels, D. W., & Tjalsma, H. (2012). Iron Availability Increases the Pathogenic Potential of Salmonella Typhimurium and Other Enteric Pathogens at the Intestinal Epithelial Interface. *PLoS ONE*, *7*(1), e29968. https://doi.org/10.1371/journal.pone.0029968

Kossiva, L., Soldatou, A., Gourgiotis, D. I., Stamati, L., & Tsentidis, C. (2013). Serum hepcidin: Indication of its role as an “acute phase” marker in febrile children. *Italian Journal of Pediatrics*, *39*(1), 25. https://doi.org/10.1186/1824-7288-39-25

Kumar, R. (2021). Impact of Societal Culture on Covid-19 Morbidity and Mortality across Countries. *Journal of Cross-Cultural Psychology*, *52*(7), 643–662. https://doi.org/10.1177/00220221211025100

Kumar, V. (2014). Robbins & Cotran Pathologic Basis of Disease. (9th ed., Robbins Pathology).

Kurekci, A. E., Atay, A. A., Sarici, S. U., Yesilkaya, E., Senses, Z., Okutan, V., & Ozcan, O. (2005). Is There a Relationship Between Childhood Helicobacter Pylori Infection and Iron Deficiency Anemia? *Journal of Tropical Pediatrics*, *51*(3), 166–169. https://doi.org/10.1093/tropej/fmi015

Larsen, C. S. (2003). Animal source foods and human health during evolution. Journal of Nutrition, 133:3893S-7.

Larsen, C. S. (1995). Biological Changes in Human Populations with Agriculture. *Annual Review of Anthropology*, 24, 185-213.

Le, C. H. H. (2016). The Prevalence of Anemia and Moderate-Severe Anemia in the US Population (NHANES 2003-2012). *PLOS ONE*, *11*(11), e0166635. https://doi.org/10.1371/journal.pone.0166635

Lee, J.-H., Wan, K.-L., Mohd-Adnan, A., & Gabaldón, T. (2012). Evolution of the ferritin family in vertebrates. *Trends in Evolutionary Biology*, *4*(1), 8–17.

Lerner, B., & Lerner, K. L. (2008). *Infectious diseases: in context*. Thomson Gale.

Li, R., Chen, X., Yan, H., Deurenberg, P., Garby, L., & Hautvast, J. G. (1994). Functional consequences of iron supplementation in iron-deficient female cotton mill workers in Beijing, China. *The American Journal of Clinical Nutrition*, *59*(4), 908–913. https://doi.org/10.1093/ajcn/59.4.908

Lightman, E., Mitchell, A., & Wilson, B. (2008). Findings. In *Poverty is making us sick: a comprehensive survey of income and health in Canada* (pp. 7–20). Essay, Wellesley Institute.

Lin, M.-H., Shu, J.-C., Huang, H.-Y., & Cheng, Y.-C. (2012). Involvement of Iron in Biofilm Formation by Staphylococcus aureus. *PLoS ONE*, *7*(3), e34388. https://doi.org/10.1371/journal.pone.0034388

Lind, T., Lönnerdal, B., Stenlund, H., Ismail, D., Seswandhana, R., Ekström, E.-C., & Persson, L.-Å. (2003). A community-based randomized controlled trial of iron and zinc supplementation

in Indonesian infants: Interactions between iron and zinc. *The American Journal of Clinical Nutrition*, *77*(4), 883–890. https://doi.org/10.1093/ajcn/77.4.883

Little, C., Alsen, M., Barlow, J., Naymagon, L., Tremblay, D., Genden, E., Trosman, S., Iavicoli, L., & van Gerwen, M. (2021). The Impact of Socioeconomic Status on the Clinical Outcomes of COVID-19; a Retrospective Cohort Study. *Journal of Community Health*, *46*(4), 794–802. https://doi.org/10.1007/s10900-020-00944-3

Lu, S.-H., & Dai, Y.-T. (2009). Normal body temperature and the effects of age, sex, ambient temperature and body mass index on normal oral temperature: A prospective, comparative study. *International Journal of Nursing Studies*, *46*(5), 661–668. https://doi.org/10.1016/j.ijnurstu.2008.11.006

Luke, D. A., & Homan, S. M. (n.d.). *Time and Change: Using Survival Analysis in Clinical Assessment and Treatment Evaluation*. 19.

Ma, I., Guo, M., Lau, C. K., Ramdas, Z., Jackson, R., & Naugler, C. (2019). Test volume data for 51 most commonly ordered laboratory tests in Calgary, Alberta, Canada. *Data in Brief*, *23*, 103748. https://doi.org/10.1016/j.dib.2019.103748

Ma, I., Guo, M., Muruve, D., Benediktsson, H., & Naugler, C. (2018). Sociodemographic associations with abnormal estimated glomerular filtration rate (eGFR) in a large Canadian city: A cross-sectional observation study. *BMC Nephrology*, *19*(1), 198. https://doi.org/10.1186/s12882-018-0991-5

Manor, O., Eisenbach, Z., Friedlander, Y., & Kark, J. D. (2004). Educational differentials in mortality from cardiovascular disease among men and women: The Israel Longitudinal Mortality Study. *Annals of Epidemiology*, *14*(7), 453–460. https://doi.org/10.1016/j.annepidem.2003.10.011

Marmot, M. (2015). *The Health Gap*. Bloomsbury.

Marx, J. (2002). Iron and infection: Competition between host and microbes for a precious element. *Best Practice & Research Clinical Haematology*, *15*(2), 411–426. https://doi.org/10.1053/beha.2002.0001

Mazess, R. B. (1975). *Biological Adaptation: Aptitudes and Acclimatization*. 10.

McKeown, R. E. (2009). The Epidemiologic Transition: Changing Patterns of Mortality and Population Dynamics. *American Journal of Lifestyle Medicine*, *3*(1_suppl), 19S-26S. https://doi.org/10.1177/1559827609335350

Mebrahtu, T., Stoltzfus, R. J., Chwaya, H. M., Jape, J. K., Savioli, L., Montresor, A., Albonico, M., & Tielsch, J. M. (2004). Low-Dose Daily Iron Supplementation for 12 Months Does Not Increase the Prevalence of Malarial Infection or Density of Parasites in Young Zanzibari Children. *The Journal of Nutrition*, *134*(11), 3037–3041. https://doi.org/10.1093/jn/134.11.3037

Meda, N., Dao, B., & Ouangré, A. (1998). HIV, maternal anemia and perinatal intervention using zidovudine. *International Journal of Gynecology & Obstetrics*, *61*(1), 65–66. https://doi.org/10.1016/S0020-7292(98)00016-2

Meghani, S., Buck, H., Dickson, V., Hammer, M., Rabelo-Silva, E. R., Clark, R., & Naylor, M. (2013). The Conceptualization and Measurement of Comorbidity: A Review of the Interprofessional Discourse. *Nursing Research and Practice*, *2012*, 1–10. https://doi.org/10.1155/2013/192782

Mendoza, E., Duque, X., Hernández Franco, J. I., Reyes Maldonado, E., Morán, S., Martínez, G., Salinas Rodríguez, A., & Martínez, H. (2019). Association between Active H. pylori Infection and Iron Deficiency Assessed by Serum Hepcidin Levels in School-Age Children. *Nutrients*, *11*(9), 2141. https://doi.org/10.3390/nu11092141

Menendez, C., Kahigwa, E., Hirt, R., Vounatsou, P., Aponte, J. J., Font, F., Acosta, C. J., Schellenberg, D. M., Galindo, C. M., Kimario, J., Urassa, H., Brabin, B., Smith, T. A., Kitua, A. Y., Tanner, M., & Alonso, P. L. (1997). Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *The Lancet*, *350*(9081), 844–850. https://doi.org/10.1016/S0140-6736(97)04229-3

Miller, J. L. (2013). Iron Deficiency Anemia: A Common and Curable Disease. *Cold Spring Harbor Perspectives in Medicine*, *3*(7), a011866–a011866. https://doi.org/10.1101/cshperspect.a011866

Milman, N., Pedersen, A. N., Ovesen, L., & Schroll, M. (2008). Hemoglobin concentrations in 358 apparently healthy 80-year-old Danish men and women. Should the reference interval be adjusted for age? *Aging Clinical and Experimental Research*, *20*(1), 8–14. https://doi.org/10.1007/BF03324741

Mitchell, B. L. (2018). Sickle Cell Trait and Sudden Death. *Sports Medicine - Open*, *4*(1), 19. https://doi.org/10.1186/s40798-018-0131-6

Mitra, A. K., Akramuzzaman, S. M., Fuchs, G. J., Rahman, M. M., & Mahalanabis, D. (1997). Community and International Nutrition Long-Term Oral Supplementation with Iron Is Not Harmful for Young Children in a Poor Community of Bangladesh 1,2. In *J. Nutr* (Vol. 127, pp. 1451–1455). https://academic.oup.com/jn/article-abstract/127/8/1451/4728965

Monga, M., Walia, V., Gandhi, A., Chandra, J., & Sharma, S. (2010). Effect of iron deficiency anemia on visual evoked potential of growing children. *Brain and Development*, *32*(3), 213–216. https://doi.org/10.1016/j.braindev.2009.02.009

Monsen, E., R., Hallberg, L., Layrisse, M., Hegsted, M., D., Cook, J., D., Mertz, W., & Finch, C., A. (1978). Estimation of available dietary iron. *The American Journal of Clinical Nutrition*, *31*, 134–141.

National Heart, Lung, and Blood Institute. (n.d.). *Iron-Deficiency Anemia*. https://www.nhlbi.nih.gov/health-topics/iron-deficiency-anemia

Naugler, C., Zhang, J., Henne, D., Woods, P., & Hemmelgarn, B. R. (2013). Association of vitamin D status with socio-demographic factors in Calgary, Alberta: An ecological study using Census Canada data. *BMC Public Health*, *13*(1), 316. https://doi.org/10.1186/1471-2458-13-316

Navaneelan, T. (2016). Deaths involving sepsis in Canada. *Health at a Glance*, *82*, 11.

Nelson, D. A., Deuster, P. A., Carter, R., Hill, O. T., Wolcott, V. L., & Kurina, L. M. (2016). Sickle Cell Trait, Rhabdomyolysis, and Mortality among U.S. Army Soldiers. *New England Journal of Medicine*, *375*(5), 435–442. https://doi.org/10.1056/NEJMoa1516257

Nilsson-Ehle, H., Jagenburg, R., Landahl, S., & Svanborg, A. (2000). Blood haemoglobin declines in the elderly: Implications for reference intervals fromage 70 to 88: Hb declines from age 70 to 88. *European Journal of Haematology*, *65*(5), 297–305. https://doi.org/10.1034/j.1600-0609.2000.065005297.x

Nyakeriga, A. M., Troye-Blomberg, M., Dorfman, J. R., Alexander, N. D., Bäck, R., Kortok, M., Chemtai, A. K., Marsh, K., & Williams, T. N. (2004). Iron Deficiency and Malaria among Children Living on the Coast of Kenya. *The Journal of Infectious Diseases*, *190*(3), 439–447. https://doi.org/10.1086/422331

Pasricha, S.-R., & Drakesmith, H. (2016). Iron Deficiency Anemia. *Hematology/Oncology Clinics of North America*, *30*(2), 309–325. https://doi.org/10.1016/j.hoc.2015.11.003

Pfeiffer, C. M., & Looker, A. C. (2017). Laboratory methodologies for indicators of iron status: Strengths, limitations, and analytical challenges. *The American Journal of Clinical Nutrition*, *106*(Supplement 6), 1606S-1614S. https://doi.org/10.3945/ajcn.117.155887

Pietrangelo, A. (2015). Pathogens, Metabolic Adaptation, and Human Diseases—An Iron-Thrifty Genetic Model. *Gastroenterology*, *149*(4), 834–838. https://doi.org/10.1053/j.gastro.2015.08.003

Porta, M. (2014). Endemic Disease. A Dictionary of Epidemiology, A Dictionary of Epidemiology.

Portugal, S., Drakesmith, H., & Mota, M. (2011). Superinfection in malaria: Plasmodium shows its iron will. *European Molecular Biology Organization*, *12*, 1233–1242. https://doi.org/10.1038/embor.2011.213

Poskitt, E. M. E. (2003). Early history of iron deficiency. *British Journal of Haematology*, *122*, 544–562. https://doi.org/10.1046/j.1365-2141.2003.04529.x

Pottie, K., Greenaway, C., Feightner, J., Welch, V., Swinkels, H., Rashid, M., Narasiah, L., Kirmayer, L. J., Ueffing, E., MacDonald, N. E., Hassan, G., McNally, M., Khan, K., Buhrmann, R., Dunn, S., Dominic, A., McCarthy, A. E., Gagnon, A. J., Rousseau, C., … coauthors of the Canadian Collaboration for Immigrant and Refugee Health. (2011). Evidence-based clinical guidelines for immigrants and refugees. *Canadian Medical Association Journal*, *183*(12), E824–E925. https://doi.org/10.1503/cmaj.090313

Powers, J. M., & Buchanan, G. R. (2014). Diagnosis and Management of Iron Deficiency Anemia. *Hematology/Oncology Clinics of North America*, *28*(4), 729–745. https://doi.org/10.1016/j.hoc.2014.04.007

Quan, H., Li, B., Couris, C. M., Fushimi, K., Graham, P., Hider, P., Januel, J.-M., & Sundararajan, V. (2011). Updating and Validating the Charlson Comorbidity Index and Score for Risk Adjustment in Hospital Discharge Abstracts Using Data From 6 Countries. *American Journal of Epidemiology*, *173*(6), 676–682. https://doi.org/10.1093/aje/kwq433

Raisinghani, N., Kumar, S., Acharya, S., Gadegone, A., & Pai, V. (2019). Does aging have an impact on hemoglobin? Study in elderly population at rural teaching hospital. *Journal of Family Medicine and Primary Care*, *8*(10), 3345. https://doi.org/10.4103/jfmpc.jfmpc_668_19

Report summary – The Direct Economic Burden of Socioeconomic Health Inequalities in Canada: An Analysis of Health Care Costs by Income Level. (2016). *Health Promotion and Chronic Disease Prevention in Canada*, *36*(6), 118–119. https://doi.org/10.24095/hpcdp.36.6.03

Riedel, S. (2005). Edward Jenner and the History of Smallpox and Vaccination. *Baylor University Medical Center Proceedings*, *18*(1), 21–25. https://doi.org/10.1080/08998280.2005.11928028

Rishi, G., Wallace, D. F., & Subramaniam, V. N. (2015). Hepcidin: Regulation of the master iron regulator. *Bioscience Reports*, *35*(3), e00192. https://doi.org/10.1042/BSR20150014

Ross, C. E., & Wu, C. (1995). The Links Between Education and Health. *American Sociological Review*, *60*(5), 719. https://doi.org/10.2307/2096319

Salive, M. E., Cornoni-Huntley, J., Guralnik, J. M., Phillips, C. L., Wallace, R. B., Ostfeld, A. M., & Cohen, H. J. (1992). Anemia and Hemoglobin Levels in Older Persons: Relationship with Age, Gender, and Health Status. *Journal of the American Geriatrics Society*, *40*(5), 489–496. https://doi.org/10.1111/j.1532-5415.1992.tb02017.x

Sapolsky, R. M. (2004). Social Status and Health in Humans and Other Animals. *Annual Review of Anthropology*, *33*(1), 393–418. https://doi.org/10.1146/annurev.anthro.33.070203.144000

Sarker, S. A., Mahmud, H., Davidsson, L., Alam, N. H., Ahmed, T., Alam, N., Salam, M. A., Beglinger, C., Gyr, N., & Fuchs, G. J. (2008). Causal Relationship of Helicobacter pylori With Iron-Deficiency Anemia or Failure of Iron Supplementation in Children. *Gastroenterology (New York, N.Y. 1943)*, *135*(5), 1534–1542.

Sazawal, S., Black, R. E., Ramsan, M., Chwaya, H. M., Stoltzfus, R. J., Dutta, A., Dhingra, U., Kabole, I., Deb, S., Othman, M. K., & Kabole, F. M. (2006). Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: Community-based, randomised, placebo-controlled trial. *The Lancet*, *367*(9505), 133–143. https://doi.org/10.1016/S0140-6736(06)67962-2

Schmaier, A.H. & Petruzzelli, L.M. (2003) Hematology for Medical Students, Lippincott Williams & Wilkins: Philadelphia, PA, pp. 35-38.

Schneiderman, N., Ironson, G., & Siegel, S. D. (2005). Stress and Health: Psychological, Behavioral, and Biological Determinants. *Annual Review of Clinical Psychology*, *1*(1), 607–628. https://doi.org/10.1146/annurev.clinpsy.1.102803.144141

Scrimshaw, N. S., & SanGiovanni, J. P. (1997). Synergism of nutrition, infection, and immunity: An overview. *The American Journal of Clinical Nutrition*, *66*(2), 464S-477S. https://doi.org/10.1093/ajcn/66.2.464S

Scully, J. L. (2004). What is a disease?: Disease, disability and their definitions. *EMBO Reports*, *5*(7), 650–653. https://doi.org/10.1038/sj.embor.7400195

Segat, L., Pontillo, A., Milanese, M., Tossi, A., & Crovella, S. (2008). Evolution of the hepcidin gene in primates. *BMC Genomics*, *9*(1), 120. https://doi.org/10.1186/1471-2164-9-120

Selye, H. (1976). Forty years of stress research: Principal remaining problems and misconceptions. *Canadian Medical Association Journal*, *115*, 53–56.

Senga, E. L., Harper, G., Koshy, G., Kazembe, P. N., & Brabin, B. J. (2011). Reduced risk for placental malaria in iron deficient women. *Malaria Journal*, *10*(1), 47. https://doi.org/10.1186/1475-2875-10-47

Shavelle, R. M., MacKenzie, R., & Paculdo, D. R. (2012). Anemia and mortality in older persons: Does the type of anemia affect survival? *International Journal of Hematology*, *95*(3), 248–256. https://doi.org/10.1007/s12185-012-1007-z

Shayeghi, M., Latunde-Dada, G., Oakhill, J., Laftah, A., Takeuchi, K., Halliday, N., . . . Mckie, A. (2005). Identification of an intestinal heme transporter. *Cell*, 122(5), 789-801.

Silventoinen, K. (2002). Health inequalities by education and age in four Nordic countries, 1986 and 1994. *Journal of Epidemiology & Community Health*, *56*(4), 253–258. https://doi.org/10.1136/jech.56.4.253

Skikne, B., & Hershko, C. (2012). Iron Deficiency. In *Iron Physiology and Pathophysiology in Humans* (pp. 251–282). Humana Press.

Smith, A., Hendrikse, R., Harrison, C., Hayes, R., & Greenwood, B. (1989). The effects on malaria of treatment of iron deficiency anaemia with oral iron in Gambian children. *Annals of Tropical Paediatrics*, *9*(1), 17–23. https://doi.org/10.1080/02724936.1989.11748589

Spottiswoode, N., Duffy, P. E., & Drakesmith, H. (2014). Iron, anemia and hepcidin in malaria. *Frontiers in Pharmacology*, *5*. https://doi.org/10.3389/fphar.2014.00125

Sprent, J. F. A. (1969). Helminth ‘‘zoonoses’’: an analysis. *Helminthologia Abstracts*, 38, 333–351.

Srikantia, S. G., Prasad, S., Bhaskaram, C., & Krishnamachari, K. A. V. R. (1976). Anemia and the immune response. *The Lancet*.

Statistics Canada. (2012a). *Calgary, Alberta (Code 4806016) and Alberta (Code 48)* (table). *Census Profile*. 2011 Census. Statistics Canada Catalogue no. 98-316-XWE. Ottawa, Ontario. Released October 24, 2012.

Statistics Canada. (2012b). Focus on Geography Series, 2011 Census. Statistics Canada Catalogue no. 98-310-XWE2011004. Ottawa, Ontario. Analytical products, 2011 Census. Released October 24, 2012.

Statistics Canada. (2012c). The Canadian Population in 2011: Age and Sex. Catalogue no. 98-311-X2011001. Ottawa, Ontario. Released May 12, 2021.

Statistics Canada. (2013). National Household Survey Profile for Calgary, CY. 2011 National Household Survey. Statistics Canada Catalogue no. 99-004-XWE.

Statistics Canada. (2015, November 27). *NHS Profile -Place name search results*. NHS Profile. https://www12.statcan.gc.ca/nhs-enm/2011/dp-pd/prof/search-recherche/frm_res.cfm?Lang=E&SearchText=calgary&SearchType=Begins&SearchPR=01&TABID=1&Geo1=CMA&Code1=825#tabs1

Statistics Canada. (2020, November 26). *Deaths, by cause: Chapter 1: Certain infectious and parasitic diseases (A00:B99)*. https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310014101&pickMembers%5B0%5D=2.1&pickMembers%5B1%5D=3.1&cubeTimeFrame.startYear=2011&cubeTimeFrame.endYear=2019&referencePeriods=20110101%2C20190101

Stefanova, D., Arezes, J., Michels, K., Dillon, B., Horwitz, M., Mehrad, B., Bulut, Y., Ganz, T., & Nemeth, E. (2016). Hepcidin Protects Against Extracellular Infections By Eliminating Non-Transferrin-Bound Iron. *Blood*, *128*(22), 260–260. https://doi.org/10.1182/blood.V128.22.260.260

Stoltzfus, R. J., Kvalsvig, J. D., Chwaya, H. M., Montresor, A., Albonico, M., Tielsch, J. M., Savioli, L., & Pollitt, E. (2001). Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: Double blind, placebo-controlled study. *BMJ*, *323*(7326), 1389–1389. https://doi.org/10.1136/bmj.323.7326.1389

Strotz, L. C., Simões, M., Girard, M. G., Breitkreuz, L., Kimmig, J., & Lieberman, B. S. (2018). Getting somewhere with the Red Queen: Chasing a biologically modern definition of the hypothesis. *Biology Letters*, *14*(5), 20170734. https://doi.org/10.1098/rsbl.2017.0734

Sultan, S. E., & Stearns, S. C. (2005). Environmentally Contingent Variation: Phenotypic Plasticity and Norms of Reaction. *Variation*, 30.

Tansarli, G. S., Karageorgopoulos, D. E., Kapaskelis, A., Gkegkes, I., & Falagas, M. E. (2013). Iron deficiency and susceptibility to infections: Evaluation of the clinical evidence. *European Journal of Clinical Microbiology & Infectious Diseases*, *32*(10), 1253–1258. https://doi.org/10.1007/s10096-013-1877-x

Thibault, H., Galan, P., Selz, F., Preziosi, P., Olivier, C., Badoual, J., & Hercberg, S. (1993). The immune response in iron-deficient young children: Effect of iron supplementation on cell-mediated immunity. *European Journal of Pediatrics*, *152*(2), 120–124. https://doi.org/10.1007/BF02072487

Timiras, M.-L., & Brownstein, H. (1987). Prevalence of Anemia and Correlation of Hemoglobin with Age in a Geriatric Screening Clinic Population. *Journal of the American Geriatrics Society*, *35*(7), 639–643. https://doi.org/10.1111/j.1532-5415.1987.tb04340.x

Touchie, C., Chief Medical Education Advisor. (2013). *Report of the Incidence and Prevalence of Diseases and Other Health Related Issues in Canada*.

Turk, E. E. (2010). Hypothermia. *Forensic Science, Medicine, and Pathology*, *6*(2), 106–115. https://doi.org/10.1007/s12024-010-9142-4

Valderas, J. M., Starfield, B., Sibbald, B., Salisbury, C., & Roland, M. (2009). Defining Comorbidity: Implications for Understanding Health and Health Services. *The Annals of Family Medicine*, *7*(4), 357–363. https://doi.org/10.1370/afm.983

Van Valen, L. (1973). A new evolutionary law. *Evolutionary Theory, 1*, 1-30.

Vokurka, M., Krijt, J., Šulc, K., & Ne, E. (2006). *Hepcidin mRNA Levels in Mouse Liver Respond to Inhibition of Erythropoiesis*. *55*, 8.

Walter, E. J., & Carraretto, M. (2016). The neurological and cognitive consequences of hyperthermia. *Critical Care*, *20*(1), 199. https://doi.org/10.1186/s13054-016-1376-4

Wander, K., Shell-Duncan, B., & Brindle, E. (2017). Lower incidence of respiratory infections among iron-deficient children in Kilimanjaro, Tanzania. *Evolution, Medicine, and Public Health*, *2017*(1), 109–119. https://doi.org/10.1093/emph/eox010

Wander, K., Shell-Duncan, B., & McDade, T. W. (2009). Evaluation of iron deficiency as a nutritional adaptation to infectious disease: An evolutionary medicine perspective. *American Journal of Human Biology*, *21*(2), 172–179. https://doi.org/10.1002/ajhb.20839

Ward, M. G., Sparrow, M. P., & Roblin, X. (2018). Therapeutic drug monitoring of vedolizumab in inflammatory bowel disease: Current data and future directions. *Therapeutic Advances in Gastroenterology*, *11*, 1756284818772786–1756284818772786.

Wei, C., Zhou, J., Huang, X., & Li, M. (2008). Effects of psychological stress on serum iron and erythropoiesis. *International Journal of Hematology*, *88*(1), 52–56. https://doi.org/10.1007/s12185-008-0105-4

Weinberg, E. D. (1975). Nutritional immunity. Host's attempt to withhold iron from microbial invaders. *Journal of the American Medical Association*, *231*(1), 39–41. https://doi.org/10.1001/jama.231.1.39

WHO/UNICEF/UNU. (2001). Iron deficiency anemia: assessment, prevention, and control. World Health Organization, Geneva: Switzerland.
Wiley, A., Allen, J., & Oxford University Press, publisher. (2017). *Medical anthropology: A biocultural approach.*

Willows, N. D., Dewailly, É., & Gray-Donald, K. (2000). Anemia and Iron Status in Inuit Infants from Northern Quebec. *Canadian Journal of Public Health*, *91*(6), 407–410. https://doi.org/10.1007/BF03404818

Wilson, M. W., DeCaro, A. J. (2013, February 14-17). *Defining, operationalizing, and assessing the relationship between stress and health in contemporary Tanzanian mothers and children.* [Conference presentation]. AAPA 2013 Annual Meeting, Knoxville, TN, United States.

Wisaksana, R., Sumantri, R., Indrati, A. R., Zwitser, A., Jusuf, H., de Mast, Q., van Crevel, R., & van der Ven, A. (2011). Anemia and iron homeostasis in a cohort of HIV-infected patients in Indonesia. *BMC Infectious Diseases*, *11*(1), 213. https://doi.org/10.1186/1471-2334-11-213

Wolfe, E. L., Barger, & A. C., Benison, S. (2000). *Walter B. Cannon, Science and Society.* Boston Medical Library.

World Health Organization. (2013). 10 facts on noncommunicable diseases. Retrieved from: https://www.who.int/features/factfiles/noncommunicable_diseases/en/

World Health Organization. (2016). *The top 10 causes of death*. Retrieved from: https://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death

World Health Organization. (2018). *Antibiotic resistance*. Retrieved from: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

World Health Organization. (2019). *Micronutrient deficiencies*. Retrieved from: https://www.who.int/nutrition/topics/ida/en/

World Health Organization. (2020a). *Constitution*. Retrieved from: https://www.who.int/about/who-we-are/constitution

World Health Organization. (2020b). *Vector-borne diseases*. Retrieved from: https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases

World Health Organization. (2021). *Malaria*. https://www.who.int/news-room/fact-sheets/detail/malaria

Xu, J.-J., Fu, S.-J., & Fu, C. (2019). Physiological and behavioral stress responses to predators are altered by prior predator experience in juvenile qingbo (*Spinibarbus sinensis*). *Biology Open*, *8*(5), bio041012. https://doi.org/10.1242/bio.041012

Zhang, A.-S., & Enns, C. A. (2009). Molecular mechanisms of normal iron homeostasis. *Hematology*, *2009*(1), 207–214. https://doi.org/10.1182/asheducation-2009.1.207

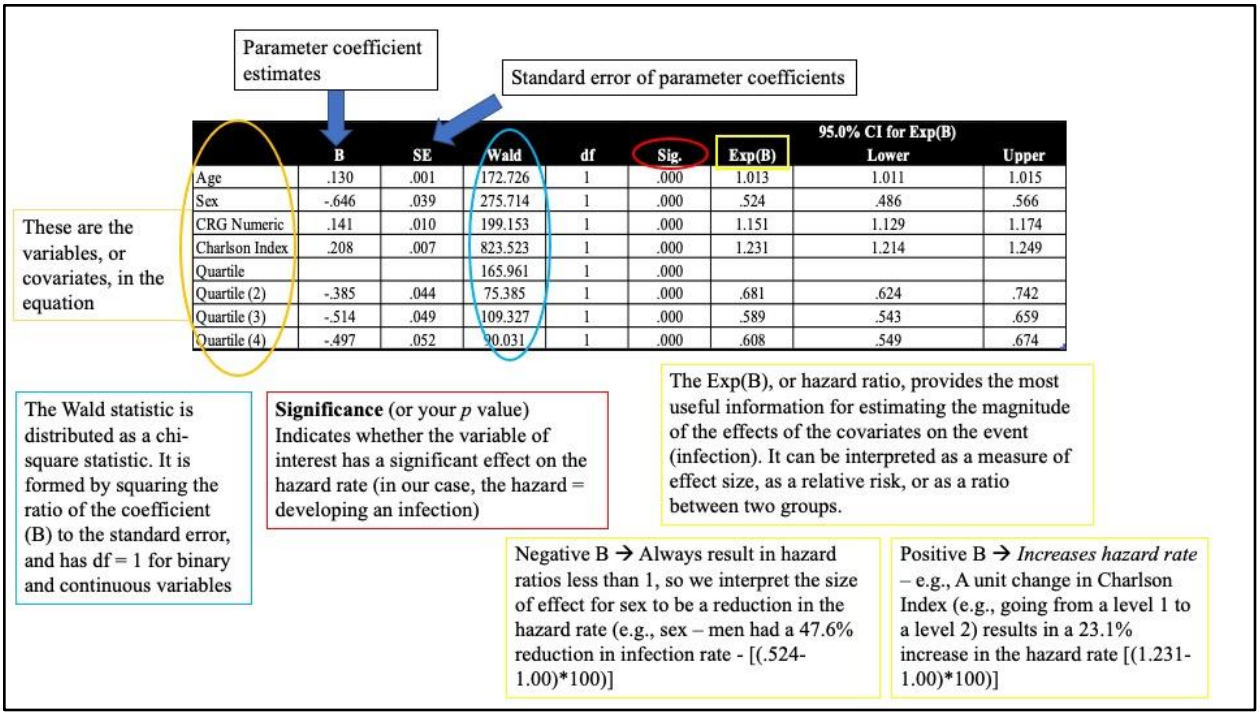
Zhang, L., & Gallo, R. L. (2016). Antimicrobial peptides. *Current Biology*, *26*(1), R14–R19. https://doi.org/10.1016/j.cub.2015.11.017

Zimmermann, M. B., & Hurrell, R. F. (2007). Nutritional iron deficiency. *The Lancet*, *370*(9586), 511–520. https://doi.org/10.1016/S0140-6736(07)61235-5

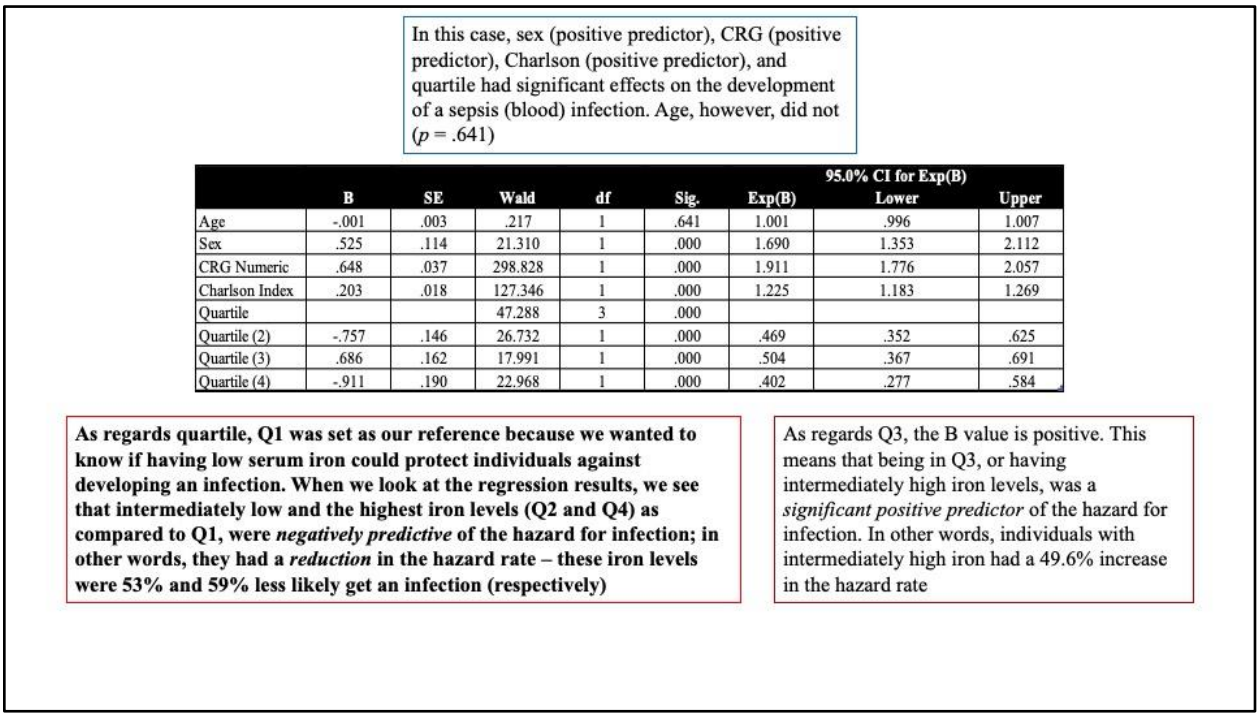
Appendix

How to Interpret Cox Regression Outputs.

The following images are included to help explain the various columns included in a cox regression output and how they are interpreted in the overarching conclusion of the results.



	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	.130	.001	172.726	1	.000	1.013	1.011	1.015
Sex	-.646	.039	275.714	1	.000	.524	.486	.566
CRG Numeric	.141	.010	199.153	1	.000	1.151	1.129	1.174
Charlson Index	.208	.007	823.523	1	.000	1.231	1.214	1.249
Quartile			165.961	1	.000			
Quartile (2)	-.385	.044	75.385	1	.000	.681	.624	.742
Quartile (3)	-.514	.049	109.327	1	.000	.589	.543	.659
Quartile (4)	-.497	.052	90.031	1	.000	.608	.549	.674



	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	-.001	.003	.217	1	.641	1.001	.996	1.007
Sex	.525	.114	21.310	1	.000	1.690	1.353	2.112
CRG Numeric	.648	.037	298.828	1	.000	1.911	1.776	2.057
Charlson Index	.203	.018	127.346	1	.000	1.225	1.183	1.269
Quartile			47.288	3	.000			
Quartile (2)	-.757	.146	26.732	1	.000	.469	.352	.625
Quartile (3)	.686	.162	17.991	1	.000	.504	.367	.691
Quartile (4)	-.911	.190	22.968	1	.000	.402	.277	.584