LARVIE, DOREEN Y., M.S. Interrelationships among Selenium, Iron Status Biomarkers and Body Weight in Young Adults. (2019)
Directed by Dr. Seth Armah. 99 pp.

Selenium deficiency is considered a risk factor for anemia of chronic inflammation, which is mediated by hepcidin. However, there are few studies providing evidence of the role of hepcidin in this relationship. In this study we investigated the interrelationships among selenium biomarkers, hepcidin concentration, and iron status among individuals with obesity compared to their normal weight counterparts, since obesity presents with low-grade chronic inflammation. A total of 59 college students (18-49 years) consisting of 27 individuals with normal weight and 32 individuals with overweight/obesity were recruited for this study. Fasting blood samples were collected for the analysis of iron status biomarkers, plasma selenoproteins (glutathione peroxidase (GPX) activity and selenoprotein P) and plasma hepcidin concentration. Subjects completed 3-day dietary records to determine average daily nutrient intakes. Regression analysis, independent t-test and Wilcoxon rank sum tests were used to determine the relationships among variables. Statistical significance was set at $p \leq 0.05$. There were no significant differences in nutrient intakes between subjects with overweight/obesity and those with normal weight ($P>0.05$). Selenoprotein P concentration, GPX activity and iron status biomarkers (serum iron, transferrin saturation and hemoglobin concentration) were lower among individuals with overweight/obesity compared with individuals with normal weight, but these differences were not significant ($p>0.05$). Regression analysis showed that the relationship between hepcidin concentration and transferrin saturation depended on body weight status (p for interaction = 0.036). Plasma GPX activity ($\beta=-0.018$, p =

0.008) and selenoprotein P concentration (β=-1.24, $p = 0.03$) were inversely associated with hepcidin concentration. In conclusion, our study showed an inverse association between selenium status and hepcidin concentration which supports the proposed role of hepcidin as mediator between selenium and iron status and also highlights the importance of selenium in addressing inflammation-related anemia. Intervention studies on the effect of selenium supplementation on hepcidin concentration and iron status in individuals with anemia of inflammation are needed to support these findings.

INTERRELATIONSHIPS AMONG SELENIUM, IRON STATUS BIOMARKERS

AND BODY WEIGHT IN YOUNG ADULTS

by

Doreen Y Larvie

A Thesis Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
2019

Approved by

____
Committee Chair

© 2019 Doreen Y Larvie

I dedicate this thesis to my family who have been a pillar of support, encouraged me to persevere and taught me the value of hard work.

APPROVAL PAGE

This thesis written by DOREEN Y LARVIE has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro

Committee Chair_________________________

Committee Members__________________________

____

____
Date of Acceptance by Committee

____
Date of Final Oral Examination

ACKNOWLEDGEMENTS

I am beholden to everyone who assisted in diverse ways and supported me to make this thesis a reality. I am indebted to Dr. Seth Armah who provided me invaluable feedback and mentorship throughout this work. I am also appreciative to my graduate committee members, Dr. Jigna Dharod and Dr. Keith Erikson who provided insightful inputs into this work. I am grateful to Dr. George Donati at the Chemistry department, Wake Forest University who helped with the use of the ICP-MS/MS for sample analysis. I am fortunate to have had Paula Cooney, the departmental research coordinator and Zachary Kincaid as phlebotomists during data collection. To research assistants, Jeanne Doherty, Sarah White, Nebyou Mulugeta, Sunshine Alvarez and Savannah Mckee who lent their time during study recruitment and data collection, I am grateful. Thank you all so much.

TABLE OF CONTENTS

Page

LIST OF TABLES vii

LIST OF FIGURES viii

CHAPTER

I. INTRODUCTION 1

Main Objective 3
Specific Objectives 3

II. LITERATURE REVIEW 5

Dietary Sources and Metabolism of Iron 5
The Role of Hepcidin in Iron Regulation 7
Hepcidin and Iron Status 8
Functions of Iron 9
DNA Replication 9
Energy Metabolism 10
Immunity 11
Other Functions 11
Iron Deficiency and Anemia 12
Inflammation 14
Types, Causes and Sources of Inflammation 14
Inflammation and Body Composition 15
Obesity, Inflammation and Iron Status 17
Diet and Inflammation 18
Selenium as a Dietary Antioxidant 18
Selenium and Inflammation 21
Selenium and Iron Status 24
Conclusion 27

III. RESEARCH ARTICLE 28

Introduction 28
Materials and Methods 30
Study Participants and Recruitment 30
Study Design 31
Anthropometry 32

Dietary and Nutrient Intake 33
Sample Analyses...33
Statistical Analysis...34
Results...37
Discussion..50
Limitations..54
Conclusion 55

REFERENCES 56

APPENDIX A. ADDITIONAL RESULTS..68

APPENDIX B. CONSENT FORM 71

APPENDIX C. SCREENING FORM 78

APPENDIX D. DIETARY RECORD INSTRUCTIONS 80

APPENDIX E. DIETARY RECORD FORM 82

LIST OF TABLES

Page

Table 3.1 Background Data of Study Participants 37

Table 3.2 Daily Nutrient Intake according to BMI Category 38

Table 3.3 Anthropometric and Biochemical Data
according to BMI Category 40

Table 3.4 Correlation among Hepcidin, Iron Status Biomarkers
and Selenoproteins in Study Participants 42

Table 3.5 Association among Hepcidin, Iron Status Biomarkers
and Selenoproteins in Study Participants 45

Table 3.6 Association between Selenoproteins and BMI
among Study Participants 47

Table 3.7 Association between Transferrin Saturation
and Hepcidin in Study Participants 49

Table A.1 Anthropometric, Biochemical, and Nutrient Data
according to Gender 68

Table A.2 Anthropometric, Biochemical, and Nutrient Data
according to Ethnicity 69

LIST OF FIGURES

Page

Figure 3.1 Study Participant Recruitment..36

CHAPTER I

INTRODUCTION

Inflammation is a biological response of the immune system to infection. There is increasing evidence that a self-destructive inflammatory action of the immune system is a secondary component of metabolic disturbance(s) in the development of chronic disease (1,2). Iron deficiency anemia remains one of the micronutrient deficiencies which can compromise immunity, increase fatigue and result in neurological deficits (3). Anemia of inflammation (AI), another form of anemia that results from chronic conditions involving inflammatory activity such as cancer, congestive heart failure, chronic kidney disease and aging independent of disease, is an emerging health concern (4). Changes associated with AI may include alterations in iron metabolism, erythrocyte life span and production and lowered transferrin saturation. Macrophage iron stores however remain replete due to its sequestration to prevent availability of iron to pathogens (5).

Apart from the chronic conditions mentioned above, inflammation is also present in a chronic low-grade form among individuals with obesity. In obesity, macrophages and immune system cells invade the adipose tissue in response to fat accumulation leading to the production of pro-inflammatory cytokines including (Interleukin 6) IL-6, (C-reactive protein) CRP, (Tumor necrosis factor alpha) TNF-α and (Nuclear factor kappa B) NF-κB (6). The production of pro-inflammatory cytokines and a consequent destruction of

adipocytes furthers a cascade of the inflammatory response that increases the risk of chronic disease (6). With the increased production of the pro-inflammatory cytokine IL-6, the synthesis of the iron regulatory hormone, hepcidin, is upregulated leading to a reduction in circulating iron and an increase in the storage of iron in macrophages and hepatocytes (7).

Hepcidin is a 25-amino acid (disulfide-rich) peptide that has been implicated to play a critical role in iron metabolism by acting as a signaling molecule and in immunity due to its antimicrobial properties (8,9). Hepcidin acts by binding to the iron transporter (ferroportin) resulting in its internalization (10). Its concentration increases in response to high iron stores and inflammation and decreases with anemia and oxidative stress. High levels of hepcidin have been associated with symptoms such as iron storage in cells of the reticuloendothelial system, reduced intestinal iron absorption and a decrease in circulating iron similar to what is observed in AI (11).

In view of the relationships among hepcidin, inflammation and iron status, studies on the potential role of antioxidant/anti-inflammatory nutrients in regulating iron status are necessary. Selenium (Se), an essential trace mineral influences inflammatory response by suppressing the release of pro-inflammatory cytokines through the antioxidant action of selenoproteins, such as glutathione peroxidase (GPX), selenoprotein P (SEPP1), and selenoprotein W (12). Thus, studies have shown suboptimal Se status during inflammation. The process by which selenium influences inflammation is subject to various levels of control in cells directly involved in the inflammatory response. In macrophages supplemented with Se, anti-inflammatory proteins were upregulated and in

chicken lymphocytes, Se pretreatment prior to hydrogen peroxide treatment up-regulated selenoprotein W and lowered the expression of TNF-α and NF-κB compared to the non-treated control cells (13,14). In hemodialysis and alcoholic liver patients, Se supplementation resulted in the down-regulation of pro-inflammatory cytokines (15,16). Despite increasing knowledge of its role in addressing inflammation, no study has investigated how selenium influences the chronic inflammation found in obesity and its relationship with hepcidin and iron status.

Main Objective

The aim of this study was to investigate the associations among selenium status, hepcidin concentration and iron status biomarkers in individuals with overweight/obesity compared to normal weight controls.

Specific Objectives

1. To compare selenium (GPX activity and selenoprotein P) and iron status biomarkers between individuals with overweight/obesity and those with normal weight adjusting for selenium intake.

Hypothesis: GPX activity, selenoprotein P concentration, transferrin saturation, serum iron and hemoglobin will be higher in individuals with normal weight compared to those with overweight/obesity

2. To investigate the relationship between the two selenium biomarkers and hepcidin concentration among study participants.

Hypothesis: Hepcidin concentration will be inversely associated with GPX activity and selenoprotein P concentration among study participants

3. To determine the associations among GPX activity and selenoprotein P and markers of circulating and functional iron in individuals with overweight/obesity and those with normal weight.

Hypothesis: GPX activity and selenoprotein P concentration will both be positively associated with transferrin saturation, serum iron and hemoglobin

CHAPTER II

LITERATURE REVIEW

Dietary Sources and Metabolism of Iron

Dietary iron comes in the form of heme and non-heme iron. Heme iron is found in poultry, red meat, liver and fish sources. Non-heme iron is found in most foods but predominantly in plant sources or their fortified alternatives including legumes, green leafy vegetables, seeds, cereals, breakfast bars, bread, nuts and whole grains (17,18). The two forms of iron are absorbed differently. Heme iron is tightly stored in a protoporphyrin ring and better absorbed by an energy mediated process. Non-heme iron is less tightly stored as ferritin and catalytic centers of proteins hence easily influenced by dietary components in food (17,19). As a result, food containing iron inhibitors and /or enhancers will impact the amount of non-heme iron absorbed (17).

Iron absorption is regulated by a person's iron status as low body iron stores will enhance intestinal iron uptake compared to an iron replete individual. The proximal part of the small intestine is adapted for iron absorption. Iron in the intestinal lumen needs to cross the apical and basolateral membranes of enterocytes for transport into the blood stream and target organs. Non-heme iron ($Fe3+$) crosses the apical membrane via the divalent metal transporter 1 (DMT1). For this to happen, it is first reduced to $Fe2+$ by duodenal cytochrome B (DcytB). Absorbed iron can be stored as ferritin or transported across the basolateral membrane via ferroportin (FPN1), an iron efflux protein.

On the other hand, the absorption of heme iron is not fully understood. Heme iron (Fe^{2+}) is presumed to be transported intact as a protoporphyrin complex across the apical membrane via endocytosis. Iron is then released from heme by heme oxygenases and stored as ferritin or transported via FPN1. Inside the enterocytes, heme and non-heme iron form part of a common iron pool (19,20). When iron needs are low, iron is stored in ferritin, a spherical intracellular protein complex holding approximately 4,500 iron atoms (21,22). However, when iron needs are high, ferroportin (encoded by the SLC40A1 gene) exports the iron, which is taken up by transferrin. This process is facilitated by hephaestin which converts the iron from Fe^{2+} to Fe^{3+} for uptake by transferrin. As a result, iron is transported to macrophages and hepatocytes from the basolateral membrane of enterocytes as Tf-Fe^{3+} (19,22).

Iron is recycled by macrophages from senescent red blood cells and hence most of the iron is not excreted from the body (23,24). The recycled iron is used to meet most of our iron need, with the exception of a small amount (1-2 mg) that has to be met through iron intake. The Dietary Reference Intakes (DRIs) are therefore set to meet this intake requirement considering the percentage absorption value. For example, the Recommended Dietary Allowance (RDA) for iron is 8 and 18mg for males and females respectively from 19 through 50 years of age (17). Proteins involved in iron metabolism include ferritin (functions in iron storage) and transferrin (delivers iron from absorption centers to all tissues) which function as iron carriers. Hepcidin (regulates iron efflux), the iron regulatory protein (IRP, regulate genes involved in iron metabolism to optimize iron availability), hemojuvelin and matriptase-2 (Mt2) (regulates the transcription of

hepcidin) are involved in iron regulation (21,25). During iron absorption, insoluble ferric iron (Fe^{3+}) is converted to its soluble intermediate, ferrous iron (Fe^{2+}) by means of ferrireductases and ferroxidases. This inter-conversion of insoluble to soluble iron increases the likelihood of generating free radicals as reactive hydroxyl species in the Fenton reaction, and hence the need for a tight homeostatic iron control. Hepcidin ensures this homeostatic control to keep iron within normal ranges in the body (22–24).

The Role of Hepcidin in Iron Regulation

Hepcidin is a protein that modulates systemic iron homeostasis and is characterized as a liver-expressed antimicrobial peptide-1. It is translated as an 84-amino acid prepropeptide and cleaved by convertases to the biologically active 25-peptide hepcidin which is released into circulation. Hepcidin is controlled at the transcriptional level and is upregulated with increased metabolic needs such as during intensive physical activity, high storage iron and inflammation and downregulated during hypoxia, iron deficiency and increased erythropoiesis (23). Hepcidin functions by binding to the iron exporter, ferroportin, inhibiting intestinal absorption and iron efflux from hepatocytes and macrophages (23,26,27).

Multiple pathways including iron status, inflammation, erythropoiesis and reactive oxygen species impact the expression of hepcidin (28). However, iron regulation through the hepcidin antimicrobial peptide (HAMP) gene is primarily influenced through two major pathways; inflammation via the IL 6-STAT3 pathway and the BMP-SMAD pathway. Intracellular and extracellular iron stores influence transcription of the HAMP gene via the BMP-SMAD (SMAD 1/5/8) pathway which is intracellularly influenced

through sensing by transferrin receptors (TfR1 and TfR2). The transmembrane protein BMP 6 in conjunction with hemojuvelin, matriptase-2 and neogenin phosphorylate the SMAD proteins enabling SMAD 4 to form a complex with BMP 6 which translocates to the nucleus and binds to the hepcidin promoter region upregulating it (29,30). SMAD 7 in the cytosol is activated by low iron stores inactivating SMAD 4 and consequently downregulating hepcidin (28). This impacts the expression of hepcidin in iron replete or deficient states via a feedback loop.

Inflammation also regulates hepcidin expression through the IL 6-hepcidin axis which activates the JAK 2/STAT 3 pathway and then hepcidin. This explains significantly higher hepcidin concentrations observed in morbidly obese women compared with their normal weight counterparts (31). The increased pro-inflammatory cytokines in inflammation also upregulates lipopolysaccharide secretion and hepcidin expression. Although it was previously thought that during inflammation hepcidin binds to ferroportin resulting in its internalization and degradation, current research suggests proteolytic ubiquitination leading to endocytosis of the hepcidin-ferroportin complex preventing iron efflux (32–34).

Hepcidin and Iron Status

A downstream effect of increased hepcidin is lowered transferrin saturation as iron is unavailable for binding onto the transferrin receptor (TfR) reducing iron delivery to tissues. Increased iron uptake onto transferrin also increases hepcidin expression through the interaction between TfR1 and TfR2 (35). Considering that hepcidin expression directly influences transferrin saturation and consequently iron available for

erythropoiesis, hepcidin has significant use as an acute marker of iron stores as upregulation leads to systemic hypoferremia and adipose tissue iron overload (35,36).

A study showed that serum hepcidin concentration was positively correlated with ferritin and negatively correlated with transferrin (37). Among adult patients in the above study, soluble transferrin receptor was also a significant predictor of serum hepcidin. These studies among others highlight the role of hepcidin as a regulator of iron status (38,39).

Functions of Iron

Iron is essential for several processes in the body. It is incorporated into hemoglobin in blood (contains two-thirds iron) and myoglobin in muscle tissue enabling these proteins to transport oxygen to tissues. Iron is also involved in dehydrogenases and cytochrome enzymes which are involved in oxidation and reduction as part of the electron transport chain during respiration. Other functions include DNA replication, cell proliferation and energy production (23,24,40).

DNA Replication

New evidence shows that enzymes such as helicases and glycosylases involved in DNA synthesis and repair have iron cofactors in the form of iron-sulfur (Fe-S) clusters at their catalytic sites. Through reduction/oxidation reactions, iron-sulfur clusters enhance the affinity of these enzymes for DNA replication. In vitro experiments show that the disruption of Fe-S cluster incorporation inhibits DNA primase activity partly as a result of unfolding of carboxy terminal domains of the catalytic subunits and also inhibits cell cycle progression to the S-phase (41). These results demonstrate the essentiality of the

Fe-S cluster for initiating genomic integrity. DNA bound polymerases by virtue of iron sulfur cluster proteins are also able to intercommunicate increasing their efficiency in DNA damage detection (42). In ovarian granuloma cells treated with Fe2+, cells accumulated at the G2/M phase showing its role in cell cycle arrest in cancer (43)

Energy Metabolism

Fe-S clusters also influence energy metabolism as they are a component of electron transport chain proteins I, II and IV. Beta cell control of mitochondrial iron is needed for membrane depolarization and closure of ATP-dependent potassium gated channels needed for insulin secretion and glucose metabolism. The AMP-ATP ratio nutrient sensor that regulates AMPK, the energy sensor, is also largely dependent on iron (44).

Iron-sulfur clusters regulate energy metabolism via cytosolic and mitochondrial aconitase. During energy metabolism, these homologs of aconitase convert citrate to isocitrate in the mitochondrion and cytosol. Mitochondrial aconitase functions in the TCA cycle. Cytosolic aconitase is regulated via the insertion of [4Fe-4S] into iron-regulatory proteins 1 (IRP1). IRPs are iron-regulated RNA-binding proteins that control mRNAs encoding critical modulators of iron storage (ferritin) and energy homeostasis (45). IRP1 is bifunctional and acts as both an IRP and a cytosolic aconitase (46–48). Only a small portion of cytosolic aconitase is recruited to IRP1 suggesting that unregulated interconversion of this large pool of cytosolic aconitase into the RNA-binding form would have negative consequences (46,47).

Immunity

Iron plays an essential role in the microbiome as gut microbiota are sustained by ingested iron (44). Iron-dependent bacteria in the gut thrive via secretion of siderophores that enable them to scavenge iron from hemoglobin or transferrin (49,50). In bacteria and fungi, the iron bound to siderophores are taken up either via receptors and transporters or directly through reductive assimilation causing the reduction of Fe^{3+} to Fe^{2+} prior to uptake. Iron bound to siderophores in soil microbes may be available for use by plants. Siderophores are also iron chelating and can deprive disease-causing pathogens of iron. In cases of iron excess, bacteria and fungi secreting siderophores increase the expression of ferritin genes thereby reducing the iron available to pathogens while still making iron available to the host (51).

Iron is also responsible for the proliferation and maturation of immune cells such as neutrophils, macrophages, and lymphocytes (52). During infection and inflammation, immune cells upregulate iron-binding proteins (lipocalin 2 and lactoferrin) which bind and inhibits the iron available for bacterial growth (53). In disease states such as cancers, defective immune cells containing excess iron increase the levels of iron available to adjoining cells via adhesion molecules leading to cell replication. However, cells without these excess amounts of iron are able to undergo programmed cell death preventing their further replication (54,55). This highlights the need for tight control of body iron stores.

Other Functions

Iron is implicated in thyroid health as it is essential for iodine uptake and involved in thyroid hormone synthesis through the activation of heme-dependent thyroid

peroxidase (56,57). Iron status markers such as red cell distribution width, hemoglobin, ferritin and mean corpuscular hemoglobin were negatively correlated with the thyroid stimulating hormone in cases of hyperthyroidism and subclinical thyroid function (57,58).

In humans, some anemias are linked to impairments in Fe-S cluster biogenesis (59). The mitochondrial iron sulfur cluster (ISC) assembly system assembles Fe-S clusters for mitochondrial proteins, and is required for the cytosolic iron sulfur cluster assembly (CIA) system. The CIA system is essential for maturation of Fe-S proteins in the cytosol and nucleus (60,61). Disruption of either the ISC or CIA system substantially impairs cell function (59).

Iron Deficiency and Anemia

NHANES data shows that there has been an increase in anemia by 3% from years 2003 to 2012 while the prevalence of moderate-severe anemia almost doubled to 2% in the same years. Iron deficiency is the leading cause of anemia in developed and low-income countries. It is the commonest nutritional deficiency worldwide and the cause for approximately half of all cases of anemia (62). Iron deficiency may occur due to increased iron needs, blood loss, decreased intake or absorption. Iron is made available for erythropoiesis via transferrin which bind iron from liver stores, gut and recycled red blood cells from macrophages. In the absence of hemorrhage, and chronic inflammatory diseases, iron requirements for tissue oxygenation and red blood cell production remain stable throughout adulthood with supply balanced by demand (63).

There are two forms of iron deficiency. Absolute iron deficiency occurs when available iron (dietary iron, iron recycled from old red blood cells) is inadequate to meet the need for erythropoiesis. Anemia develops and is evident by low iron stores, low transferrin saturation, increased total iron binding capacity, and lowered hemoglobin synthesis (23,63). On the other hand, inflammatory conditions may mask these changes in blood markers as less iron is made available to tissues in order to inhibit use by harmful pathogens. This is evidenced by lowered transferrin saturation, low total iron binding capacity and increased ferritin stores (23). This form of iron deficiency is called functional iron deficiency and like absolute iron deficiency can also lead to anemia (62). The consequences of anemia include fatigue, poor concentration, reduced productivity, poor cognitive and motor function. Economically disadvantaged individuals, pregnant women, pre-menopausal women, and children are at risk of iron deficiency.

Due to the multifaceted nature of anemia, challenges still remain in identifying accurate treatment and management therapies. Current therapies for the acute treatment of anemia include blood transfusion, erythropoietin therapy, Vitamin B-12 injections, intravenous iron therapy, intermittent iron supplementation and more recently, hepcidin inhibitors (64–66). Strategies such as iron supplementation and fortification although beneficial against severe forms of anemia may not provide for long-term management of mild or moderate anemia particularly in developing countries (67). In developing countries, the fortification of staples, use of micronutrient powders and supplementation were beneficial in improving hemoglobin concentrations. However, disadvantages

include cost-effectiveness of fortified compared to unfortified foods, distribution and traditional cooking practices (68).

In order to mitigate these challenges dietary diversification has been suggested. A multicenter study in four African countries with high prevalence of micronutrient deficiencies advocated for dietary diversification to improve micronutrient intake (69). Dietary diversity is ensuring individual access to safe and quality foods laden with adequate micro and macronutrients. However, minimal evidence for outcome measures, affordability and the paucity of food databases are disadvantages for implementation. Particularly for iron, synergy from other nutrients such as proteins are needed to enhance absorption making dietary diversification a sustainable long-term solution for anemia (67).

Inflammation

Types, Causes and Sources of Inflammation

Inflammation is a physiological mediator through which nutritional and metabolic actions interplay and exert their actions in a non-specific adaptive response of the body to pathogens, infection and/or tissue injury in order to restore homeostatic control (70). The acute inflammatory state activates macrophages triggered by innate immune receptors such as Toll-like receptors recruiting plasma proteins, leukocytes, vasoactive amines, chemokines and cytokines further down the cascade (70).

Types of inflammation may include sterile inflammation not borne from infections and occurs independent of infection to recruit chemokines and cytokines to a site of inflammation (71). Para-inflammation on the other hand presents characteristics

intermediate between hemostatic control and classic inflammatory processes which may not be characteristic of overt infection or tissue injury (70). The sustenance and persistence of the associated tissue damage in para-inflammation with the combined activation of macrophages and T-cells result in chronic inflammation leading to an altered metabolic state due to changes in homeostatic set points (70,71).

Inflammation and Body Composition

Obesity is characterized by chronic low-grade inflammation (72). Adiposity activates metabolic pathways implicated in inflammatory signaling. The release of pro-inflammatory cytokines in visceral adipose tissue may be associated with the increased secretion of TNF-α, IL-6 and adiponectin seen in chronic conditions such as obesity (73,74).

At the systemic level, IL-6 and the acute phase protein, CRP are both produced in the liver. IL-6 triggers the production of CRP and are easily detectable pro-inflammatory cytokines in serum. At the cellular level, the release of these pro-inflammatory cytokines triggers the release of neutrophils and monocytes (differentiate into macrophages). Neutrophils ingest the pathogens by the release of reactive oxygen species which potentiates the release of other cytokines increasing the inflammatory response. Some studies further show that these biomarkers may exist in other states other than chronic low-grade inflammation while others ascribe this physiological response to that evident in the chronic low-grade inflammation observed in obesity (7,75).

This subclinical inflammatory process with increased levels of pro-inflammatory cytokines was observed among severely obese individuals for gastric bypass surgery.

Researchers observed that the portal vein which drains visceral fat had a higher concentration of IL-6 compared to the peripheral artery blood showing that omental fat is an important site for the production of IL-6. IL-6 concentration was also positively correlated with CRP levels (76). Similarly, in two phases out of seven in a study among 3500 UK civil servants followed for 11 years, mean increases in CRP concentration were 3% lower in normal weight individuals as compared to overweight/obese individuals (77). In a similar trajectory of body composition and the link with inflammation, CRP and IL-6 levels were positively correlated with BMI, visceral fat, total body fat, waist circumference and waist to hip ratio ($p<0.05$) with higher levels of CRP, IL-6 and TNF-α in obese than non-obese Korean adults (n=100) (78).

Muscle strength and composition loss may also be evidence of disruption in local tissue and may promote chronic inflammation and further deteriorate muscle function (79). Muscle tissue is a source of IL-6 and has been linked to muscle wasting in mdx mice. In C2C12 myoblasts, IL-6 mRNA knockdown reduced muscle specific gene expression. Elevated IL-6 concentrations may also be associated with arthritis and dystrophy of the muscle stem cells (80).

The interplay between adiposity and inflammation is seen in obesity and evidenced by the production of inflammatory protein molecules/biomarkers. This inflammatory link from an increased percentage of body fat may be the reason for deficiencies of essential elements in obesity (81). This may also in part be due to regional fat accumulation resulting in impaired concentrations of essential nutrients (82). Regional adipose tissue distribution is a key determinant of obesity aside weight considerations. It

has been shown that this accumulation of lipids in skeletal muscle and abdominal/visceral fat may further increase the risk of insulin resistance and type 2 diabetes (83).

Obesity, Inflammation and Iron Status

Apart from iron deficiency, inflammation is another leading cause of anemia. According to the National Institute of Diabetes and Digestive and Kidney Diseases, it is the second most common cause of anemia (84). Anemia of chronic disease or anemia of inflammation, is a multifactorial malignancy induced by IL-6 via the IL-6-STAT3 pathway increasing hepcidin levels and resulting in ferroportin endocytosis and decreased iron export. During inflammation, IL-6 binds to glycoprotein l30 and the activation of this ligand increases the expression of hepcidin. As a result, there is a lowered proliferative capacity of erythroid progenitor cells due to decreased availability of iron for erythropoiesis from the increased iron sequestration. This may be evidenced by the elevated ferritin levels seen in anemia of inflammation. This increased cytokine release is similarly observed in obesity resulting in increased levels of hepcidin, decreased iron absorption and a consequent mild iron deficiency (23,85).

Obesity marked by low grade chronic inflammation thereby increases the risk of anemia of inflammation, through a hepcidin mediated-inflammatory response. The expression of pro-inflammatory cytokines in adipose tissue in obese adults and adolescents has also been linked to iron deficiency anemia (25,86). On the whole, the underlying metabolic state of obesity and the associated multiple factors including age and diet have been seen to influence iron status (87).

In a sub-analysis of the Healthy Lifestyle in Europe by Nutrition in Adolescence-Cross Sectional Study (HELENA-CSS) to evaluate the associations among obesity, inflammation and iron status in a group of European adolescents (n= 876), overweight/obese boys had higher storage iron (ferritin; $p<0.001$) and higher CRP compared to boys with normal BMI ($p = 0.02$). Among girls, CRP but not ferritin concentration was significantly higher among overweight/obese subjects compared to those with normal BMI ($p=0.002$) (88). In another study by Cheng et al., among overweight and obese women, BMI was a significant predictor of serum iron, serum ferritin and CRP levels, although they did not find a significant association between low serum ferritin levels and inflammation or hepcidin concentration (87). This contrasted with a study by Andrew et al., where researchers observed that visceral adipose tissue in individuals with obesity was associated with high serum ferritin and hepcidin concentration and inflammation (89). In this study, circulating iron concentration, NF-κB and TNF-α were also increased in individuals with obesity with and without type 2 diabetes compared to the healthy weight control group (89).

Diet and Inflammation

Selenium as a Dietary Antioxidant

Selenium, a trace mineral, is of fundamental importance to human health. It is an essential component of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defense systems of Vitamin C and other molecules, and immune function (86). The selenium content of plant-based food varies depending on the geographical location and the soil selenium content while the amounts in animal sources

such as milk, eggs and meat remain fairly consistent due to the known selenium content of controlled animal feeds. The forms of dietary selenium are selenomethionine (major dietary form) and selenocysteine which are both well absorbed. Selenocysteine and selenomethionine are the organic forms of selenium found in plants and animals. Selenocysteine is incorporated into selenoproteins through a highly regulated process while selenomethionine incorporated non-specifically into proteins such as albumin is unregulated. Other forms include selenate and selenite found in fortified foods and supplements. Selenide, a metabolite of selenium functions in other bodily reactions or is excreted in urine (17).

Selenium is an antioxidant and exerts biological activity through its incorporation as selenocysteine at the active site of selenoproteins. Glutathione peroxidase (GPX), selenoprotein P and thioredoxin reductase are selenoproteins predominantly characterized as storage sites for Se. Selenium status is measured through biomarkers linked directly to selenium function (GPX and SEPP1) and indirect measures (plasma selenomethionine concentrations which provides information on dietary intake and tissue stores). Selenium maximizes the activity of selenoproteins at plasma concentrations ranging from 80-100ng/mL (90).

GPX contains 4 selenocysteine atoms at its active site. Se deficiency results in the impaired synthesis of GPX in erythrocytes, serum and cytosol. Glutathione peroxidase is characterized in detail into four enzymes from GPX 1-4. GPX1 was the first identified biochemical marker of Se status to reduce hydrogen peroxide (91). As a result, GPX1 activity counters oxidative stress and its impaired synthesis alters erythrocyte function

(92). GPX2 is found in the epithelium of the gastrointestinal system while GPX3 is predominantly found in plasma and inhibits the action of hydrogen peroxide and lipoperoxides (93). GPX4 is essential in lipid membranes and catalyze the reduction of lipid hydroperoxides preventing lipid peroxidation. GPX variants tightly regulate adipocyte differentiation and have been associated with obesity and its associated pathologies (93).

Selenoprotein P functions in homeostasis and in the distribution of selenium. SEPP1 is produced by the liver and carries approximately 60% of plasma selenium transported to peripheral tissues (90). A storage site for a majority of the selenium in plasma, SEPP1 is a biomarker for total selenium nutritional status and consequent selenium deficiency. It has 2 domains, the N-terminal which contains 1 selenocysteine residue and the C-terminal which contains multiple selenocysteines, approximately 9 in humans (94). Individuals with BMI> $30kg/m^2$ have been observed to have low concentrations of selenoprotein P which may be due to the upregulation of pro-inflammatory cytokines in adipose tissue (95,96).

In the United Kingdom and other European Union countries, the decline in blood selenium concentration resulted in chronic disease health implications. In Finland, the determination of Se-poor soil resulted in suboptimal dietary selenium intake (25µg/day) and the likelihood of associated health problems. This led to the addition of sodium selenate to agricultural fertilizers to increase the levels of Se in soil, crops, animal feeds and consequently human foods. This mode of Se supplementation resulted in notable increases in vegetables, cereals, cow's milk and meat sources. However, its direct impact

on the health of this population remains fairly unevaluated even though the spate of specific health problems remains low (97).

The implicit importance of selenium to human health is recognized. This may in part explain the proposal by the FDA in April 2013 for the addition of the mineral Se to the list of required nutrients in infant formula. This was to avoid Se deficiency by meeting the 2µg/100kcal requirement. There is also current FDA policy for Se supplementation of livestock feeds for beef cattle and sheep (98). However, the current RDA for selenium was introduced on the basis of blood GPX activity and effective 26th July 2018, the FDA reduced the RDI for 14 nutrients including selenium which was reduced from 70µg/day to 55µg/day (99). However, optimal plasma Se needed to maximize the activity of selenoprotein P may be more appropriate (91). The current dietary Se recommendation may not completely accommodate research highlighting the functional roles of other selenoproteins aside from GPx. The levels of Se intake to optimize selenoproteins such as selenoprotein P, which contains approximately 60% of plasma Se, is estimated at 70 µg/day (97).

Selenium and Inflammation

The expression of selenoproteins may be present in the cell membrane where it mounts a defense against lipid peroxidation (97,100). As a result, selenoproteins may play a critical role in the underlying inflammation in adipose tissue (101). Selenium influences the inflammatory response by the down-regulation of pro-inflammatory cytokines (13,14). When the incorporation of selenium at the active sites of enzymes such as GPX is impaired due to selenium deficiency, the inflammatory response in chronic

conditions is exacerbated. Suboptimal selenium status is associated with an increase in IL-6 (12). Selenium deficiency in chronic inflammatory conditions also result in the decreased synthesis of selenoproteins and antioxidants such as glutathione (GSH), increasing the production of CRP and the inflammatory state (102). Selenium supplementation may restore serum selenium levels resulting in the down-regulation of CRP attenuating the inflammatory response (88,103,104).

In a study to investigate the association between serum selenium concentrations and IL-6 in the institutionalized elderly (n=336; 70-87yr), elderly populations with the highest quartile of IL-6 had 23% significantly lower selenium concentration as compared to the lowest quartile after adjustment for covariates (OR= 2.35 (95% confidence interval: 1.15-4.83), p=0.015). CRP was also significantly higher across increasing quartiles of IL-6 (p=0.005). The prevalence of selenium deficiency in this population and its association with high levels of IL-6 suggests possible benefits for monitoring Se status to reduce inflammatory states (104).

Selenium may similarly be protective in inflammatory states by the suppression of pro-inflammatory genes via the NF-κB signaling pathway in human macrophage cells. In a study to investigate the effect of selenomethionine (SeMet) on the NF-κB signaling pathway in LPS-induced macrophage cells, U937 human macrophage cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium, 10% fetal bovine serum under standard conditions. To determine the effect of SeMet on the NF-κB pathway, p-NF-κB-Luc reporter plasmid was transfected into some of the SeMet and/or LPS cells. TNF-α

and IL-6 expression were one-fold lower in SeMet+LPS transfected cells as compared to LPS non-transfected cells ($p<0.05$) (13).

Selenium deficiency in the presence of inflammation was similarly observed among alcoholic liver disease patients which may be due to the cellular injury and the initiation of inflammatory responses. A 120% higher IL-6 ($p<0.0001$) and a 28% lower serum selenium ($p=0.001$) were observed in the highest severity group of liver cirrhosis as compared to controls without cirrhosis. In all liver cirrhosis groups, the concentration of selenium was negatively correlated with IL-6 ($r= -0.38$, $p=0.03$) (15).

Oxidative stress as routinely occurs in patients after coronary artery bypass graft surgery could not be attenuated by the administration of intravenous selenium. In this study, CRP concentration was significantly lower in the selenium group compared to the control group after 48 hours ($p=0.017$). However, there were no significant differences in IL-6 and TNF- α between the selenium and control group at 48hours (102,105).

In an animal study, the effect of Se-enriched rice with high resistant starches (SRRS) in attenuating chronic inflammation compared to normal rice resistant starches (NRRS) was evaluated in high fat fed Institute of Cancer Research mice. There was a 35% and 30% lower level of IL-6 in the SRRS and NRRS group respectively as compared to diabetic controls ($p<0.05$). Lower TNF-α and CRP levels were reported with SRRS treatment compared to the NRRS and the diabetic control respectively ($p<0.05$). In this study, a 4-week administration of SRRS reduced the expression of pro-inflammatory cytokines in diabetic mice. In another study the effect of a single dose of Brazil nut, a rich dietary source of selenium, on inflammatory markers was investigated. In this

randomized crossover trial of 10 adult volunteers (23-34y), participants were randomized to 5, 20 and 50g Brazil nuts. Among subjects who consumed 50 g brazil nuts, IL-6 concentration decreased by 15% between day 1 and day 30 after the ingestion ($p<0.05$) (106).

Individuals with increased body weight have been shown to have low levels of circulating selenium in the blood (107). Guarino et al., observed that after a 52- week Se supplementation (83mcg), there was a significant decrease in visceral adiposity. This may be because selenium reduces adipocyte differentiation and adipogenesis with a consequent decrease in the size of fat cells (108). In another study, there was an approximately 20% lower dietary Se intake in overweight/obese men and women compared to their normal weight counterparts. In the same study, among women, android fat was lower (11%) in the high Se intake group compared to the low Se intake group and in men, trunk fat was also lower (16%) in the high Se intake group compared to the low Se intake group. Due to this, dietary Se intake needs to be adjusted for body weight as different body weight may have altered dietary Se needs (109).

Selenium and Iron Status

Selenoenzymes are implicated to be protective against anemia. In mice models, glutathione peroxidase was protective against oxidative stress in erythroid cells undergoing maturation, erythrocyte turnover and heme homeostasis (110). Reductions in the levels of glutathione have been implicated in defects in Fe-S cluster biogenesis (111). Selenium deficiency (plasma selenium<70ng/mL) is linked with anemia through multiple pathways namely, modulation through inflammation via hepcidin, increases in heme-

oxygenase 1 and oxidative stress (86). A recent study also implicated serum zinc as a mediator in this relationship (112).

In the pathway linking oxidative stress to the anemia observed in selenium deficiency, it is noted that the hierarchical formation and turnover of red blood cells make them susceptible to oxidative stress. Due to the availability of iron, free α-globin units of immature erythrocytes are particularly prone to the action of reactive oxygen species leading to the aggregation of Heinz bodies and the hemolysis of erythrocytes. Selenium deficiency as evidenced by low levels of redox buffer systems (GPX and thioredoxin reductase) potentiates this oxidative stress activity in erythrocytes. The lack of selenium also impacts the erythropoietic environment by making heme/iron unavailable for the maturing of erythrocytes leading to decreased erythrocyte lifespan (110).

Increased expression of heme oxygenase-1 due to selenium deficiency is also implicated in anemia. Heme-oxygenase is a membrane bound enzyme expressed in liver and spleen and essential for recycling heme to release biliverdin, iron and carbon monoxide. High levels of heme oxygenase-1 impairs the uptake of iron from transferrin for erythroid cells resulting in defective erythropoiesis (113,114). In the liver of inflammation induced rats, the expression of heme oxygenase-1 was increased after 4 and 6 hours (115). In chicken lymphocytes treated with lead to induce inflammation, selenium supplementation reduced the levels of heme oxygenase-1 (113).

The role of selenium in anemia via inflammation has not been extensively studied. As already known, selenium deficiency plays a role in the impairment of the antioxidant defense system. This leads to increased expression of reactive oxygen species

and consequently, the expression of pro-inflammatory cytokines, particularly IL-6. The activation of IL-6 is the main induction pathway in anemia of inflammation via hepcidin, suggesting hepcidin as a mediator in the relationship between selenium and anemia (86). Studies have shown that plasma selenium concentration, GPX and superoxide dismutase are negatively correlated with hepcidin in patients with chronic kidney disease (CKD) and Alzheimer's disease (116).

Several studies have been conducted to investigate the relationship between selenium and iron status. In the NHANES III data, lower serum selenium concentrations were observed among older adults with anemia compared to their non-anemic counterparts. A higher prevalence of anemia was observed in the lowest quartile of serum selenium compared to the highest quartile. After adjusting for demographics and chronic diseases in this population, serum selenium was positively associated with hemoglobin concentration (117). Among adolescent girls with poor zinc and selenium status, low serum selenium concentration was associated with anemia (118). Serum selenium concentration was also positively correlated with serum ferritin concentration among Korean female adults (119). However, among college students with low iron status without anemia, serum selenium and glutathione peroxidase concentrations were not significantly different from the control group pre and post-iron supplementation (120).

Most studies that investigate the relationship between selenoproteins and iron status have been done among subjects with CKD due to the shortened life span of erythrocytes. Among these patients, there is a resulting iron-restricted erythropoiesis due to increased hepcidin concentration and the impaired production of erythropoietin for the

bone marrow leading to defective erythropoiesis (28). In chronic kidney disease, plasma selenium and GPX concentrations were reduced (27). Supplementation with selenium significantly increased plasma selenium concentration and erythrocyte GPX activity in CKD patients at different stages of severity (121). In the 2011 to 2014 NHANES dataset among CKD patients, hemoglobin and serum iron were positively correlated with selenium (122).

Conflicting findings still remain in the association between body selenium status and anemia. However, most animal and human studies show inverse association between plasma/serum selenium and anemia.

Conclusion

This review has discussed the relationships among iron metabolism, inflammation and selenium status. We have further shown that selenium deficiency leads to impaired iron status and discussed the mechanisms through which this happens. We have also discussed how obesity is associated with iron and selenium status through inflammation. However, there is an existing gap in how selenium influences iron status through the inflammation observed in obesity.

CHAPTER III

RESEARCH ARTICLE

An Observational Study of the Interrelationships among Selenium, Iron Status and Inflammation in Young Adults

Introduction

Anemia affects a third of the world's population (123). In the US, the prevalence increased by 3% from years 2003 to 2012 (124). Anemia results from a homeostatic iron imbalance with increased destruction of erythrocytes and/or impaired synthesis (123). Iron-deficiency anemia is the most common form of anemia accounting for about half of all anemia cases, while inflammation is the second most common cause of anemia (3,84).

Inflammation refers to a biological response of the immune system to infection and is a secondary component of chronic disease that is implicated in anemia of inflammation (1,2). Inflammation accounts for about a fifth of anemia cases in older adults and among individuals with obesity, the observed chronic low-grade inflammation is implicated to result in hypoferremia (3,4,84,123). Anemia caused by inflammation is associated with alterations in iron metabolism, erythrocyte life span and production, lowered transferrin saturation and serum iron, and increased ferritin concentrations (125). The link between inflammation and anemia has been explained by the iron regulatory protein, hepcidin. Hepcidin is a 25 amino acid (disulfide-rich) peptide that plays a critical role in iron metabolism by acting as a signaling molecule and in immunity due to its

antimicrobial properties (8,9). Hepcidin acts by binding to the iron transporter (ferroportin) resulting in its ubiquitination, endocytosis and consequent degradation (42). Hepcidin concentration increases in response to high iron stores and inflammation and decreases with anemia and oxidative stress (126).

In obesity, macrophages and immune system cells invade the adipose tissue in response to fat accumulation leading to the production of pro-inflammatory cytokines including (Interleukin 6) IL-6, (6). With the increased production of the pro-inflammatory cytokine IL-6, hepcidin, is upregulated leading to a reduction in circulating iron and an increase in iron storage in macrophages and hepatocytes and a consequent mild iron deficiency (7,85).

The inflammation from increased percentage of body fat may also result in deficiencies of essential elements in obesity and antioxidant/anti-inflammatory nutrients such as selenium may be a potential agent in addressing inflammation (81,86). Selenium is incorporated as part of selenocysteine at the active site of selenoproteins (GPX, selenoprotein P and thioredoxin reductase). GPX1 contains 4 selenocysteine sites and counters oxidative stress and its impaired synthesis alters erythrocyte function. Selenoprotein P (SEPP1) is a biomarker of total selenium nutritional status and selenium deficiency and functions in the distribution of body selenium (92,94). Studies show that selenoproteins may play a critical role in the underlying inflammation in adipose tissue (81,101). Poor selenium status is also a risk factor for anemia as its deficiency contributes to the formation of methemoglobin which is unable to carry ferric iron ($Fe3+$) in its structure and consequently oxygen (127,128). Studies also show that selenium is linked

to anemia through the modulation of inflammation via the IL-6 pathway, the increased expression of heme-oxygenase 1 and oxidative stress (86,115,118,129). Other researchers site serum zinc as a potential modulator in this relationship (112).

Despite the increasing knowledge of the role of selenium in addressing inflammation, no study has investigated how selenium influences the chronic inflammation found in obesity and its relationship with hepcidin and iron status. The aim of this study was to investigate the associations among selenium status, hepcidin concentration and iron status biomarkers in individuals with overweight/obesity compared with normal weight controls. We hypothesized that selenium status will be negatively correlated with hepcidin concentration and positively correlated with iron status biomarkers among study participants.

Materials and Methods

Study Participants and Recruitment

Subjects were recruited from University of North Carolina, Greensboro (UNCG) students and staff via mass emails in the Summer of 2018. Out of a total 129 subjects who responded to the mass e-mail, 66 of them came for screening at the Cemala Foundation Human Nutrition Research Laboratories of the Nutrition Department (**Figure 3.1**). At the screening visit, potential subjects were provided with a copy of the informed consent form to read and sign if they agreed to participate in the study. The informed consent contained information on the study, and benefits and risks of participating. When they had provided consent, they filled out the screening form with information on demographics, use of vitamin and mineral supplement, medical history and any current

medication. As part of the screening procedure, duplicates of their height and weight were also measured to estimate their BMI to assess their eligibility for the study. Subjects were eligible for the study if they were 18 to 49 years of age, with a BMI of 18.5 kg/m^2 or over, with no history of inflammation associated chronic diseases such as chronic kidney disease, cancer, heart disease, diabetes mellitus, and severe/mild hypertension. In addition, they were eligible if they were non-smokers, not pregnant, not lactating and not taking any mineral/vitamin supplement or medicines that could interfere with iron and selenium status or inflammatory markers. Also, individuals who had donated blood two months prior to the start of the study were excluded.

We estimated that 58 subjects were needed to determine a significant association between selenium biomarkers and hepcidin concentration in a regression model assuming an r-squared 0.5 for regression model with up to 9 predictors at an alpha level of 0.05 and with a statistical power of 0.8. To meet this sample size, we recruited a total of 66 subjects with equal numbers of subjects having either normal weight or overweight/obesity. Out this number, 2 subjects opted out for personal reasons, 3 of them did not come in for blood draw, 1 had a BMI<18.5kg/m^2 at the screening visit and 1 was excluded from the analysis as the required volume of blood could not be drawn at the study visit. Fifty-nine subjects were used in the final analysis. The protocol for this study was approved by the Institutional Review Board of UNCG.

Study Design

The study was a cross-sectional study in which anthropometry, dietary intake data and blood samples were obtained from subjects. Subjects who qualified based on the

inclusion/exclusion criteria were provided with 3-day dietary record forms and weighing scales to weigh and record foods eaten. They were to be completed and returned at the study visit. Instructions for completing the dietary record was provided by the research assistant.

For the study visit, the study participants were required to do an overnight fast where they did not consume any food or drink (except water) 10 hours prior to the study visit. On the morning of the study visit, the dietary records were collected and reviewed by the research assistant. Their height, weight and other anthropometric measurements were taken and approximately 30 ml of venous blood was collected to measure the concentrations of iron and selenium biomarkers, hepcidin and markers of inflammation. All study participants who completed both screening and study visits, were provided $60 Walmart gift cards as compensation for participating in the study.

Anthropometry

Height, weight and body composition measurements such as visceral (abdominal) fat, and total body fat were taken. A stadiometer (Seca 213) was used to measure the standing height of the study participants following standard procedure. A weighing scale (Tanita BWB-800) was used to measure the weight in kilograms in light clothing (130).

Body composition was measured using a bioelectrical impedance analyzer (Tanita Segmental Body Composition Monitor, BC-545F) which worked by passing a very low, safe electrical current from four electrodes in contact with the hands and feet to the abdomen. The electrical signal passes through different body components and the level of resistance (impedance) to the current is measured and used in a prediction equation to

calculate estimates of their body composition. To use the bioelectrical impedance analyzer, study participants were required to remove shoe and socks from both feet, step on the metal electrodes of the analyzer and hold the handle at a horizontal level from the ground away from them. As part of this protocol study participants were also asked to be properly hydrated prior to measurement.

Dietary and Nutrient Intake

To assess dietary intake using the 3-day weighed dietary record, subjects were required to keep a log of all foods consumed on 3 non-consecutive days (2-week days and 1 weekend day) between the screening and study visits. They were provided with a food weighing scale to weigh and record all food items consumed during the selected days. For students who ate in the cafeteria or ate out, they were asked to estimate amounts compared to amounts eaten at home. They were also encouraged to note serving amounts when eating at chain restaurants or food service places with calorie information. Study participants who consumed pre-packaged foods were asked to bring along the empty packages during the study visit. Dietary records were analyzed into nutrients and food groups using the Nutrition Data System for Research (NDSR software, University of Minnesota). To ensure reliability and accuracy of dietary records, a trained research assistant reviewed the dietary information with participants on the study visit and data were reviewed by another research assistant during data entry into NDSR.

Sample Analyses

Fasting blood samples collected at the study visit were processed into serum, plasma or whole blood for the measurement of selenium, iron status and inflammatory

markers as well as plasma hepcidin. Iron status biomarkers (ferritin, hemoglobin, Unsaturated Iron Binding Capacity, Total Iron Binding Capacity, transferrin saturation and plasma iron) and CRP concentration were measured from serum or whole blood (in the case of hemoglobin) by LabCorp Laboratories (Burlington, NC). With the exception of the serum and whole blood samples that were sent to LabCorp, all other samples were stored in our laboratory at – 80 ^{o}C and analyzed upon study completion.

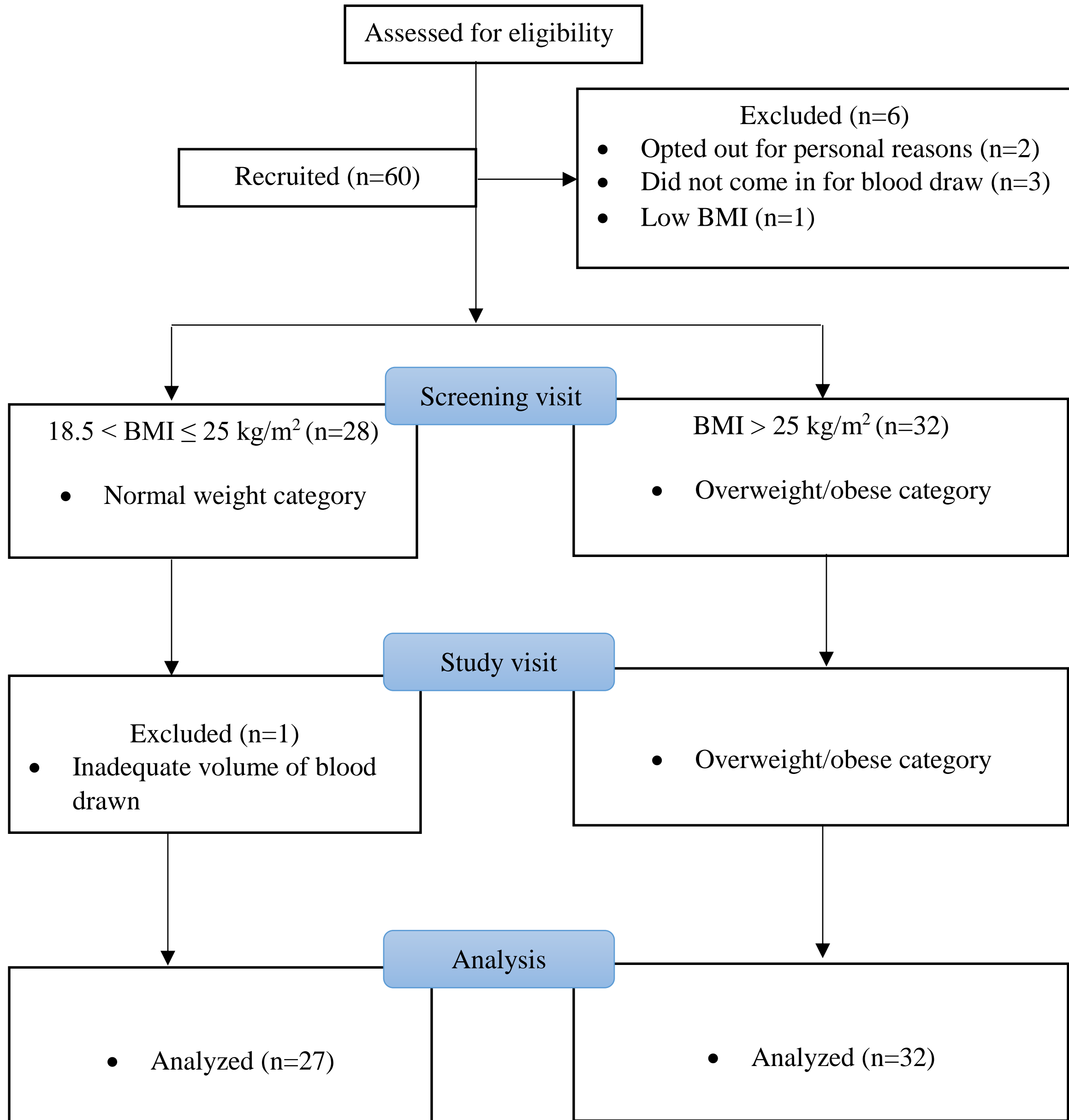
Plasma was predigested in our laboratory with trace metal grade HNO_3 overnight in a sandbath at 65 ^{o}C. The samples were then diluted with 2% HNO_3 and deionized water. The digested plasma samples were analyzed for plasma selenium concentration using the 8800 Tandem ICP-MS/MS from Agilent Technologies (Santa Clara, CA) in the lab of Dr. George Donati (Chemistry department, Wake Forest University). Plasma concentrations of hepcidin, selenoprotein P, and GPX activity were measured in our laboratory using Enzyme Linked Immunosorbent Assay (ELISA) kits. Erythrocyte GPX activity was measured from red blood cell lysate obtained after the plasma was collected and the remaining blood sample lysed. The GPX activity ELISA kit was obtained from Cayman Chemical (Ann Arbor, MI) while the selenoprotein and hepcidin kits were obtained from MyBioSource (San Diego, CA) and Peninsula Laboratories International (San Carlos, CA) respectively.

Statistical Analysis

Data were stratified according to normal or overweight/obese. Our primary outcome variables were plasma hepcidin and serum iron concentrations, while our independent variables were GPX activity and selenoprotein P. Means and standard

deviations were reported for continuous variables with normal distributions. Median and interquartile ranges were reported for nutrient intake variables. Percentages were reported for categorical variables. Ferritin, TIBC, selenoprotein P, plasma selenium, CRP, and hepcidin concentrations were log transformed to approximate normality. Student's t-test was used to compare continuous variables between the two groups. Wilcoxon rank sum test was used to determine significant differences in nutrient intakes between the two subject groups. Linear regression analysis was used to determine the associations among selenium biomarkers, iron status and hepcidin concentration adjusting for the potential confounders. Confounding variables adjusted for in the regression analysis were age, gender, BMI category, ethnicity, and dietary selenium. To determine if associations among variables differed between subjects with overweight/obesity and those with normal weight, we fitted an interaction term in the regression models and kept it in the model only when it was significant. Statistical significance was set at $p \leq 0.05$, and $0.05 < p \leq 0.1$ was considered weakly significant. The R-software (Rx64 3.5.0.ink) was used for data analysis.

Figure 3.1 Study Participant Recruitment



Results

Out of the 59 participants included in the final analysis, a majority (71%) were female and 42% were Non-Hispanic Blacks (%) **(Table 3.1)**. There were 46% of study participants with normal weight and 54% with overweight/obesity. About 19% of the participants had high C-reactive protein (CRP ≥ 3 mg/L).

Table 3.1 Background Data of Study Participants

Variables	N (%)
Gender	
Male	17 (29)
Female	42 (71)
Ethnicity	
Non-Hispanic White	18 (31)
Hispanics	6 (10)
Non-Hispanic Black	25 (42)
Other Race[2]	10 (17)
BMI category, kg/m^2	
Normal	27 (46)
Overweight/obese	32 (54)
Inflammation category	
Low (<1 mg/L)	40 (68)
Medium (≥1-3 mg/L)	8 (13)
High (≥3 mg/L)	11 (19)

Age, years[1]	21.36±2.87

[1]Values are mean ± SD
[2]Other Race are Asians, Arabs, Multiracial and Persians

Table 3.2 shows the nutrient intake between participants with normal weight and those with overweight/obesity is compared. The median caloric intake was 1875 kcal/day with no significant difference between subjects with normal weight and overweight/obesity. Similarly, the median selenium intake was not significantly different between individuals with normal weight (99.42 mcg/day) compared to individuals with overweight/obesity (102 mcg). Vitamin C intake was lower in individuals with overweight/obesity (43.37 mg/day) compared to individuals with normal weight (88 mg/day), p=0.028. All other nutrient intakes were not significantly different between the two groups (p> 0.05).

Table 3.2 Daily Nutrient Intake according to BMI Category [1]

	Normal (n=27)	Overweight/obese (n=32)	Total	p-value[2]
Caloric intake, kcal	1844 (1423, 2611)	1878 (1347, 2234)	1875 (1357, 2304)	0.667
Total fat, g	66.28 (56, 107)	80.67 (53, 98)	72.01 (54, 104)	0.982
Protein, g	70.52 (50, 97)	73.1 (52, 96)	73.16 (51, 97)	0.994
Selenium, mcg	99.42 (70, 151)	102 (83, 153)	101.82 (79, 152)	0.645
Iron, mg	17.24 (10, 20)	12.41 (10, 16)	13.33 (10, 19)	0.272
Zinc, mg	8.97 (6, 16)	9.28 (7, 12)	9.19 (6, 14)	0.886

Vitamin A, mcg	352.51 (214, 442)	318.71 (180, 427)	352.51 (190, 430)	0.667
Vitamin C, mg	88 (52, 124)	43.37 (19, 95)	57.44 (34, 103)	0.028*
Vitamin E, mg	11.22 (7, 21)	9.39 (7, 12)	10.53 (7, 13)	0.19

p<0.001 ‘***’ p<0.01 ‘**’ p<0.05 ‘*’
[1]Values are median (interquartile range)
[2]p-values are based on Wilcoxon rank sum test

As expected, the mean BMI was significantly lower among individuals with normal weight compared with individuals with overweight/obesity, p<0.001. Higher total body fat (8%) and visceral fat (4 levels) levels were observed in subjects with overweight/obesity compared to subjects with normal weight, p<0.001. There were no significant differences in iron status biomarkers and hepcidin concentration. Erythrocyte GPX activity was not significantly different in individuals with normal weight (1001±386 nmol/min/ml) compared with individuals with overweight/obesity (1077±415 nmol/min/ml), p = 0.766. Plasma GPX activity was higher among normal weight individuals compared with those with overweight/obese, however this difference was not statistically significant, p=0.169. There was similarly no significant difference in selenoprotein P or plasma selenium concentration among individuals with normal weight compared to those with overweight/obesity, p=0.347 **(Table 3.3)**

Table 3.3 Anthropometric and Biochemical Data according to BMI Category[1]

	Normal (n=27)	Overweight/obese (n=32)	Total	p-value[3]
Plasma selenium, ng/ml[2]	114.38 (90, 145)	112.06 (92, 137)	113.1 (91, 141)	0.363
Erythrocyte GPX activity, nmol/min/ml	1001±386	1077±415	1042±400	0.766
Plasma GPX activity, nmol/min/ml	84.29±18.31	79.48±18.44	81.75±18.36	0.169
SEPP1[2], ng/ml	360.77 (290, 450)	352.13 (276, 446)	356.05 (284, 450)	0.347
BMI, kg/m^2	22.43±1.71	29.28±4.14	26.15±4.73	0.0000009***
Visceral fat, level	1.83±0.81	5.79±3.04	3.98±3.04	0.000002***
Total body fat, %	25.78±8.38	33.82±8.6	30.14±9.34	0.0006**
Skeletal muscle, kg	44.86±10.11	51.34±10.77	48.37±10.89	0.021*
Hemoglobin, g/dl[5]	13.48±1.55	13.25±1.95	13.35±1.77	0.616
Ferritin, ng/ml[2]	36.2 (13, 101)	41.07 (13,136)	38.76 (13, 118)	0.677
TIBC, µg/dl[2,4]	350.37 (284, 432)	347.56 (296, 407)	348.84 (287, 420)	0.874
Transferrin saturation, %	29.33±15.21	26.59±12.32	27.85±13.66	0.456
Serum iron, µg/dl	98.37±45.67	91.06±42.05	94.41±43.51	0.528

Hepcidin, ng/ml[2]	9.95 (3, 29)	15.09 (3, 67)	12.47 (3, 47)	0.218

$p<0.001$ '***' $p<0.01$ '**' $p<0.05$ '*'
[1]Values are mean±SD
[2]Values are in geometric means (±1SD)
[3]p-values are based on Independent t-test
[4]Total Iron Binding Capacity
[5]Missing data (n=1)

Table 3.4 shows the correlations among hepcidin, iron status and selenium biomarkers. Hepcidin concentration was positively correlated with ferritin concentration (r=0.79, p<0.001), serum iron (r=0.42, p<0.01), hemoglobin concentration (r=0.52, p<0.001) and transferrin saturation (r=0.52, p<0.001), and negatively correlated with TIBC (r=-0.57, p<0.001). Selenoprotein P concentration was negatively correlated with plasma GPX activity (r=-0.32, p<0.016). C-reactive protein concentration was negatively correlated with serum iron (r=-0.28, p=0.031), and transferrin saturation (r=-0.33, p=0.011). Other significant correlations were observed among the iron status biomarkers. There were no significant correlations between selenium biomarkers and iron status biomarkers (p>0.05), neither were there any significant correlations between the selenium biomarkers and C-reactive protein or hepcidin concentrations (p> 0.05).

Table 3.4 Correlation among Hepcidin, Iron Status Biomarkers and Selenoproteins in Study Participants

	Ferritin, ng/ml[2]	Transferrin saturation, %	Serum iron, µg/dl	TIBC, µg/dl[1]	Hemoglobin,g/dl	CRP, mg/L[2]	Erythrocyte GPX activity, nmol/min/ml	Plasma GPX activity, nmol/min/ml	SEPP1, ng/ml[2]	Hepcidin, ng/ml[2]
Ferritin, ng/ml[2]	1.00	0.66***	0.51***	-0.67***	0.65***	-0.07	0.0002	0.017	0.11	0.79***
Transferrin saturation, %	0.66***	1.00	0.93***	-0.49***	0.68***	-0.33**	0.03	-0.04	0.09	0.52***
Serum iron, µg/dl	0.51***	0.93***	1.00	-0.16	0.61***	-0.28*	0.004	-0.06	0.04	0.42**
TIBC, µg/dl [1,2]	- 0.67***	-0.49***	-0.16	1.00	-0.43***	0.14	-0.15	0.01	-0.14	-0.57***
Hemoglobin, g/dl	0.65***	0.68***	0.61***	-0.43***	1.00	-0.25	0.04	-0.08	0.09	0.52***
CRP, mg/L[2]	-0.07	-0.33**	-0.26*	0.14	-0.25*	1.00	-0.05	0.09	-0.19	0.11
Erythrocyte GPX activity, nmol/min/ml	0.0002	0.03	0.004	-0.15	0.04	-0.05	1.00	-0.23	0.23	-0.003

Plasma GPX activity, nmol/min/ml	0.017	-0.04	-0.06	-0.15	0.04	0.09	-0.22	1.00	-0.32*	-0.11
SEPP1, ng/ml[2]	0.11	0.09	0.04	-0.14	0.09	-0.19	0.23	-0.32*	1.00	-0.08
Hepcidin, ng/ml[2]	0.79***	0.52***	0.42**	0.57***	0.52***	0.06	-0.003	-0.11	-0.08	1.00

$p<0.001$ '***' $p<0.01$ '**' $p<0.05$ '*'
[1]Total iron binding capacity
[2]Variables were log transformed before analysis

In multiple linear regression analysis, hepcidin concentration was significantly predicted by plasma GPX activity (β =-0.02, $p<0.01$), selenoprotein P concentration (β=-1.23, $p=0.028$) and ferritin concentration (β=1.01, $p<0.001$). Serum iron was predicted by ferritin concentration (β=13.26, $p=0.035$). Transferrin saturation was predicted by ferritin concentration ($p<0.001$), and weakly predicted by C-reactive protein ($p<0.067$). Hemoglobin concentration was significantly predicted by ferritin concentration ($p<0.01$). All the regression models were adjusted for age, gender, BMI category and ethnicity ($p<0.001$), **Table 3.5**.

Table 3.5 Association among Hepcidin, Iron Status Biomarkers and Selenoproteins in Study Participants

	Hepcidin ng/ml (n= 55) [1]			Serum iron, ug/dL (n=55)			Transferrin saturation, % (n=55)			Hemoglobin, g/dL (n=54)		
Predictors	*β*	*SE*	*p*	*β*	*SE*	*p*	*β*	*SE*	*p*	*β*	*SE*	*p*
(Intercept)	7.59	3.41	**0.031**	184.96	167.51	0.276	24.37	43.77	0.581	16.81	4.37	**<0.001**
Plasma GPX activity, nmol/min/ml	-0.02	0.01	**0.009**	-0.24	0.33	0.474	-0.03	0.09	0.705	-0.01	0.01	0.208
Ferritin, ng/ml[1]	1.01	0.12	**<0.001**	13.26	6.07	**0.035**	6.58	1.59	**<0.001**	0.51	0.16	**0.002**
C-reactive protein, mg/L[1]	0.02	0.09	0.849	-6.47	4.53	0.161	-2.23	1.18	**0.067**	-0.08	0.12	0.483
Erythrocyte GPX activity, nmol/min/ml	-0.00	0.00	0.776	-0.00	0.01	1.000	0.00	0.00	0.839	0.00	0.00	0.808
Selenoprotein P, ng/ml[1]	-1.23	0.54	**0.028**	-26.15	26.74	0.334	-4.26	6.99	0.545	-0.93	0.70	0.192
Gender												

Male	-0.38	0.32	0.245	20.92	15.80	0.193	6.07	4.13	0.149	1.94	0.41	**<0.001**
Age, years	0.01	0.04	0.887	1.65	1.85	0.376	0.37	0.48	0.454	0.05	0.05	0.343
Weight status, kg/m^2												
Overweight/obese	0.23	0.26	0.386	0.14	12.77	0.991	-0.07	3.34	0.984	0.30	0.34	0.383
Ethnicity												
Black	-0.12	0.31	0.699	-10.00	15.17	0.513	-3.92	3.96	0.328	-1.27	0.41	**0.003**
Hispanic	-0.46	0.40	0.262	-25.83	19.88	0.201	-9.34	5.19	0.079	-0.73	0.53	0.173
Other Race[2]	0.33	0.37	0.376	-4.22	18.22	0.818	-4.93	4.76	0.307	-0.54	0.49	0.273
R^2 / adjusted R^2	0.728 / 0.658			0.375 / 0.215			0.561 / 0.449			0.705 / 0.628		

[1]Variables were log transformed before analysis
[2]Other Race are Asians, Arabs, Multiracial and Persians

Plasma GPX activity was weakly predicted by gender (p=0.093) after adjustment for age, ethnicity, BMI category and dietary selenium intake, **Table 3.6**

Table 3.6 Association between Selenoproteins and BMI among Study Participants

	Erythrocyte GPX activity, nmol/min/ml (n=59)			Plasma GPX activity, nmol/min/ml (n=55)			SEPP1, ng/ml (n=59) [1]		
Predictors	*β*	*SE*	*p*	*β*	*SE*	*p*	*β*	*SE*	*p*
(Intercept)	620.85	778.81	0.429	115.90	34.23	**0.001**	6.29	0.43	**<0.001**
Ferritin, ng/ml[1]	-5.84	63.39	0.927	0.84	2.86	0.771	0.00	0.04	0.920
Dietary selenium, mcg[1]	54.06	134.08	0.689	-8.30	5.83	0.161	-0.11	0.07	0.144
Gender									
Male	-20.13	148.73	0.893	-11.59	6.75	**0.093**	0.09	0.08	0.305
Age, years	7.44	20.06	0.712	0.29	0.87	0.739	0.00	0.01	0.905
Weight status, kg/m^2									
Overweight/obese	46.81	126.31	0.713	-1.27	5.79	0.827	-0.01	0.07	0.841

Ethnicity									
Black	46.52	147.61	0.754	-3.95	6.91	0.570	0.02	0.08	0.768
Hispanic	-115.03	204.51	0.576	-8.97	9.08	0.328	0.14	0.11	0.216
Other Race[2]	24.08	180.10	0.894	9.13	8.08	0.264	0.09	0.10	0.380
R^2/Adjusted R^2	0.030 / -0.125			0.163 / 0.018			0.119 / -0.021		

[1] Variables were log transformed before analysis

[2]Other Race are Asians, Arabs, Multiracial and Persians

In **Table 3.7**, transferrin saturation was significantly predicted by ferritin (β=5.09, p=0.02) and C-reactive protein (β=-2.87, p<0.01). Hepcidin concentration had an inverse relationship with transferrin saturation in overweight/obese individuals compared to a positive association in their normal weight counterparts (β=-4.77, p for interaction= 0.036).

Table 3.7 Association between Transferrin Saturation and Hepcidin in Study Participants

	Transferrin saturation, % (n=59)		
Predictors	*β*	*SE*	*p*
(Intercept)	-7.00	10.26	0.498
Hepcidin, ng/ml[1]	4.49	2.32	0.059
Weight status, kg/m^2			
Overweight/obese	11.62	6.42	0.077
C-reactive protein, mg/L[1]	-2.87	1.04	**0.008**
Ferritin, ng/ml[1]	5.09	2.11	**0.020**
Gender			
Male	5.48	3.42	0.116
Age, years	0.28	0.44	0.524
Ethnicity			

Black	-3.57	3.34	0.291
Hispanic	-9.57	4.67	**0.046**
Other Race[2]	-3.20	4.04	0.433
Hepcidin: BMI.Cat1Overweight/obese	-4.77	2.20	**0.036**
R^2/adjusted R^2	0.612 / 0.532		

[1]Variables were log transformed before analysis
[2]Other Race are Asians, Arabs, Multiracial and Persians

Discussion

In spite of the recognized association between selenium status and anemia, to our knowledge, no study has investigated the role of hepcidin in the relationship between selenium biomarkers and iron status in obesity. The aim of this study was to investigate the associations among selenium status, hepcidin concentration and iron status biomarkers among young adults with normal weight or overweight/obesity.

Our results showed lower concentrations of functional (hemoglobin) and circulating (transferrin saturation, serum iron) iron in individuals with overweight/obesity when compared with individuals with normal weight but these differences were not statistically significant. Although some studies have reported, significantly higher concentrations of serum iron, transferrin saturation and hemoglobin in obese adolescents compared to their non-obese counterparts (20,21), most studies have demonstrated that obesity is associated with iron deficiency, hypoferremia and hyperferritinemia (131,132) .

There is evidence supporting the fact that invading macrophages in obese adipose tissue sequester iron resulting in iron overload (36). While our results showed the expected trends, it is likely that the differences were not significant because we had no individuals with morbid obesity in the overweight/obese group with most of them having overweight status.

In obesity, the increase in adipose tissue leads to low grade chronic inflammation (133). In our study, CRP was positively correlated with visceral fat levels ($r=0.57$, $p<0.001$) and total body fat percentages ($r=0.63$, $p<0.001$) (data not shown), which concurs with findings among Korean adults with obesity (78). Higher levels of adipose tissue and other comorbidities seen in morbid obesity result in increased concentration of inflammatory cytokines including Interleukin-6 (IL-6). The increased secretion of IL-6 in turn results in high concentration of hepcidin, leading to poor iron status (87). In this study, despite seeing the expected differences in hepcidin concentration between subjects with normal weight compared to those with overweight/obesity these differences were not statistically significant. This is likely because as most subjects in the latter group were overweight, there were few subjects with high levels of inflammation (19%; n=11) with only 8 of them in the overweight/obese group. Studies comparing subjects with extreme obesity to those will normal weight may show statistically significant relationships among these variables.

Researchers have observed that hepcidin concentration was inversely correlated with serum iron and positively correlated with BMI and CRP among obese adolescents (39). While we observed a positive correlation between hepcidin concentration and both

serum iron ($r=0.42$, $p<0.01$) and transferrin saturation ($r=0.52$, $p<0.001$), we demonstrated using linear regression, after fitting an interaction term in the model, that the nature of the relationship between the hepcidin and transferrin saturation depended on body weight status, with an inverse association among individuals with overweight/obesity and a positive association among subjects with normal weight. This concurs with the inflammatory effect, the consequent dysregulation and overproduction of hepcidin leading to impaired iron release observed in obesity. The converse is true as individuals with normal weight show a tight regulation and the clearance of excess amounts of hepcidin leading to effective iron homeostasis (134).

Selenium is incorporated in selenoenzymes and exerts immune and anti-inflammatory effects. Total plasma selenium reflects bound selenium in the form of selenocysteine, in the 2 predominant selenoproteins (GPX and selenoprotein P) (135). Low levels of selenium biomarkers are seen in obesity (93,136). For example, in obese mice, there was a reduction in local SEPP1 expression and this is likely because these selenoproteins which regulate oxidative stress are depleted due to the increased inflammation in obesity (137). We observed a non-significant negative association between selenoproteins (plasma GPX and selenoprotein P) and BMI while other studies have reported a significant negative correlation (118). We also observed lower concentrations of plasma selenoprotein P and GPX in individuals with overweight/obesity compared to their normal weight counterparts but this difference was not significant which may be because subjects in our study were selenium replete.

Our key finding in this study was that hepcidin concentration was predicted by selenoprotein P and plasma GPX activity in a regression model adjusting for potential confounders. The results from the model indicate that lower selenoprotein P and plasma GPX activity were associated with higher hepcidin concentration. This observation supports one of the proposed mechanisms through which selenium deficiency increases anemia risk. Petrova et al., espoused this relationship and reported higher hepcidin and lower GPX concentrations in chronic kidney patients with ischemic stroke compared with healthy adults (116). In these patients and among Alzheimer's disease patients, hepcidin concentration was negatively correlated with superoxide dismutase and GPX (116,138). To the best of our knowledge our study is the first to discover this relationship among individuals with normal weight or overweight/obesity. Other mechanisms by which selenium deficiency increases risk of anemia include increases in heme oxygenase 1, oxidative stress and through the effect of serum zinc.

It is interesting that despite observing this relationship between selenoprotein concentrations and hepcidin, we did not find a significant association between selenoproteins and iron status biomarkers as shown by other studies. For example, among rural Vietnamese adolescent girls, low serum selenium concentration was significantly associated with anemia (118). Also, in the US, low serum selenium has also been observed in adults with anemia of chronic inflammation (128). However, the non-significant association between circulating iron biomarkers and both glutathione peroxidase and selenoprotein P may be because very few subjects (17 %; n=10) in our study had anemia with 7 of these subjects in the overweight/obese category. Also, none

of these anemia cases were due to inflammation. Studies show that pathological conditions of induced anemia in mice is linked to reduced glutathione peroxidase and selenoprotein W concentrations suggesting that these associations may be more evident in anemia (129). It may also be attributed to the fact that most subjects in our study were selenium sufficient. The daily median selenium intake for subjects in this study was 100.5 mcg compared to the recommended intake of 55 mcg for adults in the US (17). Mean plasma selenium concentration of subjects was 113.1 ng/ml also well above the reference level of 70 ng/ml (90). Both plasma selenium concentration and dietary selenium intakes were not significantly different between the subjects with normal weight and those with overweight/obesity.

Limitations

Causal inference cannot be drawn from this study because it is a cross-sectional study. Also, most of our participants were selenium sufficient and very few had anemia hence the inability to observe obvious trends in the relationship between anemia and iron status as statistically significant. In addition, our study included no subjects with morbid obesity, thus only few had high CRP concentrations. In our study we measured GPX activity in erythrocytes to determine intracellular GPX pools and long-term selenium status. However, erythrocytes are collected with blood and if not treated properly may be less advantageous over plasma as a specimen for assessing selenium status. Also of note is the likely interaction of glutathione with heme iron impacting results (90). To mitigate this shortcoming, we measured GPX activity in plasma.

Conclusion

This study is novel in showing the role of hepcidin in the link between selenium status and anemia among healthy subjects with normal weight or overweight/obesity. Further studies on this relationship among individuals with morbid obesity who due to adipose tissue inflammation are prone to selenium deficiency and impaired iron status are needed. Also, using animal models of obesity will be helpful in investigating mechanistic relationships among selenium status, hepcidin and iron status. Additionally, studies are needed to investigate whether improving selenium status among individuals with selenium deficiency will improve iron status via hepcidin. This study also brings to the fore a long-term need for further research into the current DRI for selenium in populations at risk of anemia due to inflammation.

REFERENCES

1. Hunter P. The inflammation theory of disease. EMBO Rep. 2012;13:968–70.

2. Kotas ME, Medzhitov R. Homeostasis, inflammation, and disease susceptibility. Cell. 2015;160:816–27.

3. Fayet-Moore F, Petocz P, Samman S. Micronutrient status in female university students: iron, zinc, copper, selenium, vitamin B12 and folate. Nutrients. 2014;6:5103–16.

4. van Eijk LT, Kroot JJ, Tromp M, van der Hoeven JG, Swinkels DW, Pickkers P. Inflammation-induced hepcidin-25 is associated with the development of anemia in septic patients: an observational study. Critical Care. 2011;15:R9.

5. Roy CN. Anemia of inflammation. Hematology. 2010;2010:276–80.

6. Castro AM, Macedo-de la Concha LE, Pantoja-Meléndez CA. Low-grade inflammation and its relation to obesity and chronic degenerative diseases. Revista Médica del Hospital General de México. 2017;80:101–5.

7. Madu AJ, Ughasoro MD. Anaemia of Chronic Disease: An In-Depth Review. MPP. 2017;26:1–9.

8. Ganz T. Hepcidin and iron regulation, 10 years later. Blood. 2011;117:4425–33.

9. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest. 2004;113:1271–6.

10. Nemeth E. Targeting the hepcidin-ferroportin axis in the diagnosis and treatment of Anemias. Adv Hematol [Internet]. 2010 [cited 2019 Mar 24];2010. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2798567/

11. Fleming RE, Sly WS. Hepcidin: a putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease. Proc Natl Acad Sci USA. 2001;98:8160–2.

12. Duntas LH, Hubalewska-Dydejczyk A. Selenium and inflammation- potential use and future perspectives. US Endocrinology. 2015;11:97.

13. Shen Y, Yang S, Shi Z, Lin T, Zhu H, Bi F, Liu A, Ying X, Liu H, Yu K, et al. SeMet mediates anti-inflammation in LPS-induced U937 cells targeting NF-κB signaling pathway. Inflammation. 2015;38:736–44.

14. Yu D, Zhang Z, Yao H, Li S, Xu S-W. The role of selenoprotein W in inflammatory injury in chicken immune tissues and cultured splenic lymphocyte. Biometals. 2015;28:75–87.

15. Prystupa A, Kiciński P, Luchowska-Kocot D, Błażewicz A, Niedziałek J, Mizerski G, Jojczuk M, Ochal A, Sak JJ, Załuska W. Association between serum selenium concentrations and levels of proinflammatory and profibrotic cytokines-interleukin-6 and growth differentiation factor-15, in patients with alcoholic liver cirrhosis. Int J Environ Res Public Health. 2017;14:(Epub ahead of print; DOI: doi:10.3390/ijerph14040437).

16. Stockler-pinto MB, Mafra D, Moraes C, Lobo J, Boaventura GT, Farage NE, Silva WS, Cozzolino SF, Malm O. Brazil Nut (bertholletia excelsa, H.B.K.) improves oxidative stress and inflammation biomarkers in hemodialysis patients. Biological Trace Element Research. 2014;158:105–12.

17. Otten JJ, Hellwig JP, Meyers LD, editors. Dietary reference intakes: the essential guide to nutrient requirements. Washington, DC: National Academies Press; 2006. 543 p.

18. Iron in diet: MedlinePlus Medical Encyclopedia [Internet]. [cited 2018 Aug 6]. Available from: https://medlineplus.gov/ency/article/002422.htm

19. Anderson GJ, Frazer DM. Current understanding of iron homeostasis. Am J Clin Nutr. 2017;106:1559S-1566S.

20. Dunn LL, Suryo Rahmanto Y, Richardson DR. Iron uptake and metabolism in the new millennium. Trends Cell Biol. 2007;17:93–100.

21. Gulec S, Anderson GJ, Collins JF. Mechanistic and regulatory aspects of intestinal iron absorption. Am J Physiol Gastrointest Liver Physiol. 2014;307:G397-409.

22. Martins AC, Almeida JI, Lima IS, Kapitão AS, Gozzelino R. Iron Metabolism and the Inflammatory Response. IUBMB Life. 2017;69:442–50.

23. Camaschella C. Iron-deficiency anemia. N Engl J Med. 2015;372:1832–43.

24. Vandevijvere S, Michels N, Verstraete S, Ferrari M, Leclercq C, Cuenca-García M, Grammatikaki E, Manios Y, Gottrand F, Santamaría JV, et al. Intake and dietary sources of haem and non-haem iron among European adolescents and their

association with iron status and different lifestyle and socio-economic factors. Eur J Clin Nutr. 2013;67:765–72.

25. Waldvogel-Abramowski S, Waeber G, Gassner C, Buser A, Frey BM, Favrat B, Tissot J-D. Physiology of Iron Metabolism. Transfus Med Hemother. 2014;41:213–21.

26. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem. 2001;276:7806–10.

27. Krause A, Neitz S, Mägert HJ, Schulz A, Forssmann WG, Schulz-Knappe P, Adermann K. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett. 2000;480:147–50.

28. Ueda N, Takasawa K. Role of hepcidin-25 in chronic kidney disease: Anemia and beyond. Curr Med Chem. 2017;24:1417–52.

29. Yadin D, Knaus P, Mueller TD. Structural insights into BMP receptors: Specificity, activation and inhibition. Cytokine Growth Factor Rev. 2016;27:13–34.

30. Goh JB, Wallace DF, Hong W, Subramaniam VN. Endofin, a novel BMP-SMAD regulator of the iron-regulatory hormone, hepcidin. Sci Rep. 2015;5:13986.

31. Auguet T, Aragonès G, Berlanga A, Martínez S, Sabench F, Binetti J, Aguilar C, Porras JA, Molina A, Del Castillo D, et al. Hepcidin in morbidly obese women with non-alcoholic fatty liver disease. PLoS ONE. 2017;12:e0187065.

32. Enriori PJ, Evans AE, Sinnayah P, Cowley MA. Leptin resistance and obesity. Obesity (Silver Spring). 2006;14 Suppl 5:254S-258S.

33. Chung B, Matak P, McKie AT, Sharp P. Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. J Nutr. 2007;137:2366–70.

34. Lee P, Peng H, Gelbart T, Beutler E. The IL-6- and lipopolysaccharide-induced transcription of hepcidin in HFE-, transferrin receptor 2-, and beta 2-microglobulin-deficient hepatocytes. Proc Natl Acad Sci USA. 2004;101:9263–5.

35. Hare DJ. Hepcidin: a real-time biomarker of iron need. Metallomics. 2017;9:606–18.

36. Nikonorov AA, Skalnaya MG, Tinkov AA, Skalny AV. Mutual interaction between iron homeostasis and obesity pathogenesis. J Trace Elem Med Biol. 2015;30:207–14.

37. Ćulafić J, Kolarović J, Pezo L, Čabarkapa V, Nikolić S, Stojadinović A, Solarov MB. Serum concentration of hepcidin as an indicator of iron reserves in children. J Med Biochem. 2018;37:456–64.

38. Zaritsky J, Young B, Wang H-J, Westerman M, Olbina G, Nemeth E, Ganz T, Rivera S, Nissenson AR, Salusky IB. Hepcidin—a potential novel biomarker for iron status in chronic kidney disease. Clin J Am Soc Nephrol. 2009;4:1051–6.

39. Nazif HK, El-Shaheed AA, El-Shamy KAI, Mohsen MA, Fadl NN, Moustafa RSI. Study of serum hepcidin as a potential mediator of the disrupted iron metabolism in obese adolescents. Int J Health Sci (Qassim). 2015;9:172–8.

40. McClung JP. Iron, zinc, and physical performance. Biol Trace Elem Res. 2019;188:135–9.

41. Klinge S, Hirst J, Maman JD, Krude T, Pellegrini L. An iron-sulfur domain of the eukaryotic primase is essential for RNA primer synthesis. Nat Struct Mol Biol. 2007;14:875–7.

42. Puig S, Ramos-Alonso L, Romero AM, Martínez-Pastor MT. The elemental role of iron in DNA synthesis and repair. Metallomics. 2017;9:1483–500.

43. Chen M-J, Chou C-H, Shun C-T, Mao T-L, Wen W-F, Chen C-D, Chen S-U, Yang Y-S, Ho H-N. Iron suppresses ovarian granulosa cell proliferation and arrests cell cycle through regulating p38 mitogen-activated protein kinase/p53/p21 pathway. Biol Reprod. 2017;97:438–48.

44. Backe MB, Moen IW, Ellervik C, Hansen JB, Mandrup-Poulsen T. Iron regulation of pancreatic beta-cell functions and oxidative stress. Annual Review of Nutrition. 2016;36:241–73.

45. Gourley BL, Parker SB, Jones BJ, Zumbrennen KB, Leibold EA. Cytosolic aconitase and ferritin are regulated by iron in caenorhabditis elegans. J Biol Chem. 2003;278:3227–34.

46. Anderson CP, Shen M, Eisenstein RS, Leibold EA. Mammalian iron metabolism and its control by iron regulatory proteins. Biochim Biophys Acta. 2012;1823:1468–83.

47. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: Regulation of mammalian iron metabolism. Cell. 2010;142:24–38.

48. Maio N, Rouault TA. Iron-sulfur cluster biogenesis in mammalian cells: New insights into the molecular mechanisms of cluster delivery. Biochim Biophys Acta. 2015;1853:1493–512.

49. Ng O. Iron, microbiota and colorectal cancer. Wien Med Wochenschr. 2016;166:431–6.

50. Andrews SC, Robinson AK, Rodriguez-Quinones F. Bacterial iron homeostasis. Oxford Academic. 27:215–37.

51. Verbon EH, Trapet PL, Stringlis IA, Kruijs S, Bakker PAHM, Pieterse CMJ. Iron and immunity. Annu Rev Phytopathol. 2017;55:355–75.

52. Soyano A, Gómez M. Role of iron in immunity and its relation with infections. Arch Latinoam Nutr. 1999;49:40S-46S.

53. Cherayil BJ. The role of iron in the immune response to bacterial infection. Immunol Res. 2011;50:1–9.

54. Fargion S, Valenti L, Fracanzani AL. Hemochromatosis gene (HFE) mutations and cancer risk: Expanding the clinical manifestations of hereditary iron overload. Hepatology. 2010;51:1119–21.

55. Gozzelino R, Andrade BB, Larsen R, Luz NF, Vanoaica L, Seixas E, Coutinho A, Cardoso S, Rebelo S, Poli M, et al. Metabolic adaptation to tissue iron overload confers tolerance to malaria. Cell Host & Microbe. 2012;12:693–704.

56. Luo J, Hendryx M, Dinh P, He K. Association of iodine and iron with thyroid function. Biol Trace Elem Res. 2017;179:38–44.

57. Szczepanek-Parulska E, Hernik A, Ruchała M. Anemia in thyroid diseases. Pol Arch Intern Med. 2017;127:352–60.

58. Li S, Gao X, Wei Y, Zhu G, Yang C. The relationship between iron deficiency and thyroid function in chinese women during early pregnancy. J Nutr Sci Vitaminol. 2016;62:397–401.

59. Sheftel A, Stehling O, Lill R. Iron–sulfur proteins in health and disease. Trends Endocrinol Metab. 2010;21:302–14.

60. Paul VD, Lill R. Biogenesis of cytosolic and nuclear iron–sulfur proteins and their role in genome stability. Biochimica et Biophysica Acta. 2015;1853:1528–39.

61. Lill R. Function and biogenesis of iron–sulphur proteins. Nature. 2009;460:831–8.

62. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. The Lancet. 2016;387:907–16.

63. Miller JL. Iron deficiency anemia: a common and curable disease. Cold Spring Harb Perspect Med. 2013;3.

64. Fernández-Gaxiola AC, De-Regil LM. Intermittent iron supplementation for reducing anaemia and its associated impairments in adolescent and adult menstruating women. Cochrane Database Syst Rev. 2019;1:CD009218.

65. Iorember F, Aviles D. Anemia in nephrotic syndrome: approach to evaluation and treatment. Pediatr Nephrol. 2017;32:1323–30.

66. Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and management. Blood. 2018;131:505–14.

67. Nair MK, Augustine LF, Konapur A. Food-based interventions to modify diet quality and diversity to address multiple micronutrient deficiency. Front Public Health. 2015;3:277.

68. Ramsay LC, Charles CV. Review of iron supplementation and fortification. Topics in Public Health [Internet]. 2015 [cited 2019 Feb 28]; Available from: https://www.intechopen.com/books/topics-in-public-health/review-of-iron-supplementation-and-fortification

69. Harika R, Faber M, Samuel F, Mulugeta A, Kimiywe J, Eilander A. Are low intakes and deficiencies in iron, vitamin A, zinc, and iodine of public health concern in Ethiopian, Kenyan, Nigerian, and South African children and adolescents? Food Nutr Bull. 2017;38:405–27.

70. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454:428–35.

71. Van Linthout S, Tschöpe C. Inflammation – cause or consequence of heart failure or both? Curr Heart Fail Rep. 2017;14:251–65.

72. Yuan H, Wang W, Chen D, Zhu X, Meng L. Effects of a treatment with Se-rich rice flour high in resistant starch on enteric dysbiosis and chronic inflammation in diabetic ICR mice. J Sci Food Agric. 2017;97:2068–74.

73. Zhou X, Wang F, Yang H, Chen J, Ren Y, Yuan Z, Wang X, Wang Y. Selenium-enriched exopolysaccharides produced by Enterobacter cloacae Z0206 alleviate adipose inflammation in diabetic KKAy mice through the AMPK/SirT1 pathway. Mol Med Rep. 2014;9:683–8.

74. McNelis JC, Olefsky JM. Macrophages, immunity, and metabolic disease. Immunity. 2014;41:36–48.

75. Del Giudice M, Gangestad SW. Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. Brain Behav Immun. 2018;70:61–75.

76. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes. 2007;56:1010–3.

77. Fransson EI, Batty GD, Tabák AG, Brunner EJ, Kumari M, Shipley MJ, Singh-Manoux A, Kivimäki M. Association between change in body composition and change in inflammatory markers: An 11-year follow-up in the Whitehall II Study. J Clin Endocrinol Metab. 2010;95:5370–4.

78. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-α and IL-6. Diabetes Res Clin Pract. 2005;69:29–35.

79. Calvani R, Marini F, Cesari M, Buford TW, Manini TM, Pahor M, Leeuwenburgh C, Bernabei R, Landi F, Marzetti E. Systemic inflammation, body composition, and physical performance in old community-dwellers. J Cachexia Sarcopenia Muscle. 2017;8:69–77.

80. Londhe P, Guttridge DC. Inflammation induced loss of skeletal muscle. Bone. 2015;80:131–42.

81. Karatela S, Ward NI. Trace elements and human obesity: An overview. Manipal Journal of Nursing and Health Science. 2016;2:10.

82. Zavala G, Long KZ, García OP, Caamaño M del C, Aguilar T, Salgado LM, Rosado JL. Specific micronutrient concentrations are associated with inflammatory cytokines in a rural population of Mexican women with a high prevalence of obesity. Br J Nutr. 2013;109:686–94.

83. Goodpaster BH, Krishnaswami S, Resnick H, Kelley DE, Haggerty C, Harris TB, Schwartz AV, Kritchevsky S, Newman AB. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. Diabetes Care. 2003;26:372–9.

84. Anemia of Inflammation & Chronic Disease | NIDDK [Internet]. National Institute of Diabetes and Digestive and Kidney Diseases. [cited 2018 Sep 6]. Available from: https://www.niddk.nih.gov/health-information/blood-diseases/anemia-inflammation-chronic-disease

85. Wang Y, Wang H, Hegde V, Dubuisson O, Gao Z, Dhurandhar NV, Ye J. Interplay of pro- and anti-inflammatory cytokines to determine lipid accretion in adipocytes. Int J Obes (Lond). 2013;37:1490–8.

86. Petkova-Marinova TV, Ruseva BK, Atanasova BD. Selenium deficiency as a risk factor for development of anemia. J Biomed Clin Res. 2017;10:9–17.

87. Cheng HL, Bryant CE, Rooney KB, Steinbeck KS, Griffin HJ, Petocz P, O'Connor HT. Iron, hepcidin and inflammatory status of young healthy overweight and obese women in Australia. PLoS ONE. 2013;8:e68675.

88. Ferrari M, Cuenca-García M, Valtueña J, Moreno LA, Censi L, González-Gross M, Androutsos O, Gilbert CC, Huybrechts I, Dallongeville J, et al. Inflammation profile in overweight/obese adolescents in Europe: an analysis in relation to iron status. Eur J Clin Nutr. 2015;69:247–55.

89. Andrews M, Soto N, Arredondo-Olguín M. Association between ferritin and hepcidin levels and inflammatory status in patients with type 2 diabetes mellitus and obesity. Nutrition. 2015;31:51–7.

90. Combs GF. Biomarkers of selenium status. Nutrients. 2015;7:2209–36.

91. Brown K, Arthur J. Selenium, selenoproteins and human health: a review. Public Health Nutr. 2001;4:593–9.

92. Barbosa KBF, Volp ACP, Marques-Rocha JL, Ribeiro SMR, Navarro-Blasco I, Zulet MÁ, Martínez JA, Bressan J. Modulators of erythrocyte glutathione peroxidase activity in healthy adults: An observational study. Redox Rep. 2014;19:251–8.

93. Matoušková P, Hanousková B, Skálová L. MicroRNAs as potential regulators of glutathione peroxidases expression and their role in obesity and related pathologies. Int J Mol Sci. 2018;19.

94. Burk RF, Hill KE. Selenoprotein P – expression, functions, and roles in mammals. Biochim Biophys Acta. 2009;1790:1441–7.

95. Méplan C. Selenium and chronic diseases: A nutritional genomics perspective. Nutrients. 2015;7:3621–51.

96. Méplan C, Nicol F, Burtle BT, Crosley LK, Arthur JR, Mathers JC, Hesketh JE. Relative abundance of selenoprotein P isoforms in human plasma depends on genotype, Se intake, and cancer status. Antioxid Redox Signal. 2009;11:2631–40.

97. Fairweather-Tait SJ, Bao Y, Broadley MR, Collings R, Ford D, Hesketh JE, Hurst R. Selenium in human health and disease. Antioxid Redox Signal. 2011;14:1337–1383.

98. Economic Impact Analyses of FDA Regulations - Summary: The Addition of Minimum and Maximum Levels of Selenium to Infant Formula and Related Labeling Requirements (Final Rule). Version current 23 June 2015. [Internet]. [cited 2018 Apr 8]. Available from: https://www.fda.gov/aboutfda/reportsmanualsforms/reports/economicanalyses/ucm496769.htm (accessed 8 April 2018)

99. Food and Drug Administration, HHS. Food labeling: revision of the nutrition and supplement facts labels. final rule. Fed Regist. 2016;81:33741–999.

100. Rayman MP. Selenium and human health. The Lancet. 2012;379:1256–68.

101. Zhang W, Zhang R, Wang T, Jiang H, Guo M, Zhou E, Sun Y, Yang Z, Xu S, Cao Y, et al. Selenium inhibits LPS-induced pro-inflammatory gene expression by modulating MAPK and NF-κB signaling pathways in mouse mammary epithelial cells in primary culture. Inflammation. 2014;37:478–85.

102. Asemi Z, Jamilian M, Mesdaghinia E, Esmaillzadeh A. Effects of selenium supplementation on glucose homeostasis, inflammation, and oxidative stress in gestational diabetes: randomized, double-blind, placebo-controlled trial. Nutrition. 2015;31:1235–42.

103. Mutakin M, Meiliana A, Wijaya A, Kobayashi K, Yamazaki C, Kameo S, Nakazawa M, Koyama H. Association between selenium nutritional status and metabolic risk factors in men with visceral obesity. J Trace Elem Med Biol. 2013;27:112–6.

104. Tseng C–., Ho C–., Hsu H–., Lin C–., Li C–., Li T–., Liu C–., Lin C–., Lin W. Selenium is inversely associated with interleukin-6 in the elderly. J Nutr Health Aging. 2013;17:280–4.

105. Sedighinejad A, Imantalab V, Mirmansouri A, Mohammadzadeh Jouryabi A, Kanani G, Nassiri Sheikhani N, Haghighi M, Atrkarroushan Z, Biazar G. Effects of low-dose selenium on the inflammatory response in coronary artery bypass graft surgery: a clinical trial. Iran Red Crescent Med J. 2016;18:e37918.

106. Colpo E, Dalton Vilanova C, Reetz LGB, Duarte MMMF, Farias ILG, Meinerz DF, Mariano DOC, Vendrusculo RG, Boligon AA, Dalla Corte CL, et al. Brazilian nut consumption by healthy volunteers improves inflammatory parameters. Nutrition. 2014;30:459–65.

107. Fantuzzi G, Braunschweig C, editors. Adipose tissue and adipokines in health and disease [Internet]. 2nd ed. Humana Press; 2014 [cited 2018 May 21]. Available from: //www.springer.com/us/book/9781627037693

108. Guarino G, Corte DP, Fontana S, Strollo F, Cecaro M, Gentile S, Ragozzino G. Selenium supplementation in obese patients with subclinical hypothyroidism and type 2 diabetes. J Nutr Health Sci [Internet]. 2018 [cited 2018 Sep 6];5. Available from: /paper/Selenium-Supplementation-in-Obese-Patients-with-and-Guarino-Corte/b415aa27a8216009cb35f0a2b6cdcb0335dfb611

109. Wang Y, Gao X, Pedram P, Shahidi M, Du J, Yi Y, Gulliver W, Zhang H, Sun G. Significant beneficial association of high dietary selenium intake with reduced body fat in the CODING study. Nutrients. 2016;8.

110. Liao C, Carlson BA, Paulson RF, Prabhu KS. The intricate role of selenium and selenoproteins in erythropoiesis. Free Radic Biol Med. 2018;

111. Cardenas-Rodriguez M, Chatzi A, Tokatlidis K. Iron–sulfur clusters: from metals through mitochondria biogenesis to disease. J Biol Inorg Chem. 2018;23:509–20.

112. Houghton LA, Parnell WR, Thomson CD, Green TJ, Gibson RS. Serum zinc Is a major predictor of anemia and mediates the effect of selenium on hemoglobin in school-aged children in a nationally representative survey in New Zealand. J Nutr. 2016;146:1670–6.

113. Sun GX, Chen Y, Liu CP, Li S, Fu J. Effect of selenium against lead-induced damage on the gene expression of heat shock proteins and inflammatory cytokines in peripheral blood lymphocytes of chickens. Biol Trace Elem Res. 2016;172:474–80.

114. Garcia-Santos D, Hamdi A, Saxova Z, Fillebeen C, Pantopoulos K, Horvathova M, Ponka P. Inhibition of heme oxygenase ameliorates anemia and reduces iron overload in a β-thalassemia mouse model. Blood. 2018;131:236–46.

115. Tron K, Novosyadlyy R, Dudas J, Samoylenko A, Kietzmann T, Ramadori G. Upregulation of heme oxygenase-1 gene by turpentine oil-induced localized inflammation: involvement of interleukin-6. Lab Invest. 2005;85:376–87.

116. Petrova J, Manolov V, Hadjidekova S, Yonova D, Bogov B, Vasilev V, Tzatchev K, Petrova M, Vazelov E, Georgieva I, et al. Quantification of hepcidin, Se, Zn, SOD and GPX in chronic kidney disease patients with ischemic stroke. Austin J Cardiovasc Dis Atherosclerosis. 2017;4:1032.

117. Semba R, Ricks M, Ferrucci L, Xue Q-L, Guralnik J, Fried L. Low serum selenium is associated with anemia among older adults in the United States. Eur J Clin Nutr. 2009;63:93–9.

118. Van Nhien N, Yabutani T, Khan NC, Khanh LNB, Ninh NX, Chung LTK, Motonaka J, Nakaya Y. Association of low serum selenium with anemia among adolescent girls living in rural Vietnam. Nutrition. 2009;25:6–10.

119. Lee O, Chung Y, Moon J. Iron status according to serum selenium concentration and physique in young female adults. Korean J Nutr. 2010;43:114–22.

120. Mcanulty LS, Gropper SS, Mcanulty SR, Keith RE. Iron depletion without anemia is not associated with impaired selenium status in college-aged women. Biol Trace Elem Res. 2003;91:125–36.

121. Sedighi O, Zargari M, Varshi G. Effect of selenium supplementation on glutathione peroxidase enzyme activity in patients with chronic kidney disease: a randomized clinical trial. Nephrourol Mon. 2014;6:e17945.

122. Pan C-F, Lin C-J, Chen S-H, Huang C-F, Lee C-C. Association between trace element concentrations and anemia in patients with chronic kidney disease: a cross-sectional population-based study. J Investig Med. 2019;

123. Patel KV. Epidemiology of Anemia in Older Adults. Seminars in Hematology. 2008;45:210–7.

124. Le CHH. The prevalence of anemia and moderate-severe anemia in the US population (NHANES 2003-2012). PLoS ONE. 2016;11:e0166635.

125. Roy CN. Anemia of inflammation. Hematology. 2010;2010:276–80.

126. Nemeth E. Targeting the Hepcidin-Ferroportin Axis in the Diagnosis and Treatment of Anemias [Internet]. Advances in Hematology. 2010 [cited 2018 Aug 20]. Available from: https://www.hindawi.com/journals/ah/2010/750643/

127. Gürgöze MK, Denizmen Aygün A, Ölçücü A, Doğan Y, Yılmaz E. Plasma selenium status in children with iron deficiency anemia. J Trace Elem Med Biol. 2004;18:193–6.

128. Walston J, Xue Q, Semba RD, Ferrucci L, Cappola AR, Ricks M, Guralnik J, Fried LP. Serum antioxidants, inflammation, and total mortality in older women. Am J Epidemiol. 2006;163:18–26.

129. Liao C, Hardison RC, Kennett MJ, Carlson BA, Paulson RF, Prabhu KS. Selenoproteins regulate stress erythroid progenitors and spleen microenvironment during stress erythropoiesis. Blood. 2018;blood-2017-08-800607.

130. Centers for Disease Control and Prevention. National Health and Nutrition Health Survey. Anthropometry procedures manual [Internet]. Available from: Version

current January 2007. Internet.
https://wwwn.cdc.gov/nchs/data/nhanes3/manuals/anthro.pdf (accessed 7 September 2018)

131. Cheng HL, Bryant C, Cook R, O'Connor H, Rooney K, Steinbeck K. The relationship between obesity and hypoferraemia in adults: a systematic review. Obes Rev. 2012;13:150–61.

132. Hamza RT, Hamed AI, Kharshoum RR. Iron homeostasis and serum hepcidin-25 levels in obese children and adolescents: relation to body mass index. Horm Res Paediatr. 2013;80:11–7.

133. Sun CC, Vaja V, Babitt JL, Lin HY. Targeting the hepcidin-ferroportin axis to develop new treatment strategies for anemia of chronic disease and anemia of inflammation. Am J Hematol. 2012;87:392–400.

134. Sangkhae V, Nemeth E. Regulation of the iron homeostatic hormone hepcidin. Adv Nutr. 2017;8:126–36.

135. Hurst R, Armah CN, Dainty JR, Hart DJ, Teucher B, Goldson AJ, Broadley MR, Motley AK, Fairweather-Tait SJ. Establishing optimal selenium status: results of a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr. 2010;91:923–31.

136. Hosseini B, Saedisomeolia A, Allman-Farinelli M. Association between antioxidant intake/status and obesity: A systematic review of observational studies. Biol Trace Elem Res. 2017;175:287–97.

137. Bellinger FP, Raman AV, Reeves MA, Berry MJ. Regulation and function of selenoproteins in human disease. Biochem J. 2009;422:11–22.

138. Manolov V, Hadjidekova S, Petrova J, Vasilev V, Petrova M, Traykov L, Tzatchev K, Marinov B, Emilova R, Bogov I. The role of iron homeostasis in alzheimer's disease. J Alzheimers Neurodegener Dis. 2017;3:1–4.

APPENDIX A

ADDITIONAL RESULTS

Hemoglobin concentration was significantly higher in males (15.39±1.06g/dl) compared to females (12.58±1.3 g/dl). Consequently, ferritin was significantly higher in males than females. Total body fat percentages were 14% significantly higher in females (34.14±6.85) than males (20.25±7.1). There were no significant differences in GPX activity and Selenoprotein P in males compared to females, **Table A.1**

Table A.1 Anthropometric, Biochemical and Nutrient Data according to Gender[1]

	Male	Female	p-value[3]
Plasma selenium, ng/ml[2]	116.03 (95, 141)	111.9 (90, 140)	0.546
GPX lysate activity, nmol/min/ml	1030.02±372.79	1046.87±415.28	0.88
GPX plasma activity, nmol/min/ml	77.03±22.14	83.36±16.9	0.341
SEPP1, ng/ml[2]	380.64 (298, 483)	346.56 (276, 437)	0.184
BMI, kg/m^2	26.67±4.79	25.94±4.74	0.596
Dietary selenium, mcg[4,5]	108.73 (66, 166)	104.34 (75, 158)	0.72
Hemoglobin, g/dl	15.39±1.06	12.58±1.3	0.0000001***
Ferritin[2], ng/ml	98.47 (49, 196)	26.57(9, 74)	0.00001***
Total body fat, %	20.25±7.1	34.14±6.85	0.000001***
Visceral fat, level	5.03±4	3.56±2.48	0.173
Hepcidin, ng/ml[2]	25.56 (10, 62)	9.33 (2, 36)	0.002**

[1]Values are mean±SD
[2]Values are in geometric means (±1SD)
[3]p-values are based on Independent t-test

[4]p-values are based on Wilcoxin rank sum test
[5]Values are median (interquartile range)

When subjects were categorized based on ethnicity (Table A.2), individuals with overweight/obesity were significantly higher among Non-Hispanic Blacks (28.34±4.82kg/m^2) compared to Non-Hispanic Whites (23.82±3.66 kg/m^2) (Table 4). Overall, total body fat percentages were 10% significantly higher in Non-Hispanic Whites and Other races compared with Non-Hispanic Blacks ($p<0.01$). Visceral fat levels were approximately 3 levels higher in Non-Hispanic Blacks compared with Non-Hispanic Whites. ANOVA showed significantly lower hemoglobin concentrations among Non-Hispanic Blacks (12.6±1.5g/dl) compared with Other Races (14.65±1.26g/dl). Geometric means for ferritin were significantly higher in Other Races compared to the three ethnic groups. Post hoc analysis showed no significant differences in dietary selenium, GPX activity and selenoprotein P among all ethnic groups **(Table A.2)**.

Table A.2 Anthropometric, Biochemical and Nutrient Data according to Ethnicity[1]

	Non-Hispanic White	Hispanic	Non-Hispanic Black	Other Race[3]
Plasma selenium, ng/ml[2]	120.77 (98, 148)	118.95 (96, 148)	107.06 (87, 130)	112.38 (86, 145)
Erythrocyte GPX activity, nmol/min/ml	1013.69±421.17	908.18±475.86	1091.96±378.54	1048.44±411.82

Plasma GPX activity, nmol/min/ml	83.13±16.98	74.94±20.88	79.57±16.18	89.02±23.63
SEPP1, ng/ml[2]	345.22 (279, 424)	392.66 (281, 545)	344.51 (273, 433)	385.44 (308, 478)
BMI, kg/m^2	23.82±3.66[a]	24.42±1.57[ab]	28.34±4.82[b]	25.88±5.4[ab]
Dietary selenium, mcg[2]	103.61 (74, 145)	98.8 (65, 150)	107.98 (67, 172)	107.48 (63, 185)
Hemoglobin, g/dl	13.81±2.1[ab]	13.03±0.84[ab]	12.6±1.5[a]	14.65±1.26[b]
Ferritin, ng/ml[2]	35.82 (12, 110)[a]	26.08 (8, 81)[a]	30.19 (10, 89)[a]	105.92 (56, 198)[b]
Total body fat, %	25.66±8.21[a]	29.38±8.68[ab]	35.54±6.95[b]	25.15±10.75[a]
Visceral fat, level	2.5±1.85[a]	2.42±0.8[ab]	5.26±3.34[b]	4.4±3.47[ab]
Hepcidin, ng/ml[2]	11.51 (3, 45)[ab]	5.7 (3, 12)[a]	10.51 (3, 38)[ab]	35.35 (11, 110)[b]

[1]Mean values with different superscripts are significantly different ($p<0.05$), based on Tukey's post-hoc test

[2]Values are geometric means (±1SD)

[3]Other Race are Asians, Arabs, Multiracial and Persians

APPENDIX B

CONSENT FORM

Project Title: Selenium and Inflammation study

Student Principal Investigator: Doreen Larvie

Faculty Advisor: Seth Armah (PhD)

Participant's Name: ________________________

What are some general things you should know about research studies?

You are being asked to take part in a research study. Your participation in the study is voluntary. You may choose not to join, or you may withdraw your consent to be in the study, for any reason, without penalty. This research does not relate to any course credits and participating or not participating in the research will not affect in any way the relationship between the principal investigator and students or other university employees. Research studies are designed to obtain new knowledge. This new information may help people in the future. There may be a direct benefit to you for being in the research study. There also may be risks to being in research studies. If you choose not to be in the study or leave the study before it is done, it will not affect your relationship with the researcher or the University of North Carolina at Greensboro. Details about this study are discussed in this consent form. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. If you have any questions

about this study at any time, you should ask the researchers named in this consent form. Their contact information is below.

What is the study about?

This is a research project. Your participation is voluntary. This study is being conducted to investigate the association among selenium status and iron markers in blood and body weight. Obesity is associated with chronic low-grade inflammation. Inflammation (the body's response to injury) is one of the main causes of anemia. This happens because inflammation increases the concentration of hepcidin in the blood. Hepcidin is a protein that controls the release of iron for making red blood cells. Selenium (an essential nutrient required in small amounts) may reduce inflammation because it is an antioxidant. This study will investigate the relationship among selenium, iron markers and body weight to determine if adequate selenium status may reduce inflammation, decrease hepcidin levels and reduce the risk of anemia due to inflammation.

Why are you asking me?

You are being invited to participate in this study because you meet the following criteria: adult 18-49 years old, with a BMI of 18.5 kg/m^2 or over; not used any mineral or vitamin supplement (including selenium) in the past one month and have not donated blood in the past two months, not a smoker, not pregnant and not lactating, have no history of any chronic disease such as cancer, diabetes, kidney disease or any autoimmune disease; not having a chronic infection; not using any drug that reduces or increases inflammation and not a professional athlete.

What will you ask me to do if I agree to be in the study?

If you agree to participate in this study, you will be required to go through a screening procedure where your height and weight will be measured and you will complete a screening form to assess your eligibility for the study. The screening procedure will take approximately 30 minutes. In the screening form, you will provide information such as current medication, use of mineral/vitamin supplement, and pregnancy status (for females). If you qualify based on this information, you will be provided with a 3-day dietary record form and a food frequency questionnaire to complete and return at your study visit. Instructions for completing the dietary record and food frequency questionnaire will be provided by a research assistant. The food frequency questionnaire will require information on how often you have consumed selenium rich foods over the past 2 years while the dietary record will require you to keep a log of all foods consumed on selected days (2week days and 1 weekend day) between your screening and study visit. You will be provided with a food weighing scale to weigh and record foods consumed. The study visit will be scheduled between you and the research assistant.

For your study visit, you will be asked to do an overnight fast. You will not consume any food or drink (except water) 10 hours prior to your visit. On the morning of your study visit, the dietary record and the food frequency questionnaire will be collected and reviewed by the research assistant. Your height, weight and body composition measurements such as visceral (abdominal) fat, and total body fat will also be taken. Your body composition will be measured by means of a bioelectrical impedance analyzer

which works by passing a very low, safe electrical current from four electrodes in contact with the hands and feet to the abdomen. The electrical signal passes through different body components and the level of resistance (impedance) to the current will be measured and used in a prediction equation to calculate estimates of your body composition. To use the bioelectrical impedance analyzer, you will be required to remove shoe and socks from both feet, step on the metal electrodes of the analyzer and hold the handle at a horizontal level from the ground away from you. Within 20seconds, your body composition measurements will be available to view and you can step off the analyzer. Venous blood sample will also be collected by a trained technician. Approximately 2 table spoons of blood (30 ml) of blood will be collected to measure the levels of iron markers, hepcidin concentration and markers of inflammation. The study visit will 30 minutes.

What are the risks to me?

The Institutional Review Board at the University of North Carolina at Greensboro has determined that participation in this study poses minimal risk to participants. While participating in this study, you may experience slight discomfort from blood draw. You may also experience some bruising after the blood draw. Risk of infection is very minimal. There is also possible risk of feeling lightheaded after blood draw. However, to minimize these risks, a trained technician who knows how to monitor for these possible risks will draw the blood samples. To avoid infection, single-use phlebotomy equipment will be used only once, and contaminated equipment will also be avoided. Appropriate hand-hygiene materials (soap and water or alcohol rub), well-fitting non-sterile gloves,

single-use disposable needles will be used. The volume of blood to be collected at each visit (two table spoons) does not pose any health risk.

Are there any benefits to society as a result of me taking part in this research?

It is hoped that the information gained in this study may benefit society by helping understand how selenium status is associated with inflammation and how that can be used to address anemia due to inflammation among overweight and obese individuals.

Are there any benefits to *me* for taking part in this research study?

If you decide to participate in this study, there will be no direct benefit to you.

Will I get paid for being in the study? Will it cost me anything?

You will not have any costs from participating in this study. At end of the study, you will receive a $60 gift card. Compensation will be given after the study visit. Since this is a cross-sectional study, there will be no monetary compensation for individuals who do not complete the study.

How will you keep my information confidential?

All information obtained in this study is strictly confidential unless disclosure is required by law. The questionnaires and data records will be locked up in secure filing cabinets in the office of the faculty advisor and will be accessible to only the student principal investigator and the research staff approved by the UNCG IRB. All participant names will be replaced with numeric codes (ID). These codes will be used to identify blood samples to ensure confidentiality. Also, soft copies of data will be kept on a password protected computer to avoid access by inappropriate persons. Only one file (the key) on the password-protected computer, a back-up file on a CD/DVD, and one hard

copy will have your name linked to your ID. All electronic copies of the data will be stored in the UNCG box account for the study e-mail. Identifying information will be destroyed 5 years after completion of the study. De-identified blood samples will be stored at the University of North Carolina Greensboro. Some of the de-identified blood samples (including only study ID and date of birth) will be sent to an outside laboratory for analysis. De-identified data and blood samples after the research study will be stored at UNCG and will be re-analyzed if there is a need for further testing to better understand the relationship among selenium, iron biomarkers and body weight. If the results are published, your identity will remain confidential. If you do not qualify for the study, all information collected from you during screening such as medical history information, weight and height measurements, pregnancy and lactation status, and age will be shredded within a month except your signed informed consent form.

What if I want to leave the study?

You have the right to refuse to participate or to withdraw at any time, without penalty. If you do withdraw, it will not affect you in any way. If you choose to withdraw, you may request that any of your data which has been collected be destroyed unless it is in a de-identifiable state. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

What about new information/changes in the study?

If significant new information relating to the study becomes available which may relate to your willingness to continue to participate, this information will be provided to

you. If you have questions, want more information or have suggestions, please contact (Dr. Seth Armah, who may be reached at 336-256-0324 or s_armah@uncg.edu).

If you have any concerns about your rights, how you are being treated, concerns or complaints about this project or benefits or risks associated with being in this study please contact the Office of Research Integrity at UNCG toll-free at (855)-251-2351.

Voluntary Consent by Participant:

By signing this consent form you are agreeing that you read, or it has been read to you, and you fully understand the contents of this document and are openly willing consent to take part in this study. All of your questions concerning this study have been answered. By signing this form, you are agreeing that you are 18 years of age or older and are agreeing to participate, in this study described to you by ____________________

Signature: _______________________Date: ____/____/____

UNCG IRB
Approved Consent Form
Valid from: 5/29/18 to **4/7/19**

APPENDIX C

SCREENING FORM

Date: _______________

Interviewer: __________________ Subject ID: ________________

1. Gender: female _____ male ________
2. Ethnicity: Asian ___ Hispanic ______ Non-Hispanic White _____ Non-Hispanic Black ____
   Multiracial ______ Other (please specify) _________________
3. Date of birth____________(mm/dd/yyyy);
4. Height (m) (i)___________ (ii) ___________ Average __________
5. Weight (kg) (i)___________ (ii) ___________ Average ___________ BMI (kg/m^2) ______________
6. If female, are you currently pregnant? Yes ___ no___ or breastfeeding? yes ____ no _______
7. Do you currently have any acute or chronic infection? yes____ no_____
8. Have you ever been diagnosed with a chronic condition or disease?
   Yes______ No________
   Describe if yes:
   ____
9. Are you currently taking any medications or drugs? yes_______ no_______
   Describe if yes: ____________________________________

10. Do you smoke? yes ______ no _______

11. Are you taking any vitamin/mineral supplements (including selenium)? __no

    __yes; If yes, which ones? ___________________

    Are you willing to not take any vitamin/mineral supplements during the study period? yes____ no_____

12. Have you donated blood in the past two months? yes____ no_____

    Are you willing to not donate blood during the study period? yes ___no ____

13. Are you a professional athlete? yes____ no_____

Eligible for inclusion ___ no ___ yes

APPENDIX D

DIETARY RECORD INSTRUCTIONS

TIME

Please record the time at which the meal was consumed.

FOOD ITEM DESCRIPTION

1. Indicate the following where necessary:
   - whether food was salted or not,
   - whether it was fresh, frozen, or canned,
2. If food was prepared at home, please write recipe at the back of the page and how much food was made compared to what was consumed.
3. Attach food labels where available
4. Indicate preparation method example: raw, boiled, baked, fried, etc.
5. List brand names where applicable
6. Record added items such as ketchup; condiments, margarine; butter; salt
7. Record all beverages consumed; indicated where applicable whether the beverage was

- caffeinated or not.
- sweetened or not

AMOUNT

Record only amount you actually consume

1. You can use household measures such as cup, teaspoons, table spoons. In that case please level the cup or the spoons
2. Cups or fluid ounces are both acceptable measuring units for beverages
3. Weigh solid foods that cannot be measured with cups or spoons. Weigh all solid foods in grams
4. Weigh the edible portion whenever possible. In situations where that is impossible, indicate that the weight or size includes refuse.

Using the weighing scale

1. Turn on the scale and make sure the unit is set to grams (g).
2. Place a paper plate on the scale.
3. Set the scale to zero by pressing on the Tare button.
4. Place the food item on the paper plate and read the weight.

APPENDIX E

DIETARY RECORD FORM

Complete the dietary record for three non-consecutive days (Two weekdays and a weekend day). Indicate the day and date on the dietary record form. For each day, record all foods and beverages consumed indicating brand names where necessary. To estimate serving size, you will be provided with a weighing scale to weigh foods consumed as much as possible. You may also use serving size indicated on packaged foods. Record the time at which each food was consumed.

Dietary Record Form- Day One

Subject ID___________

Day of the week ________________________ Date __________________________

Time	Food Item Description	Amount

Dietary Record Form- Day Two

Subject ID___________

Day of the week _______________________ Date _________________________

Time	Food Item Description	Amount

Dietary Record Form- Day Three

Subject ID____________

Day of the week _________________________ Date ___________________________

Time	Food Item Description	Amount