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THE EFFECTS OF IRON STATUS ON MOOD, STRESS, AND QUALITY OF LIFE IN WOMEN OF REPRODUCTIVE AGE

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ABSTRACT

BACKGROUND: Results from studies examining the relationship between iron and stress, iron and mood, and iron and quality of life in women of reproductive age (WRA) are inconsistent. We hypothesized that iron supplementation in iron deficient WRA would improve overall mood and quality of life and decrease feelings of stress compared to placebo in iron sufficient participants.

METHODS: WRA were recruited from Penn State University (n=33) and upon their initial visit, asked to complete questionnaires assessing the affective outcomes of mood via the Profile of Mood States (POMS), stress via the Perceived Stress Scale (PSS), and quality of life via the WHO Quality of Life (WHOQOL-BREF) and the SF36v2 Quality of Life (SF36v2). Their iron status was also measured via hemoglobin (Hb), ferritin (Ft), transferrin receptor (TfR), hematocrit (Hct), and alpha-1-acid-glycoprotein (AGP) at the initial visit through a blood screening. Participants categorized as iron sufficient (Hb > 12.0 g/dL, Ft > 20 μg/L, TfR < 8 mg/L) were instructed to take placebo, gelatin pills, daily for 4 months and participants categorized as iron depleted but not anemic (Hb > 12.0 g/dL, Ft < 20 μg/L, TfR > 8) were instructed to take 60 mg ferrous sulfate daily for 4 months. Participants were blinded to their treatment. After 4 months, participants returned and their iron status was re-assessed and they completed the same questionnaires (POMS, PSS, WHOQOL-BREF, and SF36v2). Statistical analyses included ANOVA/ANCOVA for cross-sectional analyses within a time point as well as an analysis in difference over time (subtracting baseline from endline), and Stepwise Regression. Data were analyzed using these statistical tests with groups categorized as iron deficient and iron

sufficient, as well as categorized as responders and non-responders based on changes in ferritin (Ft) for one analysis and hemoglobin (Hb) for another.

RESULTS: Iron had a significant role in improving the affective outcomes stress, mood, and quality of life. Specifically, iron treatment in iron deficient WRA improved feelings of Physical Functioning and Social Functioning and decreased feelings of Bodily Pain, Stress, Tension, Anger, Depression, Confusion and overall Mood Disturbance compared to placebo given to iron sufficient women. Participants whose Hb status improved over time (irrespective of treatment) experienced improvements in Social Functioning, Physical Health, and Social Health and decreases in Bodily Pain, Role limitations due to Emotional health, Stress, Tension, Anger, Fatigue, Depression, Confusion and overall Mood Disturbance compared to placebo. Participants whose Ft status improved (irrespective of treatment) experienced improvements in Social Functioning, Physical Health, Psychological Health, and Social Health and decreases in Bodily Pain, Role limitations due to Emotional health, Tension, Anger, Depression, and overall Mood Disturbance compared to placebo.

CONCLUSIONS: This study contributes to the evidence that iron, specifically treatment with iron via supplementation, is a promising mechanism for improving the affective outcomes of mood, stress, and quality of life in iron deficient WRA. However, given our sample size, more studies are needed to truly understand the effects of iron on affective outcomes in WRA.

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Chapter 1

Iron Nutrition

Iron and Females of Reproductive Age

Iron is an element abundant on earth and essential in the functioning of every living organism. Despite its abundance, iron metabolism is complicated. Iron, upon interaction with oxygen, forms oxides that are insoluble and thus not able to be taken up by organisms (Abbaspour et al. 2014), meaning that much of the abundance of iron is unable to be used by organisms. Iron is required for the synthesis of the proteins hemoglobin and myoglobin which help transport oxygen from the lungs to the tissues of the body. Iron is also involved in the formation of heme and other enzymes that assist in electron transfer and oxidation reductions that occur in a number of bodily processes (McDowell 2003). Iron also plays a role in DNA synthesis, metabolic energy, and cellular respiration (Loreal et al. 2014). Iron exists in many forms in the body, approximately 60% is found in the hemoglobin in erythrocytes and 25% is contained in a readily mobilizable iron store while the remaining 15% is bound to the myoglobin in muscle tissue and in the enzymes involved in oxidative metabolism and other cellular functions (Trumbo et al. 2001).

Iron is particularly important to women of reproductive age as they monthly lose blood and therefore, iron, through their menstrual cycle and is of particular importance for women preparing to have children. Iron requirements are higher for pregnant women and ensuring that women who may become pregnant have adequate iron status is essential for both their own

health and their future baby's health. The exact amount of iron required and recommended varies according to sex and age and current available iron status, though it is necessary no matter the sex or age. The amount of iron required for males and females remains the same until the individuals reach 14 years of age. At 14 years of age, the iron requirement is larger for females than males until 51 years of age. At 51 years of age, the iron requirements for males and females no longer differ because 51 years is the average age at which women reach menopause, where a woman's ovaries decrease the production of hormones and therefore the menstrual cycle stops and women lose less blood, decreasing iron requirements (Kaufert 1988). The current recommended dietary allowance (RDA), which is defined as the "average daily level of intake sufficient to meet the nutrient requirements of nearly all healthy individuals" of iron for females aged 14 to 18 years is 15 mg while the recommendation increases for women aged 19-50 years to 18 mg. The amount required for pregnant women is significantly higher as well: 27 mg (Institute of Medicine 2001).

Dietary iron has two forms: heme and non-heme. Non-heme iron is present in both animal and plant-based foods while heme iron is present in animal source foods, coming from hemoglobin or myoglobin. Heme iron is more easily absorbed in the body compared to non-heme because non-heme iron enters the common pool of iron in the digestive tract before it is absorbed and absorption of that iron depends on the body's iron status, whereas heme iron is able to be absorbed directly through the intestine without any transformations (Hurell, Egli 2010). Foods containing non-heme iron include nuts, beans, vegetables, and fortified grains. Heme iron is most richly available in lean meat and seafood. In the United States, about half of the dietary iron comes from fortified grains (U.S. Department of Agriculture 2013). The USDA Nutrient Database website provides a full list of foods and their nutrient content.

Iron Metabolism

Iron Storage and Loss

Iron in the human body is incorporated into proteins as a part of heme, iron-sulfur clusters, or other functional groups (Evstatiev et al. 2012). Due to previously mentioned scarce iron bioavailability, the human body efficiently stores and recycles iron. About 2 grams of iron (the majority) is contained in hemoglobin and is recycled via macrophages during erythrophagocytosis. Iron-recycling macrophages and liver hepatocytes are major storage sites for iron. The remaining cells in the body contain much smaller amounts of iron needed for various functions as previously mentioned. The pool of circulating iron (bound to transferrin) in the blood is small, about 2 to 4 mg (compared with 2 g in hemoglobin) and is turned over every few hours to meet daily iron requirements to support erythropoiesis and other needs (20 to 25 mg). One to two mg of iron is provided through daily iron absorption and is balanced by a daily one to two mg unregulated loss through the shedding of skin and blood loss. Urinary iron excretion is minimal due to iron typically being bound to the protein transferrin and the kidney working to reclaim iron (Yang et al. 2003, Dev, Babitt 2017). Overall, iron losses are minimal so dietary iron uptake and the release of iron from recycling macrophages and hepatocytes both work in order to regulate the body's iron balance (Dev, Babitt 2017).

Iron Absorption and Regulation

Iron can be absorbed from the diet in an inorganic form, in a heme form, or as ferritin. The absorption of iron in the inorganic form is the process that is best understood. Dietary inorganic

iron is typically present in the oxidized or ferric (Fe^{3+}) form but must be reduced to the ferrous (Fe^{2+}) form to be absorbed into the intestinal cell (enterocyte). This reduction reaction is facilitated by duodenal cytochrome B which requires ascorbic acid (vitamin C) (McKie et al. 2001). After reduced, iron in the ferrous (Fe^{2+}) form is transported across the apical membrane of the enterocyte via Divalent Metal Transporter 1 (DMT1) (Worthen et al. 2014).

Once in the enterocyte, iron can be used for the cell's metabolic processes, stored in the enterocyte in ferritin or hemosiderin, or exported across the basolateral membrane for use in other cells of the body. Iron's storage in ferritin allows for more control over the delivery of iron to the exporters on the basolateral membrane because iron that cannot be used immediately in the body, as controlled by hepcidin, can be stored in ferritin for later use when the body does need iron (Dev et al. 2017). Iron, still in the Fe^{2+} form, in order to be exported, must go through ferroportin, the only known cellular iron exporter, to enter the blood stream. Ferroportin is regulated via hepcidin. Hepcidin binds ferroportin when iron in the blood stream is sufficient in order to promote its internalization and degradation so as not to allow any more iron out of the enterocyte (Nemeth et al. 2004). Ferroportin exports iron from the enterocyte in the Fe^{2+} form after which it is oxidized by ceruloplasmin and hephestin to Fe^{3+} to be taken up by transferrin (Drakesmith et al. 2015). Transferrin then delivers iron to the transferrin receptor protein that is expressed on the surface of cells to take iron into other cells (besides enterocytes) for use.

Implications of Iron Nutrition

There are a number of diseases that have proven to be associated with iron homeostasis disorders, but two particular homeostasis problems are more prevalent and lead to a larger

number of iron-related problems: iron deficiency which can lead to anemia and iron overload or hemochromatosis.

Iron Deficiency and Iron Deficiency Anemia

It is estimated that about 7.6% of women in the US are anemic (Le 2016). Anemia leads to an increased risk of maternal and child mortality, impaired cognitive and physical development for children, reduced productivity and physical performance in adults, and cognitive declines for the elderly (Lopez et al. 2016). The WHO estimated that in the world, 50% of anemia cases are attributable to iron deficiency (de Benoist et al. 2008). While this statistic is not unique to the United States, iron deficiency and iron deficiency anemia are still quite prevalent. In the United States, it is estimated that about 11% of females aged 16-19 years, and 11% of females aged 20-49 years have iron deficiency. For females aged 16-19 years, 3% have iron deficiency anemia (IDA) and females aged 20-49 years, 5% have IDA (Looker et al. 1997). Causes of iron deficiency include lack of adequate iron in the diet to meet the body's iron needs, malabsorption of iron in the body, increased iron requirements during pregnancy, increased iron requirements for rapidly growing children, and blood loss for a number of reasons (Lopez et al. 2016). Iron deficiency anemia can also, in more rare forms, result from mutations in the genes of DMT1, ceruloplasmin, and transferrin involved in iron uptake in the duodenum, mobilization of iron from body stores, or erythroid iron uptake or utilization (Mims et al. 2005, Beutler et al. 2000, Harris et al. 1995). Iron deficiency typically refers to a reduction in total body iron levels, but more severe iron deficiency can lead to anemia, distinguishable by a reduction in the levels of circulating hemoglobin thereby limiting erythropoiesis (Dev et al. 2017).

Iron Overload and Hemochromatosis

Iron overload occurs when iron exceeds the capacity of transferrin to bind and carry it through the blood. This leads to the presence of non-transferrin bound iron which is dangerous because it is highly reactive. The non-transferrin bound iron can be taken up by the liver, heart, and endocrine glands, where it may fuel dangerous oxidative damage and organ dysfunction.

Iron overload most commonly results from hereditary hemochromatosis. Hereditary hemochromatosis results from mutations in genes responsible for inducing hepcidin expression in response to iron: the human hemochromatosis protein HFE, transferrin receptor, and hemojuvelin (Roetto et al. 2003, Feder et al. 1996, Camaschella et al. 2000, Papanikolaou et al. 2004). These gene mutations result in the inability of the hepatocyte to detect high iron levels and stimulate the transcription of hepcidin. Hepcidin levels then decrease leaving ferroportin unregulated causing continued dietary absorption of iron and release of iron from macrophages despite adequate iron, leading to the accumulation of iron in a number of tissues (Drakesmith et al. 2005).

Measuring Iron Status

The current study aimed to understand the effects of iron deficiency and supplementation on stress, mood, and quality of life. Iron deficiency, in the absence of anemia, was assessed in the current study. Iron deficiency (ID) is diagnosed via multiple biomarkers assessed in the blood, but there is currently no international consensus on which biomarkers are best for assessing iron status (Ferrari et al. 2011). For the current study, iron status was assessed with hemoglobin (Hb) and hematocrit (Hct), ferritin (Ft), and transferrin receptor (TfR). Additionally, alpha-1-acid

glycoprotein (AGP) was assessed to account for inflammation. Body iron was also calculated using the following equation from Cook et al. 2003 (Figure 1):

$$Body\ iron\ \left(\frac{mg}{kg}\right) = -\ \frac{\left[\log 10\left(soluble\ transferrin\ receptor\left[\frac{mg}{L}\right] x\ \frac{1000}{ferritin}\left[\frac{\mu g}{L}\right]\right) -\ 2.8229\right]}{0.1207}$$

Figure 1. Body Iron Equation

Chapter 2

Measuring Affective Outcomes: Stress, Mood, and Quality of Life

Stress: Perceived Stress Scale (PSS)

The Perceived Stress Scale (PSS) (Cohen et al. 1983) is the most commonly used psychological instrument for measuring stress. It measures the degree to which occurrences in someone's life are deemed stressful. The scale was designed to test how unpredictable, uncontrollable, and overloaded the lives of respondents are. The scale also asks questions directly regarding current levels of experienced stress. The scale was designed with questions and response options that are easy to understand and was intended to be used in community samples with a minimum of a junior high school education.

A study published by Cohen et al. (1983) compared the validity of the PSS scale to previously commonly used life-event scales. The hypothesis was that the PSS scale would be a better predictor of health outcomes than life-event scales. In this study, researchers found that the PSS was a better predictor of health and health-related outcomes and was found to be highly correlated with depressive symptoms but measured a different and independent predictive construct. PSS was found to differ from the other life-event scales in that it asks about a shorter period of time (one month) rather than six to twelve months like the life-event scales typically ask. The PSS was found to be best predictive for one to two months following administration whereas life-event scales are typically predictive anywhere from several months to several years. This is important because perceived level of stress should be influenced by varying daily hassles, major events, and changes in coping resource availability which tend to vary over a short period

of time. The PSS can also be used to determine whether stress, as evaluated through the PSS is a risk factor in behavior diseases or disorders (Cohen et al. 1983).

Mood: Profile of Mood States (POMS)

The assessment of moods has proven challenging and a number of problems have emerged in literature related to personality assessment and test construction. The Profile of Mood States or POMS was developed through exploratory factor analysis (Reddon et al. 1985) which is typically used to uncover the underlying structure of a larger set of variables (Norris et al. 2009). In terms of its use for developing a questionnaire, exploratory factor analysis is used to identify a set of underlying constructs that may affect the measured variables (Fabrigar et al. 1999).

Quality of Life: World Health Organization Quality of Life (WHOQOL-BREF)

Throughout history, health measurement has gone beyond simple measures of morbidity and mortality to include the measurement of disease impact and the measurement of disease on the impairment of daily activities and behavior, perceived health measures, and disability/functional status measures.

An important first step in the development of the assessment of quality of life was developing the definition for and characteristics of 'quality of life.' The majority of quality of life researchers agree that quality of life is subjective but the subjectivity can be broken down into levels. The WHOQOL Group refers to levels of questions and believe that questions can ask for information about functioning (how many 'y' did you 'x'), global evaluations of functioning (how well did you 'x'), and highly personalized evaluations of functioning (how satisfied are you

with ‘x’). The WHOQOL group argues that while a person’s report of functioning is an important indicator of health status, questions about global evaluations of behaviors, states and capacities, and satisfaction/dissatisfaction with behaviors, states, and capacities are what give the full picture of quality of life. Quality of life is also multidimensional, at minimum it includes physical, psychological and social dimensions and some even include dimensions for pragmatic or empirical reasons. The WHOQOL group also includes a spiritual dimension, or a dimension that assesses the respondent’s perception of the meaning of life or overall beliefs that structure and qualify experiences. Additionally, there is evidence that these four domains (physical, psychological, social, and spiritual) are values that are universal across cultures. It is also hypothesized and agreed upon that quality of life includes positive and negative dimensions and therefore assessments must ask about both positive and negative perceptions. Given all of these elements, the WHOQOL Group defined quality of life as “individuals’ perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns.” The group goes on to note that it is a “broad concept encompassing the complexity of individuals’ physical health, psychological state, independence level, social relationships, personal beliefs, and relationships to important features of the environment” (WHOQOL Group 1996). The assessment is designed to understand the effects of health interventions on quality of life (not detail symptoms, diseases, or conditions (WHOQOL Group 1996).

After testing the assessment, 100 items were selected for the Trial Version: 4 items for the 24 facets of quality of life and four items related to overall quality of life and general health (Table 1).

Table 1: WHOQOL-BREF Domains

Domain	Facets within Domains
1. Physical Health	• Activities of daily living • Dependence on medicinal substances and medical aids • Energy and fatigue • Mobility • Pain and Discomfort • Sleep and Rest • Work capacity
2. Psychological	• Bodily image and appearance • Negative feelings • Positive feelings • Self-Esteem • Spirituality/Religion/Personal beliefs • Thinking, learning, memory, and concentration
3. Social Relationships	• Personal relationships • Social support • Sexual activity
4. Environment	• Financial resources • Freedom, physical safety and security • Health and social care: accessibility and quality • Home environment • Opportunities for acquitting new information and skills • Participation in and opportunities for recreation/leisure activities • Physical environment (pollution/noise/traffic/climate) • Transport
The WHOQOL Group. (1996) WHOQOL-BREF: Introduction Administration, Scoring, and Generic Version of the Assessment. Field Trial Version. Programme on Mental Health. World Health Organization. Geneva, Switzerland.	

The WHOQOL-100 allows for detailed assessment of the individual facets related to quality of life listed in Table 1. For more practical use, the WHOQOL-BREF Field Trial Version was developed as a short form assessment. The WHOQOL-BREF assessment contains 26

questions: one for each of the 24 facets listed in Table 1 and two questions related to overall quality of life and general health. The WHOQOL-BREF was used in this study.

Quality of Life: SF36 Health Survey (SF36v2)

The domains chosen for the SF36 Health Survey are designed to represent the health domains most frequently measured by other commonly used health surveys and those believed to be most affected by disease and health conditions. The SF36 items are representative of multiple operational indicators of health such as behavioral function and dysfunction, distress and well-being, objective reports, and subjective ratings, and favorable and unfavorable self-evaluations of general health status. Ten years after SF36's original uses, improvements were needed to address problems of how questions and response choices were worded, and to address the shortcomings of the role-functioning scales. The updates resulted in the SF36v2.

There are eight domain scales in the SF36v2. Physical Functioning is a ten-item scale reflecting levels of and kinds of limitations between the extremes of physical activities. Role-Physical is a four-item scale looking at limitations related to physical health including the limitations in the kind of work or other usual daily activities, reductions in time spent on work or other regular activities, difficulty performing work or typical activities and finally accomplishing less than desired. Bodily Pain includes two items pertaining to the intensity of bodily pain and measuring the extent of pain interference on activity. The General Health scale has five items: rating of health and four items addressing the respondent's expectations of his/her health. Vitality is a four-item measure also known as energy level and fatigue that captures differences in subjective well-being. The two-item Social Functioning scale addresses the impact of physical

health and emotional problems on social activities. The Role-Emotional scale is a three-item scale that assesses role limitations in response to emotional problems. Finally, the Mental Health scale consists of five items including items addressing anxiety, depression, loss of behavioral/emotional control, and psychological well-being.

Scores are also summarized and reported as a Physical Component Summary or Mental Component Summary. The component score measures were designed to reduce an eight-scale survey to a more easily presentable format without losing information. The Physical Component Summary includes the scores for all eight SF36v2 health domain scales and yields an overall measure of physical health while the Mental Component Summary also consists of scores on all eight domain scales but provides an overall measure of mental health.

The SF36v2 was designed to be able to be used in various populations and to allow for comparisons between populations. The SF36v2 scales have become valid health measures for documenting disease burden. The SF36v2 has also proven helpful in clinical trials as often medical researchers require some sort of patient reported outcomes measures and the SF36 is the leading measure in clinical trials. The SF36 can also be helpful in providing practical solutions to the challenges of measuring disease management (QualityMetric Incorporated 2009).

Chapter 3

Review of Current Literature on Iron and Affective Outcomes: Stress, Mood, and Quality of Life in Women of Reproductive Age

The connection between nutritional status, specifically micronutrient status and cognition has been well established in a number of populations: infants, children, and pregnant women, but fewer studies have been done analyzing the importance of micronutrient nutrition, specifically iron nutrition, in non-pregnant women of reproductive age (WRA). In WRA, affective consequences of iron deficiency have been suspected as, individuals encountering WRA often in their line of work report that common symptoms of iron deficiency in WRA are irritability, apathy, fatigue, depressive symptoms, and difficulty concentrating. A review published by Murray-Kolb in 2011 analyzed the available literature in understanding the relationships between emotion and iron, quality of life and iron, cognition and iron, and behavior and iron and concluded that evidence of a relationship between iron status and cognitive function for women of reproductive age is starting to accumulate but there is little empirical evidence regarding WRA (Murray-Kolb 2011).

Iron and Stress

Iron has been shown to be associated with various elements of cognition and mental health but few studies have directly linked iron and stress outcomes. A study published in 2005 by Beard et al. aimed to understand if iron deficiency anemia affects postpartum emotions and cognition. Three groups were compared: nonanemic controls, anemic mothers receiving placebo,

and anemic mothers receiving daily iron. Results showed that iron treatment resulted in 25% improvement on scores from depression and stress scales in mothers who were previously iron deficient but treated with iron. Analyses also showed a strong association between iron variables and behavior variables including stress. While the study population is not identical to that of this study, the results are still relevant in showing a relationship between iron and stress (Beard et al. 2005).

Previous Uses of the Perceived Stress Scale Related to Iron Intake/Status

A study comparable to the current study population is a study published by Blanton et al. in 2013 examining the association of body iron concentrations with cognitive executive planning function (analyses controlled for Perceived Stress) in a population of college women. Participants were recruited from the students at University of California, Davis and included females between the ages of 19 and 30 years with a BMI less than or equal to 29.9 kg/m^2. After the exclusion criteria, the number of participants was 42. Iron and cognition were assessed. The specific cognitive tests used are not applicable to this study. The Perceived Stress Scale was used as a covariate in a repeated measures analysis. The average PSS score among the women was 10.6, slightly lower than the aforementioned norm values (13.7 for females, 14.2 for anyone aged 18-29). While this study does not directly measure the relationship between iron and stress, it does present a picture of Perceived Stress. The study reports a significant slowing of planning speed during a test of central executive function in women with reduced body iron (without anemia) (Blanton et al. 2013).

Iron and Mood

Numerous micronutrients have been shown to be associated with mood. Particularly, deficiencies of thiamin (Benton et al. 1997) and iron (McClung et al. 2009, Vahdat Shariatpanaahi et al. 2007, Vaucher et al. 2012, Patterson et al. 2000, Verdon et al. 2003) have been associated with poorer mood. Iron deficiency anemia is associated with reports of poor mood, lethargy, and problems sustaining attention. A low iron status, even without anemia, has been associated with feelings of depression (Benton and Donohoe 1999). Two studies (Rangan et al. 1998) and (Fordy and Benton 1994) showed that despite overall results pointing to little association between psychological distress and iron status, populations of iron-deficient females taking oral contraceptives showed more symptoms of distress such as depression, irritability, and difficulty concentrating. A more recent study (McClung et al. 2009) has shown a relationship between iron status and mood in soldiers during basic training. A study by Beard et al. in 2005 showed that there is a relationship between iron status and depression, stress, and cognitive functioning in African mothers during the postpartum period. Another study by Corwin, Murray-Kolb, and Beard in 2003 showed that there was an association between hemoglobin concentration and postpartum depressive symptoms where women with anemia had higher depressive symptoms scores compared to women with normal hemoglobin levels.

Previous Uses of the Profile of Mood States Related to Iron Intake/Status

The POMS scale has been used in the past to measure the various mood states as they relate to iron and other nutrients. A study published by McClung et al. 2009 reported a relationship between iron supplementation, iron status, and improved mood. The objective of the study was

to understand whether iron supplementation could prevent reductions in iron status (evidence has shown that iron status declines during initial military training) and improve physical performance and mood in female soldiers during basic combat training. The study was an 8-week randomized, double-blind, placebo-controlled trial that included 219 solider volunteers who took either 100 mg ferrous sulfate or placebo daily. Iron status and mood using Profile of Mood States were assessed before and after basic combat training. Results of the study showed that iron supplementation protected against iron status deterioration in the soldiers and that iron supplementation resulted in significantly improved vigor scores on the Profile of Mood States (McClung et al. 2009).

Iron and Quality of Life

The relationship between iron status and quality of life is better studied in scientific literature (compared to iron and stress/mood). A review by Ross et al. in 2003 aimed to understand the effect of treatment for anemia on health-related quality of life. The conclusion of the review was that treatment of anemia with erythropoiesis-stimulating protein improved selected quality of life domains in patients with renal insufficiency or cancer-related anemia. More specific to iron nutrition, in two studies looking at iron deficiency in patients with chronic heart failure, iron deficiency was a determinant for health-related quality of life. A study by Enjuanes et al. in 2014 pointed out that patients with chronic heart failure presented significant impaired health related quality of life and that iron deficiency is a comorbidity in chronic heart failure. They also reported that the quality of life outcomes are strongly determined by iron deficiency regardless of how iron depletion affects hemoglobin levels. The second study by

Comin-Colet et al. in 2013 found that increased levels of soluble transferrin receptor were associated with impaired quality of life outcomes. More specific to this study's population, a paper by Grondin et al. in 2008 explored iron deficiency's relationship with quality of life but had only one significant finding: that iron deficiency was linked with perceived general health. More information is needed to study the relationship between iron status and quality of life in women of reproductive age in the U.S. (Murray-Kolb 2011).

Previous Uses of WHOQOL-BREF and SF36v2 Related to Iron Intake/Status

As mentioned previously, there are few studies analyzing the relationship between iron status and quality of life in women of reproductive age (Murray-Kolb 2011). No other studies have used the WHOQOL-BREF questionnaire to study this relationship in WRA.

The SF36v2 has been used in many different settings including settings to diagnose symptoms of disease. In the case of this particular study, the SF36v2 was used to assess the relationship between iron status and health related quality of life. Two studies have looked at the relationship between iron interventions and health related quality of life using the SF36v2. A study published by Beck et al. in 2012 also analyzed the relationship between iron status and self-perceived health, well-being, and fatigue in female University students in New Zealand. The sample size for the study included 233 women aged 18-44 years attending Massey University in Auckland, New Zealand. The women had blood drawn which was assessed for serum ferritin, hemoglobin, and c-reactive protein. Additionally, the women completed the SF36v2 General Health Survey along with a fatigue inventory, and anthropometric measures, demographics, lifestyle, and medical history were also collected. The study, however, found no significant

differences between participants characterized as iron depleted and those characterized as iron sufficient according to serum ferritin values greater than or equal to 20 micrograms per liter and hemoglobin greater than or equal to 120 grams per liter indicating good iron status. This study did not include any type of intervention (Beck et al. 2013).

A study published by Ando et al. in 2006, on the other hand, examined the relationship between iron status and health-related quality of life by prescribing daily iron tablets for 3 months to Japanese women of reproductive age who had been recently diagnosed with iron deficiency anemia. The SF-36v2 survey was completed by 92 participants at baseline, 1 month, and 3 months during their supplementation period. The results showed that at baseline, participants had significantly lower vitality and general health scores than the Japanese national norms. At 1 month, a significant improvement was seen in all of the domain scores except role-emotional. At 3 months, all eight scores were close to or greater than the Japanese national norms. Physical functioning and vitality scores of patients with a lower hemoglobin level at baseline showed particular dramatic improvement. The conclusion of the study was that iron supplementation in Japanese iron deficient anemic WRA improves hemoglobin levels, physical function, vitality, and general health perception (Ando et al. 2006).

Chapter 4

Methods

Study Overview

This study aimed to understand the relationship between iron nutrition and affective variables in women of reproductive age. The aims of the study were two-fold: 1) to analyze the differences between iron deficient and iron sufficient participants in terms of mood, stress, and quality of life as measured by the Profile of Moods (POMS), Perceived Stress Scale (PSS), WHO Quality of Life (WHOQOL), and the SF36v2, and 2) to analyze how iron supplementation affected scores on these scales. Participants were supplemented based on their iron status as measured through a blood draw at the beginning of the study. Data were collected at two different time points. Blood was taken and questionnaires were completed upon recruitment and then again after 4 months of receiving iron treatment or placebo. The study occurred in three phases, each analyzing three different sets of participants for the same outcomes. The current thesis is an analysis of existing data collected by other individuals from the Murray-Kolb Lab.

Recruitment

Participants were recruited through the posting of flyers around the University Park campus of Penn State and interested participants were requested to contact the Murray-Kolb Lab. Participants were screened for eligibility in the initial phone call. Inclusion criteria required that the participant was a female between the age of 18 and 35 years (woman of reproductive age), was in general good health, was either iron deficient (not anemic) or iron sufficient, was able to

speak English, and was willing to abstain from taking any supplements other than the one required by the study. Participants were excluded from the study if they were pregnant or lactating, currently taking a supplement that they were unwilling to discontinue, had a hemoglobin level less than 120 g/L at blood screening, had a history of gastrointestinal or hematological disorders, or used psychoactive drugs. Participants were given $20 cash as compensation for completing baseline testing and an additional $30 cash for completing endline testing.

Blood Collection, Iron Assessment, and Supplementation

Participants' completed a baseline venous blood draw at the Penn State Clinical Research Center (CRC) in Noll Lab where approximately 8 milliliters of blood was drawn from an antecubital vein into two vacutainer tubes from each participant. Hemoglobin (Hb) and hematocrit (Hct) were measured via Coulter Counter using whole blood. Ferritin (Ft) and transferrin receptor (TfR) were measured via ELISA (Ramco, Inc) and alpha-1-acid-glycoprotein (AGP) was measured via radialimmunodiffusion (Kent Laboratories) using serum. AGP is predicted to have an effect on iron outcomes when it is greater than 100 mg/dL and 6 participants in this study had an AGP value greater than 100 mg/dL. After initial blood screening, participants were categorized as either iron sufficient (Hb > 12.0 g/dL, Ft > 20 µg/L, TfR < 8 mg/L) or iron depleted (Hb >12.0 g/dL, Ft < 20 µg/L, TfR > 8). Participants categorized as iron depleted received 60 mg ferrous sulfate daily for 4 months while participants categorized as iron sufficient received placebo, gelatin pills, daily for 4 months. Following the four-month

supplementation/placebo protocol, participants returned to the CRC for another blood draw that was assayed for the same variables. Participants were blinded to the treatment.

Anthropometrics/Health History

At the baseline visit, interested participants were given the consent form to read, ask questions about, and sign if they were willing to continue with the study. Upon receiving consent, participants' blood was drawn and they filled out the anthropometric/health history form answering questions about their height, weight, age, smoking/drinking habits, birth control use, and questions about their menstrual cycle. They were again asked a few questions about pregnancy, and about medications/supplements to confirm their eligibility. They then answered questions about previous medical diagnoses and completed the mood, stress, and quality of life questionnaires (described below).

Questionnaires/Assessment of Mood, Stress and Quality of Life

Participants were asked to fill out four questionnaires to obtain information about their perceived stress, quality of life, health and well-being, and mood state at baseline and endline (four months after the supplementation). The Perceived Stress Scale (PSS) asked questions about stress level and the Profile of Moods (POMS) asked questions attempting to understand what the participant's total mood disturbance was. Both the SF36v2 and WHO Quality of Life (WHOQOL) were assessing the participants' understanding and perception of their own quality of life. The questionnaires are available in Appendix B.

Perceived Stress Scale Scoring

The PSS contains 10 questions that ask the respondents how often they felt a certain way in the past month. Answers are indicated on a scale of 0-4. Six of the questions are negatively stated and four are positively stated. The positively stated questions are reverse coded such that 0=4, 1=3, 2=2, 3=1, and 4=0. After reverse coding, the responses are added together and the sum represents the overall stress score. Thus overall, the higher the score on the Perceived Stress Scale, the more stress the respondent feels. The norm as defined by a mean of 1406 tested females was 13.7 and for ages 18-29, both male and female, the norm as defined by a mean of 645 respondents tested was 14.2. The norms were calculated from the L Harris Poll where information was collected from 2,387 respondents in the US (Cohen et al. 1983).

Profile of Mood States Scoring

The Profile of Mood States (POMS) is a 65-item questionnaire designed to assess six dimensions of the mood construct: Anger, Confusion, Depression, Tension, Fatigue, and Vigor. The standard form gives the option to the administrator whether to ask the respondent to answer according to how they have been feeling during the past week, including today, how they feel right now or something else entirely. In the current study, the women were asked how they have been feeling in the past week. The response options for all sixty-five questions are on a Likert scale labeled 1 to 5 including options for “Not at all,” “A little,” “Moderately,” “Quite a bit,” and “Extremely.” Each question is categorized according to the six dimensions of the mood construct mentioned above. Seven out of the sixty-five questions remain unscored. The remaining fifty-eight questions are associated with a particular mood construct. Ultimately, the raw scores for

each question are added together according to their categorization and a raw score for each mood construct is formed: tension, depression, anger, fatigue, confusion, and vigor. Those raw scores can be used individually or put together to develop a Total Mood Disturbance score which is obtained by adding the scores for tension, depression, anger, fatigue, and confusion and subtracting the score for vigor. Because of this formula, a higher Total Mood Disturbance score indicates more mood disturbance (more Tension, Depression, Anger, Fatigue and Confusion and less Vigor). Similarly, a higher raw Tension, Depression, Anger, Fatigue, Confusion, or Vigor score indicates that the respondent is tenser, more depressed, angrier, more fatigued, more confused, or more vigorous (McNair et al. 1992).

WHO Quality of Life Scoring

The WHOQOL-BREF responses provide a quality of life profile. Four domain scores are derived and two questions are examined separately: the overall perception of quality of life and overall perception of health (questions 1 and 2 respectively). The four domains represent the respondent's perception of his/her Physical Health, Psychological Health, Social Health, and Environmental Health and correspond with the domains listed in Table 1. Response options are listed on a Likert scale with a '1' representing the most negative perception and '5' the most positive perception. The questions are categorized according to their domain (but those categorizations are not presented to the subject) and then the raw scores for each of the questions are averaged within their assigned domain. The averages are then multiplied by four in order to make them comparable to the scores for the WHOQOL-100. Scores are scaled positively meaning that a higher score indicates higher quality of life (The WHOQOL Group, 1996).

SF36v2 Health Survey Scoring

The SF36v2 is meant to be self-administered for individuals who are at least 18 years old. The form uses four-week recall and therefore at least four weeks must pass before the SF-36v2 can be administered a second time. The SF36v2 asks 11 main questions but question 3 has 10 parts, question 4 has 4 parts, question 5 has 3 parts, question 9 has 9 parts and question 11 has 4 parts. This makes for a total of 36 responses. The questions are also asked on a Likert scale and each question is associated with an SF36v2 scale/domain. The SF36v2 is scored through a QualityMetric™ computer-based scoring software. The software operates by recoding the item response values and summing them for each item in the various scales to obtain the raw score and then the raw score is transformed to a 0-100 score which is transformed to a z-score which is transformed to a T-Score. The Physical Component Score (PCS) and Mental Component Score (MCS) are obtained by multiplying each domain z-score by a scale-specific physical or mental factor score coefficient and summing all of the resulting products and converting that total to a T-Score (QualityMetric Incorporated 2009).

There are three approaches to interpreting the scoring software's T-Scores: the norm-based, content-based, or criterion-based interpretations. For norm-based interpretations, the individual and summary T-scores can be compared to general population norms or disease-specific norms. Generally, for individual respondents, T-scores of 45 or greater indicate at least average overall functioning or well-being in each of the domains compared to U.S. general population. T-scores less than 45, then, indicate impaired well-being or functioning as it is related to the general U.S. population. Responses in the 40-44 range need further investigation such as considering the confidence interval around the score and the use of age, gender, or disease-based norms in order to understand if those scores are related to impaired functioning or

well-being. For content-based interpretations, the frequency distribution of specific responses to SF-36v2 items across score levels of the domain scales and component summary measures are analyzed. For example, knowing that more than 75% of the general U.S. population scoring below 30 on the PCS measure have difficulty performing work or other activities all or most of the time is useful in analyzing what scores less than 30 on the PCS measure mean for the current studied population. Finally, the criterion-based interpretation analyzes relationships between the particular measures and other variables, criteria, measured at the same time or after a period of time. The established strategy for evaluating the SF36v2 score meaningfulness is to link component summary measure and domain scale T-scores to important benchmarks like the ability to work, or healthcare utilization and showing how differences in scores can help predict clinical and social events (QualityMetric Incorporated 2009).

According to Stirling University's Framework for Measuring Impact, quality of life is personal and therefore it does not make sense to compare scores to normative values. They suggest that the higher the scores on each of the indicators, the better the quality of life (Stirling IT 2012).

Statistical Analysis

Sample size during each phase of the study varied greatly. Phase one, completed outside of the Murray-Kolb Lab, enrolled the most participants (n= 65) but had the least complete data as 46 participants did not have complete data at endline. For phase two, 10 participants were analyzed at baseline and endline, and for phase three, 9 participants were analyzed at baseline and endline. In order to make analyses comparable, only participants who had complete data at

baseline and endline were included in the analyses: 14 from Phase 1, 10 from Phase 2, and 9 from Phase 3 for a total of 33. All statistical analyses were completed in SAS Version 9.4.

Analysis of variance (ANOVA/ANCOVA) were run to examine the mean differences between the scores on various questionnaire subscales by the iron sufficient group, who received placebo, and the iron deficient group, receiving iron treatment, at both baseline and endline. Covariates were identified from correlation tables. Health History (demographic) characteristics that were correlated at $p < 0.1$ with the questionnaire subscales were included as covariates. Each subscale had its own covariates that differed between baseline and endline as the correlation tables changed (although most were the same at both time points). The difference covariates were all of the covariates controlled for at both baseline and endline for each subscale (see Appendix A for a list of covariates used in each analysis).

The ANOVA was completed at baseline using a sample size of 33. It was also completed using the "difference" variables (baseline subtracted from endline for each blood and questionnaire variable) with a sample size of 33. The ANCOVA was completed at baseline and endline and with difference values with a sample size varying from 29 to 33 depending on the covariates used for each questionnaire outcome (some demographic characteristics were missing for a few participants). Stepwise regressions were completed to understand how the Health History variables may predict the questionnaire outcomes.

For secondary analyses, participants were categorized as being ferritin responders or non-responders and hemoglobin responders or non-responders, irrespective of initial iron status and treatment. A 'responder' was defined as having experienced an increase in the biomarker (ferritin or hemoglobin) larger than the known day-to-day variation for that biomarker (26.8% for ferritin

and 4.4% for hemoglobin) (Borel et al. 1991). Once categorized as responders (Ft or Hb) or non-responders, the same statistical tests as above were completed.

Chapter 5

Main Analysis Results

This chapter begins by presenting the basic characteristics of the study population categorized according to iron status beginning with health history (demographic) characteristics such as age, BMI, drinking and smoking habits, vitamin/medication use, and difference between the participants' last menses and the date of their study examination. It then describes the participants' blood profile and scores on the various questionnaires. After this preliminary information, the results for the main analyses, comparing the treated vs. untreated groups are presented including an analysis of variance and analysis of variance with covariates, an analysis of variance comparing each group's change over time with and without covariates, repeated measures, and stepwise regression.

Following the presentation of the main analyses, a presentation of the secondary analyses, where participants were re-categorized as ferritin responders or non-responders and hemoglobin responders or non-responders according to their change in ferritin or hemoglobin above the normal daily change predicted by Borel et al. 1991 will be presented.

Basic Characteristics

Demographic Characteristics

There were no significant differences between iron sufficient (IS) and iron deficient (ID) groups for age, BMI, smoking, drinking, difference between last menses and date of study examination, use of medication, and use of vitamins/herbal supplements (Table 2). The iron deficient group had a slightly higher mean BMI (IS: 22.9 and ID: 24.6) and percentage of obese participants than the iron sufficient group, although neither were significantly different between groups (Table 2).

Table 2: Demographic Characteristics by Group

Variable		Iron Sufficient (n=15)	Iron Deficient (n=18)
Age	Mean (SD)	21.5 (2.5)	21 (2.5)
	Minimum	18	18
	Maximum	27	29
BMI	Mean (SD)	22.9 (2.8)	24.6 (4.3)
	Underweight # (%)	0 (0%)	1 (5.6%)
	Normal # (%)	12 (80%)	10 (55.6%)
	Overweight # (%)	3 (20%)	5 (27.8%)
	Obese # (%)	0 (0%)	2 (11.1%)
Smoke # (%)	Yes	1 (6.7%)	0 (0%)
	No	14 (93.3%)	15 (100%)
Drinks per Week # (%)	0 to 2	7 (46.7%)	10 (55.6%)
	3 to 4	4 (26.7%)	5 (27.8%)
	>5	4 (26.7%)	3 (16.7%)
Difference between last menses and date of study examination	Mean # of Days (SD)	19.2 (25.1)	14.6 (9.7)
	≤ 1 Week (0-7 days)	5 (35.7%)	3 (20%)
	Between 1&2 Weeks (8-14 days)	4 (28.6%)	6 (40%)
	Between 2&4 Weeks (15-28 days)	4 (28.6%)	4 (26.7%)
	> 4 Weeks (29+ days)	1 (7.1%)	2 (13.3%)
Medication # (%)	Yes	4 (26.7%)	3 (16.7%)
	No	11 (73.3%)	15 (83.3%)
Vitamins/Herbals # (%)	Yes	5 (33.3%)	9 (50%)
	No	10 (66.7%)	9 (50%)

Blood Characteristics

Table 3 presents the baseline and endline blood characteristics for the participants. The participants were categorized as iron sufficient vs. iron deficient according to their Ft concentrations. As expected, determined by a t-test, there were significant differences at baseline between iron sufficient and iron deficient groups for the Hb, Ft, and body iron values (Table 3). At endline, there were still significant differences between iron sufficient and iron deficient groups for Hb and Ft but body iron values improved enough in the iron supplemented group such that they were no longer significantly different between iron sufficient and iron deficient groups.

Looking within the groups between time points, there was a significant increase in Ft and body iron values for the iron deficient group between baseline and endline, after treatment with an iron supplement, indicating improved iron status. Ft significantly increased in the iron deficient group but not to the level to be categorized as iron sufficient at endline. As previously mentioned, body iron increased significantly for the iron deficient group enough to no longer see a difference in body iron scores between the iron sufficient and iron deficient groups at endline (Table 3).

Table 3: Blood Variables by Group (Baseline and Endline)

		Baseline				Endline			
Blood Variables		n	Iron Sufficient	n	Iron Deficient	n	Iron Sufficient	n	Iron Deficient
Hemoglobin g/dL	Mean (SD)	15	14.0 (1.6)[a]	18	12.6 (1.2)[b]	15	13.7 (0.6)[a]	18	13.1 (1.1)[b]
	Range		12.2-18.5		10.1-14.3		12.1-15.0		10.4-15.0
Hematocrit %	Mean (SD)	15	41.9 (2.2)	18	40.1 (3.6)	15	41.8 (2.2)	16	40.7 (3.2)
	Range		38.5-46.0		33.0-46.0		37.8-45.8		33.3-46.0
Ferritin µg/L	Mean (SD)	15	58.6 (24.5)[a]	18	8.3 (4.8)[b]	12	56.4 (27.0)[a]	11	19.9 (14.9)[b,*]
	Range		25.1-99.3		1.5-19.7		6.0-101.5		1.8-52.3
Transferrin Receptor mg/L	Mean (SD)	14	4.8 (2.5)	15	5.3 (2.0)	10	5.4 (2.1)	9	4.6 (1.2)
	Range		1.1-11.1		3.0-9.5		2.1-9.8		2.8-5.8
Body Iron mg/kg	Mean (SD)	14	7.8 (2.8)[a]	15	-0.4 (3.2)[b]	10	6.3 (3.9)	9	3.7 (3.4)*
	Range		2.0-12.5		-7.3-4.7		-3.2-10.6		-3.0-8.8
AGP mg/dL	Mean (SD)	15	56.9 (21.8)	16	71.3 (29.6)	10	67.9 (28.3)	9	69.3 (21.9)
	Range		21.9-110.4		34.8-111.2		36.9-118.5		39.8-97.2
Differing superscripts within a row represent significant differences ($p < 0.05$) between groups within a time point.									
* represents significant differences ($p<0.05$) within a group, across time									

Main Analyses

The main analyses of this study aimed to understand the relationship between groups categorized according to iron status: iron sufficient (treated with placebo) vs. iron deficient (treated with supplements).

Iron Sufficient vs. Iron Deficient Analysis of Variance (ANOVA/ANCOVA)

Analysis of variance (ANOVA) was done in order to compare differences between the iron sufficient and iron deficient groups at both baseline and endline. As illustrated in Table 4, at baseline, the only significant ($p < 0.05$) differences between iron sufficient and iron deficient groups were at baseline for the Confusion subscale of the Profile of Moods questionnaire. Iron deficient participants had a significantly higher Confusion score compared to iron sufficient participants indicating iron deficient participants reported more confusion at baseline. After controlling for covariates, differences between groups for Confusion were attenuated ($p=0.0542$). After treatment, there was no difference between groups on the Confusion subscale score. However, after controlling for covariates, scores differed significantly between groups ($p < 0.0001$) at endline. The iron sufficient group had a higher score for Confusion compared to the iron deficient group after treatment (Table 4).

In the Tension subscale of the Profile of Moods questionnaire, there was an almost significant ($p < 0.1$) difference at baseline between iron sufficient and iron deficient groups with the iron deficient group indicating more tension. No covariates were identified for tension at

baseline. At endline, after controlling for the covariates, the model became significant ($p < 0.0001$). However, despite the decrease, the iron deficient group still had significantly higher scores on the Tension subscale compared to the iron sufficient group at endline (Table 4).

At endline, there was an almost significant difference ($p < 0.1$) between iron sufficient and iron deficient groups in the Environmental Health subscale of the WHO Quality of Life questionnaire. The iron sufficient group, on average, indicated a higher quality of environmental health compared to the iron deficient group. No covariates were identified, and therefore, an ANCOVA was not run for this outcome (Table 4).

After controlling for covariates, two subscales on the SF36v2 questionnaire were significantly different at baseline between the iron deficient and iron sufficient groups: Bodily Pain ($p=0.0005$) and Physical Component Score ($p=0.034$). For both, the iron sufficient group had a higher average score compared to the iron deficient group (Table 4).

From the WHOQOL questionnaire at baseline, two variables were almost significant between groups after controlling for covariates. Physical Health, ($p=0.0729$) after controlling for covariates became almost significant with the iron deficient group having a higher average score compared to the iron sufficient group. There also was an almost significant difference ($p=0.0743$) between the groups for Psychological Health after controlling for covariates. The iron sufficient group had a higher average Psychological Health score compared to the iron deficient group (Table 4).

From the POMS questionnaire at baseline, after controlling for covariates, there was a significant difference ($p=0.0523$) between groups for scores on the Depression subscale. The iron deficient group indicated a higher average score for depression compared to the iron sufficient group (Table 4).

At endline, after the addition of covariates into the model, there was a significant difference ($p=0.0498$) between groups for the Physical Health subscale of the WHOQOL with the iron sufficient group having a higher average score compared to the iron deficient group after treatment. Significant differences ($p=0.0596$) also occurred between the groups for scores on Psychological Health of the WHOQOL. The iron sufficient group had a slightly higher average score compared to the iron deficient group after treatment (Table 4).

The remaining three significant variables at endline, after the addition of covariates, are from the POMS questionnaire. There were significant differences between groups for the Anger ($p<0.0001$) and Depression ($p=0.0525$) subscales, and the Total Mood Disturbance summary value ($p=0.0005$). The iron sufficient group had a higher score on the Anger and Depression subscales compared to the iron deficient group but the iron deficient group had a higher Total Mood Disturbance score compared to the iron sufficient group (Table 4).

Table 4: Main Analysis of Variance (ANOVA)

			Baseline			Endline		
Subscale	Variable		Iron Sufficient (n=15)	Iron Deficient (n=18)	p-value	Iron Sufficient (n=15)	Iron Deficient (n=18)	p-value
SF36v2	Physical Functioning	Mean (SD)	96.7 (4.5)	93.3 (13.7)	0.3752	97.0 (4.9)	95.3 (10.2)	0.5551
		Range	90.0-100.0	45.0-100.0		85.0-100.0	60.0-100.0	
	Role Physical	Mean (SD)	88.3 (17.7)	89.6 (20.7)	0.8547	92.1(16.1)	91.3 (17.0)	0.8963
		Range	50.0-100.0	18.8-100.0		50.0-100.0	37.5-100.0	
	Bodily Pain	Mean (SD)	84.9 (16.9)	79.4 (24.5)*	0.4642	85.7(21.9)	86.9 (19.2)	0.873
		Range	52.0-100.0	0.0-100.0		31.0-100.0	22.0-100.0	
	General Health	Mean (SD)	73.4 (17.7)	73.2 (13.7)	0.9743	72.5 (17.5)	68.7 (14.3)	0.5031
		Range	37.0-100.0	47.0-90.0		30.0-100.0	37.0-87.0	
	Vitality	Mean (SD)	55.8 (19.1)	51.0 (15.0)	0.4263	57.1 (19.0)	57.6 (18.3)	0.9325
		Range	12.5-87.5	31.3-81.3		18.8-87.5	6.3-81.3	
	Social Functioning	Mean (SD)	86.7 (17.3)	79.2 (21.0)	0.2781	86.7 (13.7)	86.8 (15.1)	0.9784
		Range	50.0-100.0	37.5-100.0		62.5-100.0	62.5-100.0	
	Role Emotional	Mean (SD)	76.7 (21.6)	81.9 (17.9)	0.4488	83.3 (17.5)	83.8 (16.5)	0.9384
		Range	33.3-100.0	41.7-100.0		50.0-100.0	50.0-100.0	
	Mental Health	Mean (SD)	73.0 (17.3)	72.5 (12.5)	0.924	75.0 (12.7)	68.1 (17.8)	0.2142
		Range	20.0-90.0	50.0-90.0		45.0-95.0	30.0-90.0	
	Physical Component Score	Mean (SD)	57.3 (6.0)	55.7 (6.8)*	0.5012	57.0 (4.0)	57.1 (7.4)	0.9623
		Range	41.8-64.8	33.4-66.6		48.5-61.4	32.2-66.9	
	Mental Component Score	Mean (SD)	46.3 (10.6)	46.3 (7.4)	0.9855	48.0 (7.2)	46.5 (9.5)	0.6344
		Range	16.0-60.6	30.3-57.0		32.0-59.5	23.6-58.5	
WHO Quality of Life	Physical Health	Mean (SD)	12.6 (2.4)	12.9 (1.8)	0.6763	13.1 (2.0)	12.7 (1.4)*	0.4609
		Range	9.1-16.6	9.7-16.6		9.1-16.0	10.9-16.6	
	Psychological Health	Mean (SD)	13.9 (2.3)	13.8 (1.7)	0.8906	14.1(1.9)	14.0 (1.8)*	0.8918
		Range	9.3-17.3	11.3-17.3		10.7-17.3	10.0-17.3	
	Social Health	Mean (SD)	15.7 (3.2)	15.6 (2.9)	0.8687	17.1 (2.1)	15.7 (3.3)	0.1749
		Range	8.0-20.0	10.7-20.0		13.3-20.0	9.3-20.0	
	Environmental Health	Mean (SD)	15.6 (2.6)	15.0 (2.1)	0.4676	16.3 (2.1)	14.9 (6.0)	0.0905
		Range	9.5-19.0	11.0-19.0		11.0-19.5	11.0-18.0	
PSS	Perceived Stress Scale	Mean (SD)	15.6 (6.0)	17.9 (5.7)	0.2577	14.9 (6.1)	14.3 (6.0)	0.7668
		Range	4.0-26.0	9.0-31.0		6.0-28.0	5.0-25.0	
Profile of Moods (POMS)	Tension	Mean (SD)	7.1 (5.6)	10.7 (6.0)	0.0822†	8.9 (7.3)	9.3 (4.5)*	0.8693
		Range	1.0-19.0	1.0-26.0		1.0-32.0	2.0-16.0	
	Anger	Mean (SD)	6.3 (7.0)	9.2 (7.8)	0.2662	6.3 (8.2)	4.7 (4.8)*	0.4894
		Range	0.0-23.0	1.0-27.0		0.0-35.0	0.0-15.0	
	Fatigue	Mean (SD)	6.3 (6.2)	8.1 (6.1)	0.3956	8.9 (7.8)	6.9 (4.6)	0.388
		Range	0.0-21.0	1.0-22.0		0.0-25.0	0.0-15.0	
	Depression	Mean (SD)	11.1 (8.7)	13.0 (9.4)*	0.5621	11.5 (10.2)	11.1 (8.0)*	0.9112
		Range	0.0-25.0	0.0-40.0		0.0-32.0	0.0-25.0	
	Vigor	Mean (SD)	9.2 (6.8)	10.4 (6.6)	0.5992	11.9 (6.7)	12.3 (7.0)	0.8646
		Range	0.0-18.0	0.0-26.0		2.0-21.0	4.0-26.0	
	Confusion	Mean (SD)	5.3 (3.7)[a]	8.1 (4.0)[b*]	0.0461	7.9 (6.4)	7.5 (2.8)	0.7964
		Range	0.0-14.0	4.0-16.0		3.0-25.0	3.0-15.0	
	Total Mood Disturbance	Mean (SD)	16.7 (29.8)	32.1 (32.7)	0.1729	22.3 (36.9)	22.6 (21.2)*	0.9786
		Range	-18.0-71.0	-2.0-126.0		-12.0-122.0	-5.0-63.0	

Differing superscripts within a row represent significant differences ($p<0.05$) between groups within a time point

* represents significant differences ($p<0.05$) between groups within a given time point after controlling for covariates

Analysis of Variance (ANOVA/ANCOVA) Evaluating the Difference in Scores between Time Points

Baseline scores were subtracted from endline scores to obtain a difference value for each participant. In these analyses, baseline scores were controlled for. An ANOVA using these difference values aims to recognize if there are significant differences in change over time between the iron sufficient and iron deficient groups (those receiving placebo vs. iron supplement).

As shown in Table 5, from the SF36v2, the changes over time on the subscales Physical Functioning ($p=0.0014$), Role Physical ($p=0.0337$), Bodily Pain ($p=0.0280$), General Health (0.0057), and Role-Emotional (0.0005) all were significantly different between groups both with (p-values listed above) and without covariates (p-values in Table 5) controlled for. There was a larger increase in scores on Physical Functioning and Bodily Pain in the ID group compared to the IS group. There was a larger increase in scores on the Role Physical and Role Emotional for the IS group compared to the ID group. The ID group decreased scores for General Health more than IS decreased. Two subscales, Mental Health and the Mental Component Score were significantly different before controlling for covariates, but after controlling for covariates no longer showed significant differences between groups. While Social Functioning was not significantly different when run without covariates, after controlling for covariates, there was a significant difference ($p<0.0001$) in changes over time between the groups. Social Functioning increased significantly more for the ID group over time compared to the IS group.

From the WHOQOL questionnaire, the subscales Physical Health ($p=0.0010$), Social Health ($p=0.0013$), and Environmental Health ($p=0.0359$) were significantly different in changes over time between groups both before and after controlling for covariates. Scores for Physical

Health and Environmental Health both decreased in the ID group while they increased slightly for the IS group. The IS group also had a larger increase in Social Health compared to the ID group despite treatment with iron. Psychological Health was significantly different between groups before but not after controlling for covariates.

The Perceived Stress score was significantly different ($p=0.0319$) both before and after controlling for covariates. The ID group decreased their scores for Stress significantly more than the IS group.

All of the POMS subscales were significantly different both before and after controlling for covariates. For the ID group (who received treatment) the scores for Tension ($p<0.0001$), Anger ($p<0.0001$), Fatigue ($p=0.0062$), Depression ($p<0.0001$), Confusion ($p=0.0031$), and Total Mood Disturbance ($p<0.0001$) all decreased over time while they increased for the IS group. Vigor, however, increased more for the IS group compared to the ID group (Table 5).

Table 5: Difference (score at endline minus score at baseline) Analysis of Variance (ANOVA)

Subscale	Dependent Variable	p-value		n	Mean	SD
SF36v2	Physical Functioning	0.0004	IS	15	0.333	6.11
			ID	18	1.946*	7.51
	Role Physical	0.0012	IS	15	3.75	20.6
			ID	18	1.736*	14.8
	Bodily Pain	0.0065	IS	15	0.8	25.1
			ID	18	7.5*	14.9
	General Health	0.0107	IS	15	-0.933	17.5
			ID	18	-4.5*	14.7
	Vitality	0.1179	IS	15	1.25	15.9
			ID	18	6.597	13.8
	Social Functioning	0.3289	IS	15	0	17.7
			ID	18	7.639	25.1
	Role Emotional	0.001	IS	15	6.666	17.6
			ID	18	1.851*	18.6
	Mental Health	0.0184	IS	15	2	15.4
			ID	18	-4.444	14.2
	Physical Component Score	0.0094	IS	15	-0.246	7.49
			ID	18	1.393	3.48
	Mental Component Score	0.0229	IS	15	1.637	7.42
			ID	18	0.26	7.18
WHO Quality of Life	Physical Health	0.0003	IS	15	0.4952	1.51
			ID	18	-0.254*	1.68
	Psychological Health	0.0372	IS	15	0.178	1.34
			ID	18	0.185	1.44
	Social Health	0.0008	IS	15	1.333	2.02
			ID	18	0.148*	3.93
	Environmental Health	0.0359	IS	15	0.667	1.36
			ID	18	-0.111*	1.6
PSS	Perceived Stress	0.058	IS	15	-0.667	4.58
			ID	17	-2.882*	6.06
Profile of Moods (POMS)	Tension	0.0003	IS	15	1.867	8.19
			ID	18	-1.444*	6.77
	Anger	<.0001	IS	15	0.067	10.3
			ID	18	-4.5*	7.97
	Fatigue	0.0062	IS	15	2.6	6.97
			ID	18	-1.167*	5.02
	Depression	0.0013	IS	15	0.333	12.7
			ID	18	-1.889*	8.65
	Vigor	0.005	IS	15	2.667	8.19
			ID	18	1.833*	6.93
	Confusion	0.0171	IS	15	2.6	4.82
			ID	18	-0.611*	4.43
	Total Mood Disturbance	0.0022	IS	15	5.6	28.8
			ID	18	-9.444*	30.1
* represents significant difference ($p < 0.05$) between groups after controlling for covariates						
IS= Iron Sufficient						
ID=Iron Deficient						

Stepwise Regression (Ft and TfR Model): Baseline

A stepwise regression analysis identifies which variables are potential predictors of the questionnaire outcomes. Two different models were run for stepwise regression: a model that included TfR and Ft with the rest of the covariates and a model that included body iron with the rest of the covariates. They were run separately because body iron is calculated from TfR and Ft.

For the Ft and TfR model, at baseline, there were 11 questionnaire variables with predictors. From the SF36v2 questionnaire: AGP and medication use were significant predictors of Physical Functioning, Bodily Pain, and Physical Component Score. AGP and medication use were negatively associated, meaning that as AGP or medication use increased, scores on Physical Functioning, Bodily Pain, or Physical Component Score decreased. Medication use was also a significant predictor of and negatively associated with Role Physical. AGP and Hematocrit were significant predictors of General Health. They were also negatively associated with General Health. TfR was a significant predictor of Role Emotional. They were positively associated meaning that as TfR values increased, scores on Role Emotional also increased. There were no significant predictors for Vitality, Social Functioning, Mental Health, or the Mental Component Score. From the WHOQOL questionnaire, vitamin use was a significant predictor of and negatively associated with Environmental Health. There were no significant predictors for Physical, Psychological, or Social Health. There were no significant predictors for Perceived Stress. From POMS: Vitamin use was a significant predictor of and positively associated with Anger and Total Mood Disturbance. Ft was a significant predictor of and negatively associated with Tension. As Ft levels increased, scores for Tension decreased (Table 6).

Table 6: Main Analysis Baseline Stepwise Regression (Ft and TfR Model)

Timepoint	Questionnaire	Dependent Variable	Step	Predictor Variable	Paramater (beta)	Partial R-Square	Predictor p-value	Model p-value	Model R-Square	Sample Size
Baseline	SF36v2	Physical Functioning	1	AGP	-0.16	0.1542	0.0522	0.0102	0.4686	25
			2	med	-11.32	0.1426	0.0463			
			3	Hct	1.35	0.0902	0.0933			
			4	vit	-6.93	0.0816	0.0949			
		Role Physical	1	med	-31.25	0.2536	0.0103	0.0103	0.34	25
			2	smoke	-26.04	0.0864	0.1037			
		Bodily Pain	1	med	-30.18	0.2437	0.0122	0.0012	0.5769	25
			2	AGP	-0.38	0.2025	0.0096			
			3	drink	-20.94	0.0758	0.0823			
			4	Ft	0.18	0.055	0.1225			
		General Health	1	AGP	-0.4	0.2669	0.0082	0.0027	0.5397	25
			2	Hct	-3.12	0.1211	0.0488			
			3	drink	17.34	0.0874	0.0753			
			4	med	-10.36	0.0643	0.1101			
		Vitality	1	AGP	-0.23	0.1386	0.0669	0.0669	0.1386	25
		Social Functioning	No variable met 0.15 significance level.							
		Role Emotional	1	TfR	3.26	0.1718	0.0394	0.0394	0.1718	25
		Mental Health	1	TfR	1.57	0.1155	0.0965	0.0965	0.1155	25
		Physical Component Score	1	med	-10.95	0.2706	0.0077	<0.0001	0.5784	25
			2	AGP	-0.15	0.3078	0.0006			
		Mental Component Score	1	TfR	1.01	0.1095	0.1062	0.1062	0.1095	25
	WHOQOL	Physical Health	1	med	1.93	0.1503	0.0555	0.0555	0.1503	25
		Psychological Health	1	BMI	-0.24	0.1165	0.095	0.0592	0.2927	25
			2	TfR	0.24	0.0978	0.1122			
			3	med	1.24	0.0785	0.1418			
		Social Health	1	vit	-2	0.1335	0.0725	0.0415	0.2512	25
			2	smoke	-4.11	0.1177	0.0764			
		Environmental Health	1	vit	-1.84	0.1851	0.0318	0.0318	0.1851	25
	PSS	Perceived Stress	1	vit	5.11	0.1455	0.0599	0.0349	0.3305	25
			2	med	-4.62	0.1027	0.097			
			3	BMI	0.61	0.0823	0.1231			
	POMS	Tension	1	Ft	-0.07	0.1509	0.055	0.0297	0.2736	25
			2	vit	4.13	0.1227	0.067			
		Anger	1	vit	5.78	0.1538	0.0525	0.0525	0.1538	25
		Fatigue	1	AGP	0.12	0.105	0.0804	0.0124	0.3063	25
			2	smoke	-10.03	0.0666	0.1485			
			3	Hb	1.43	0.0684	0.1396			
			4	Ft	-0.06	0.0663	0.1338			
		Depression	1	TfR	1.44	0.118	0.0927	0.064	0.2211	25
			2	smoke	-15.25	0.103	0.1021			
		Vigor	1	med	-4.23	0.1242	0.0841	0.0777	0.2072	25
			2	Hct	-0.58	0.0831	0.1432			
		Confusion	1	vit	2.83	0.1503	0.0555	0.04	0.2536	25
			2	med	-3.23	0.1033	0.0949			
		Total Mood Disturbance	1	vit	26.54	0.1629	0.0454	0.0454	0.1629	25

med=Medication Use (Yes or No) vit=Vitamin Use (Yes or No) smoke=Smoking (Yes or No)

Stepwise Regression (Ft and TfR Model): Endline

At Endline, 14 variables had significant predictors. From SF36v2, vitamin use, BMI and TfR were significant predictors of Physical Functioning. Vitamin use and BMI were negatively associated with Physical Functioning and TfR was positively associated. Vitamin use was significantly predictive of and negatively associated with scores on Role Physical. BMI was predictive of and negatively associated with scores for General Health. Smoking was negatively associated with and a significant predictor of Role Emotional. There were no significant predictors for Bodily Pain, Vitality, Social Functioning, Mental Health, and the Physical and Mental Component Scores (Table 7).

From the WHOQOL questionnaire, TfR was a significant predictor of and positively associated with Physical Health. Smoking was negatively associated with and significantly predictive of Psychological Health, and BMI was negatively associated with and significantly predictive of Social Health. There were no significant predictors for Environmental Health. From the Perceived Stress Scale, AGP was significantly predictive of and positively associated with Stress (Table 7).

For the POMS questionnaire, smoking was a significant predictor of and positively associated with Tension, Anger, Depression, Confusion, and Total Mood Disturbance. As smoking increases, scores for those subscales increase. Medication use and AGP were also significant predictors of Tension. Medication use was negatively associated, and AGP positively associated with Tension. AGP was also significantly predictive of and negatively associated with Depression. AGP and Vitamin use are significant predictors of and positively associated with Vigor. Fatigue had no significant predictors (Table 7).

Table 7: Main Analysis Endline Stepwise Regression (Ft and TfR Model)

Timepoint	Questionnaire	Dependent Variable	Step	Predictor Variable	Paramater (beta)	Partial R-Square	Predictor p-value	Model p-value	Model R-Square	Sample Size
Endline	SF36v2	Physical Functioning	1	vit	-5.3	0.2583	0.0531	0.0045	0.7512	15
			2	BMI	-0.8	0.2154	0.0468			
			3	TfR	0.91	0.1893	0.0303			
			4	pdd	-0.04	0.0882	0.0891			
		Role Physical	1	vit	-18.75	0.375	0.0152	0.0152	0.375	15
		Bodily Pain	No variable met 0.15 significance level.							
		General Health	1	BMI	-1.7	0.4575	0.0056	0.0056	0.4575	15
		Vitality	1	AGP	-0.37	0.1773	0.1181	0.1181	0.1773	15
		Social Functioning	1	med	14.77	0.231	0.0697	0.0697	0.231	15
		Role Emotional	1	smoke	-36.9	0.2625	0.0509	0.0509	0.2625	15
		Mental Health	No variable met 0.15 significance level.							
		Physical Component Score	No variable met 0.15 significance level.							
		Mental Component Score	1	AGP	-0.15	0.1574	0.1432	0.1432	0.1574	15
	WHOQOL	Physical Health	1	TfR	0.43	0.3028	0.0336	0.0253	0.4581	15
			2	med	1.48	0.1553	0.0884			
		Psychological Health	1	smoke	-3.62	0.3238	0.0268	0.0268	0.3238	15
		Social Health	1	BMI	-0.61	0.3228	0.0271	0.0198	0.4801	15
			2	TfR	0.61	0.1573	0.081			
		Environmental Health	1	vit	-3.17	0.1841	0.1105	0.0655	0.3652	15
			2	drink	-4.17	0.181	0.0891			
	PSS	Perceived Stress	1	AGP	0.17	0.3819	0.0141	0.0141	0.3819	15
	POMS	Tension	1	smoke	18.21	0.8041	<0.0001	<0.0001	0.9656	15
			2	med	-4.6	0.0623	0.0357			
			3	Ft	-0.1	0.0386	0.0581			
			4	AGP	0.1	0.0605	0.0019			
		Anger	1	smoke	30.21	0.9236	<0.0001	<0.0001	0.9364	15
			2	BMI	0.36	0.0128	0.1468			
		Fatigue	1	AGP	0.13	0.1949	0.0994	0.0994	0.1949	15
		Depression	1	smoke	33.89	0.2606	0.0518	0.0073	0.5595	15
			2	AGP	-0.27	0.2989	0.0145			
		Vigor	1	AGP	0.18	0.2563	0.0542	0.012	0.5214	15
			2	vit	7.43	0.2651	0.0242			
		Confusion	1	smoke	14.12	0.3187	0.0283	0.0293	0.4446	15
			2	TfR	-1.21	0.1259	0.125			
		Total Mood Disturbance	1	smoke	109.93	0.6261	0.0004	0.0004	0.6261	15

med=Medication Use (Yes or No) vit=Vitamin Use (Yes or No) smoke=Smoking (Yes or No)

Stepwise Regression (Body Iron Model): Baseline

Results of the stepwise regression using body iron were similar to those using Ft and TfR. At baseline, the models that did not change include, from SF36v2, Physical Functioning, Role Physical, General Health, Vitality, Social Functioning, from WHOQOL Physical Health, Social Health, Environmental Health, from POMS, Anger, Vigor, Confusion, and Total Mood Disturbance and Perceived Stress (Table 6).

At baseline, for SF36v2, the Bodily Pain and Physical Component Score models changed, but had the same significant predictors: medication use and AGP, negatively associated with both. The remaining models that changed: Role Emotional, Mental Health, and Mental Component Score no longer had significant predictors. From WHOQOL, only Social Health had a new model, but no predictors were significant (Table 8).

For the POMS questionnaire at baseline, body iron was a significant predictor of both Tension and Depression. Body iron was negatively associated with Tension and Depression, as body iron increased, Tension and Depression scores decreased (Table 8).

Table 8: Main Analysis Baseline Stepwise Regression (Body Iron Model)

Timepoint	Questionnaire	Dependent Variable	Step	Predictor Variable	Paramater (beta)	Partial R-Square	Predictor p-value	Model p-value	Model R-Square	Sample Size
Baseline	SF36v2	Physical Functioning	1	AGP	-0.16	0.1542	0.0522	0.0102	0.4686	25
			2	med	-11.32	0.1426	0.0463			
			3	Hct	1.35	0.0902	0.0933			
			4	vit	-6.93	0.0816	0.0949			
		Role Physical	1	med	-31.25	0.2536	0.0103	0.0103	0.34	25
			2	smoke	-26.04	0.0864	0.1037			
		Bodily Pain	1	med	-31.67	0.2437	0.0122	0.0007	0.6004	25
			2	AGP	-0.39	0.2025	0.0096			
			3	drink	-17.6	0.0739	0.0687			
			4	bodyiron	1.27	0.0804	0.0729			
		General Health	1	AGP	-0.4	0.2669	0.0082	0.0027	0.5397	25
			2	Hct	-3.12	0.1211	0.0488			
			3	drink	17.34	0.0874	0.0753			
			4	med	-10.36	0.0643	0.1101			
		Vitality	1	AGP	-0.23	0.1386	0.0669	0.0669	0.1386	25
		Social Functioning	No variable met 0.15 significance level.							
		Role Emotional	No variable met 0.15 significance level.							
		Mental Health	No variable met 0.15 significance level.							
		Physical Component Score	1	med	-11.29	0.2706	0.0077	<0.0001	0.6427	25
			2	AGP	-0.14	0.3078	0.0006			
			3	bodyiron	0.34	0.0643	0.0655			
		Mental Component Score	No variable met 0.15 significance level.							
	WHOQOL	Physical Health	1	med	1.93	0.1503	0.0555	0.0555	0.1503	25
		Psychological Health	1	BMI	-0.23	0.1165	0.095	0.0799	0.2927	25
			2	med	1.31	0.0888	0.1313			
		Social Health	1	vit	-2	0.1335	0.0725	0.0415	0.2512	25
			2	smoke	-4.11	0.1177	0.0764			
		Environmental Health	1	vit	-1.84	0.1851	0.0318*	0.0318	0.1851	25
	PSS	Perceived Stress	1	vit	5.11	0.1455	0.0599	0.0349	0.3305	25
			2	med	-4.62	0.1027	0.097			
			3	BMI	0.61	0.0823	0.1231			
	POMS	Tension	1	bodyiron	-0.53	0.2609	0.0091	0.0072	0.3617	25
			2	vit	3.76	0.1008	0.0757			
		Anger	1	vit	5.78	0.1538	0.0525	0.0525	0.1538	25
		Fatigue	1	AGP	0.14	0.105	0.0804	0.0139	0.3063	25
			2	smoke	-11.81	0.0666	0.1485			
			3	Hb	1.06	0.0684	0.1396			
		Depression	1	bodyiron	-0.87	0.1955	0.0269	0.0241	0.2872	25
			2	AGP	-0.11	0.0917	0.1066			
		Vigor	1	med	-4.23	0.1242	0.0841	0.0777	0.2072	25
			2	Hct	-0.58	0.0831	0.1432			
		Confusion	1	vit	2.83	0.1503	0.0555	0.04	0.2536	25
			2	med	-3.23	0.1033	0.0949			
		Total Mood Disturbance	1	vit	26.54	0.1629	0.0454	0.0454	0.1629	25
med=Medication Use (Yes or No) vit=Vitamin Use (Yes or No) smoke=Smoking (Yes or No)										

Stepwise Regression (Body Iron Model): Endline

At endline, the models that did not change are Bodily Pain, General Health, Vitality, Social Functioning, Role Emotional, Mental Health and the Mental and Physical Component scores, Psychological and Environmental Health, Perceived Stress, Anger, Fatigue, Depression, Vigor, and Total Mood Disturbance (Table 7).

For Physical Functioning, BMI and vitamin use were predictive in the same way. Body iron was also significantly predictive of and negatively associated with Physical Functioning. For Tension, smoking and medicine use were still predictive in the same way, but body iron became significantly predictive of and negatively associated with Tension. For Role Physical and Confusion, what loaded into the model changed, but the significant predictors did not. Physical Health had no significant predictors in the Body Iron model (Table 9).

Table 9: Main Analysis Endline Stepwise Regression (Body Iron Model)

Timepoint	Questionnaire	Dependent Variable	Step	Predictor Variable	Paramater (beta)	Partial R-Square	Predictor p-value	Model p-value	Model R-Square	Sample Size
Endline	SF36v2	Physical Functioning	1	vit	-5.52	0.2583	0.0531	0.0082	0.7176	15
			2	BMI	-0.58	0.2154	0.0468			
			3	body iron	-0.35	0.1732	0.0404			
			4	pdd	-0.04	0.0707	0.1446			
		Role Physical	1	vit	-19.09	0.375	0.0152	0.0148	0.5046	15
			2	body iron	-1.28	0.1296	0.1017			
		Bodily Pain	No variable met 0.15 significance level.							
		General Health	1	BMI	-1.7	0.4575	0.0056	0.0056	0.4575	15
		Vitality	1	AGP	-0.37	0.1773	0.1181	0.1181	0.1773	15
		Social Functioning	1	med	14.77	0.231	0.0697	0.0697	0.231	15
		Role Emotional	1	smoke	-36.9	0.2625	0.0509	0.0509	0.2625	15
		Mental Health	No variable met 0.15 significance level.							
		Physical Component Score	No variable met 0.15 significance level.							
		Mental Component Score	1	AGP	-0.15	0.1574	0.1432	0.1432	0.1574	15
	WHOQOL	Physical Health	1	med	1.82	0.2448	0.0608	0.0608	0.2448	15
		Psychological Health	1	smoke	-3.62	0.3238	0.0268	0.0268	0.3238	15
		Social Health	1	BMI	-0.48	0.3228	0.0271	0.0314	0.4383	15
			2	med	1.92	0.1155	0.1422			
		Environmental Health	1	vit	-3.17	0.1841	0.1105	0.0655	0.3652	15
			2	drink	-4.17	0.181	0.0891			
	PSS	Perceived Stress	1	AGP	0.17	0.3819	0.0141	0.0141	0.3819	15
	POMS	Tension	1	smoke	18.58	0.8041	<0.0001	<0.0001	0.9462	15
			2	med	-4.57	0.0623	0.0357			
			3	AGP	0.1	0.0291	0.108			
			4	body iron	-0.44	0.0507	0.0119			
		Anger	1	smoke	30.21	0.9236	<0.0001	<0.0001	0.9364	15
			2	BMI	0.36	0.0128	0.1468			
		Fatigue	1	AGP	0.13	0.1949	0.0994	0.0994	0.1949	15
		Depression	1	smoke	33.89	0.2606	0.0518	0.0073	0.5595	15
			2	AGP	-0.27	0.2989	0.0145			
		Vigor	1	AGP	0.18	0.2563	0.0542	0.012	0.5214	15
			2	vit	7.43	0.2651	0.0242			
		Confusion	1	smoke	13.57	0.3187	0.0283	0.0283	0.3187	15
		Total Mood Disturbance	1	smoke	109.93	0.6261	0.0004	0.0004	0.6261	15
med=Medication Use (Yes or No) vit=Vitamin Use (Yes or No) smoke=Smoking (Yes or No)										

Chapter 6

Secondary Analysis Results

A secondary analysis was run in order to recategorize participants to study iron's effects on mood, stress, and quality of life in a different way. Our hypothesis was that changes in iron status would be related to changes in affective variables. Therefore, we can analyze the data based on changes in iron status, irrespective of treatment group assignment. Physiologically, we know that iron changes daily despite treatment type. Therefore, if we are to categorize participants as responders or non-responders in terms of changes in iron status, we want to account for the daily variation that occurs in iron status markers and define "responders" as those who experience a change in iron status (ferritin or hemoglobin) above and beyond the known day-to-day variation (Borel et al. 1991).

The groups analyzed here are categorized as responders vs. non-responders according to changes in Ft (FtR vs. FtNR) and responders vs. non-responders according to changes in Hb (HbR vs. HbNR). Analysis of Variance at given time points as well as examining the difference between baseline and endline (both with and without covariates) will be presented for FtR vs. FtNR and HbR vs. HbNR.

Ferritin Responders vs. Non-Responders Analyses

Analysis of Variance (ANOVA/ANCOVA)

An analysis of variance was done in order to understand if any difference existed between the responders and non-responders, determined by changes in Ft, on any of the questionnaire

subscales. Table 10 represents the means and p-values at both time points. There were no significant differences between groups on any of the subscale scores at baseline. With the addition of covariates, the WHOQOL Physical Health subscale showed significant differences between FtR and FtNR at p=0.0457. Non-responders had a higher average score for Physical Health compared to responders at baseline.

At endline, there were no significant differences between groups on any of the subscale scores when running unadjusted analyses. After the addition of covariates, scores on the POMS Anger subscale became significantly different between groups with responders having a significantly lower score than non-responders ($p<0.0001$). Scores on several other subscales became almost significantly different between groups after controlling for covariates: SF36v2 General Health, where non-responders scored higher than responders (p=0.057) Physical Health on the WHOQOL was higher for responders than for non-responders (p=0.068). WHOQOL Psychological Health was higher for the responders vs. non-responders (p=0.073); finally, on the POMS questionnaire, 3 subscales became significant after covariate adjustment: Tension (p=0.0001), Confusion (p=0.0451), and Total Mood Disturbance (p=0.0008), where non-responders had a higher average score on all 3 subscales compared to responders (Table 10).

Table 10: Responders vs. Non-Responders (according to Ft) Analysis of Variance (ANOVA)

			Baseline			Endline		
	Variable		Responder (Ft) n=12	Non-Responder (Ft) n=11	p	Responder (Ft) n=12	Non-Responder (Ft) n=11	p
SF36v2	Physical Functioning	Mean (SD)	97.9 (3.3)	95.0 (6.7)	0.195	98.3 (4.4)	96.8 (5.1)	0.456
	Role Physical	Mean (SD)	93.8 (12.5)	89.8 (17.3)	0.532	96.4 (7.8)	94.9 (15.0)	0.768
	Bodily Pain	Mean (SD)	87.4 (15.8)	83.5 (18.4)	0.583	90.8 (11.8)	85.9 (21.9)	0.504
	General Health	Mean (SD)	74.0 (15.3)	75.9 (17.1)	0.781	72.9 (15.4)	77.5 (8.3)*	0.386
	Vitality	Mean (SD)	52.1 (17.5)	57.4 (17.9)	0.481	62.0 (21.1)	56.3 (17.7)	0.49
	Social Functioning	Mean (SD)	79.2 (20.9)	84.1 (19.4)	0.566	89.6 (15.8)	88.6 (13.1)	0.878
	Role Emotional	Mean (SD)	76.4 (23.3)	83.3 (17.9)	0.434	84.0 (16.5)	81.8 (21.7)	0.785
	Mental Health	Mean (SD)	69.6 (19.1)	74.1 (10.4)	0.497	69.2 (18.6)	70.5 (13.5)	0.852
	Physical Component Score	Mean (SD)	58.9 (4.3)	56.6 (6.7)	0.339	59.1 (3.3)	58.6 (3.5)	0.695
	Mental Component Score	Mean (SD)	43.7 (11.5)	47.8 (7.5)	0.326	46.9 (10.4)	46.5 (7.9)	0.907
WHO Quality of Life	Physical Health	Mean (SD)	12.3 (1.9)	13.5 (2.1)*	0.196	12.6 (1.2)	13.5 (2.0)*	0.176
	Psychological Health	Mean (SD)	14.0 (2.1)	13.9 (2.2)	0.896	14.1(1.6)	13.8 (1.8)*	0.686
	Social Health	Mean (SD)	15.4 (3.8)	16.2 (2.8)	0.577	15.9 (3.2)	16.5 (3.2)	0.661
	Environmental Health	Mean (SD)	14.5 (2.0)	16.2 (2.6)	0.09481	15.0 (1.9)	16.4 (2.7)	0.188
PSS	Perceived Stress Scale	Mean (SD)	16.8 (4.5)	16.1 (6.8)	0.786	15.0 (6.0)	15.4 (7.2)	0.896
Profile of Moods (POMS)	Tension	Mean (SD)	9.7 (5.8)	7.0 (5.7)	0.278	9.2 (4.5)	9.3 (8.7)*	0.971
	Anger	Mean (SD)	8.1 (7.3)	7.9 (7.7)	0.956	3.1 (3.8)	8.5 (10.1)*	0.10021
	Fatigue	Mean (SD)	7.1 (5.4)	4.6 (4.9)	0.271	6.5 (6.3)	8.3 (7.7)	0.549
	Depression	Mean (SD)	12.3 (7.1)	14.5 (7.9)	0.488	12.1 (8.9)	14.8 (10.7)	0.511
	Vigor	Mean (SD)	9.9 (6.1)	7.1 (6.0)	0.277	10.6 (5.5)	10.7 (6.9)	0.956
	Confusion	Mean (SD)	6.7 (2.1)	5.6 (4.9)	0.516	7.3 (2.6)	9.5 (7.4)*	0.345
	Total Mood Disturbance	Mean (SD)	24.9 (24.5)	17.7 (31.8)	0.549	19.3 (21.2)	28.1 (43.4)*	0.54
* represents significant differences ($p<0.05$) between groups within a given time point after controlling for covariates								

Analysis of Variance (ANOVA/ANCOVA): Evaluating the Difference in Scores between Time Points

This analysis again includes the difference values obtained from subtracting the baseline scores from the endline scores for each participant. An ANOVA using these difference values aims to understand if there is a significant difference between responders and non-responders (according to ferritin) in their changes over time. Table 11 represents the average changes over time for each subscale in each group as well as the p-value determining whether the difference was significant. Baseline values were controlled for in this analysis.

Scores on SF36v2 Physical Functioning (p=0.0031), Role Physical (p=0.0174), Bodily Pain (p=0.0082), and General Health (p=0.0028), Role Emotional (p=0.0127), and Physical Component Score (p=0.0001) changed significantly differently over time between the responder and non-responder groups according to Ft both with (p-values listed above) and without (p-values listed in Table 11) controlling for covariates. The non-responder group had a larger increase in scores on Physical Functioning, Role Physical, and General Health, compared to the responder group. The responder group had a larger increase in scores for Bodily Pain and Social Functioning compared to the non-responder group. For the Role Emotional scores, the responders increased while the non-responders decreased. The scores for Vitality and Mental Component Score were no longer significantly different between groups after controlling for covariates. Differences in scores for Mental Health remained insignificant after the addition of covariates.

Physical Health (p=0.0270), Psychological Health (p=0.0396), and Social Health (p=0.0217) scores from the WHOQOL questionnaire also had significantly different changes over time between the responder and non-responder groups with (p-values listed above) and

without (p-values listed in Table 11) controlling for covariates. For all three subscales, responders increased their scores significantly more than non-responders. There were no significant differences with or without controlling for covariates for the Environmental Health subscale.

The POMS questionnaire subscales Tension ($p < 0.0001$), Anger ($p < 0.0001$), Depression ($p=0.0125$), and Vigor ($p=0.0247$) scores were significantly different in their changes over time between the responder and non-responder groups both with (p-values listed above) and without (p-values listed in Table 11) controlling for covariates. The responders' scores for Tension, Anger, and Depression on average, all decreased while the non-responders' scores on average increased. The non-responders, however, had a larger increase in scores for Vigor compared to the responders group. Both Confusion, and Total Mood Disturbance showed significant differences between groups in change over time after controlling for covariates. The responders had an average decrease on scores for Total Mood Disturbance compared to the non-responders whose scores actually increased. While both groups increased scores on Confusion, responders also had a lower average increase on scores for Confusion compared to the non-responders. There were no significant differences for scores on the Fatigue subscale with or without covariates (Table 11).

Table 11: Difference (score at endline minus score at baseline) Analysis of Variance (ANOVA) (Ft R vs. NR)

		Variable		Responder (Ft) n=12	Non-Responder (Ft) n=11	p
Difference	SF36v2	Physical Functioning	Mean (SD)	0.4 (5.8)	1.8 (7.5)*	0.001
		Role Physical	Mean (SD)	2.6 (11.1)	5.1 (22.7)*	0.0002
		Bodily Pain	Mean (SD)	3.4 (14.0)	2.5 (29.8)*	0.0034
		General Health	Mean (SD)	-1.1 (10.4)	1.6 (15.4)*	0.0035
		Vitality	Mean (SD)	9.9 (16.3)	-1.1 (14.2)	0.0983
		Social Functioning	Mean (SD)	10.4 (19.1)	4.5 (25.8)*	<0.0001
		Role Emotional	Mean (SD)	7.6 (19.0)	-1.5 (18.6)*	0.0172
		Mental Health	Mean (SD)	-0.4 (17.9)	-3.6 (11.2)	0.1112
		Physical Component Score	Mean (SD)	0.2 (3.6)	2.0 (8.2)*	<0.0001
		Mental Component Score	Mean (SD)	3.2 (8.0)	-1.3 (5.8)	0.0453
	WHO Quality of Life	Physical Health	Mean (SD)	0.2 (1.6)	0.1 (1.4)*	0.0074
		Psychological Health	Mean (SD)	0.1 (1.6)	-0.1 (1.2)*	0.0101
		Social Health	Mean (SD)	0.4 (3.9)	0.2 (2.1)*	0.0398
		Environmental Health	Mean (SD)	0.5 (2.3)	0.1 (0.6)	0.2088
	PSS	Perceived Stress Scale	Mean (SD)	-1.8 (4.4)	-0.7 (4.5)	0.623
	Profile of Moods (POMS)	Tension	Mean (SD)	-0.5 (6.3)	2.3 (9.8)*	0.0157
		Anger	Mean (SD)	-5.0 (8.5)	0.5 (11.4)*	0.0012
		Fatigue	Mean (SD)	-0.6 (4.3)	3.6 (7.6)	0.1591
		Depression	Mean (SD)	-0.3 (6.2)	0.3 (15.0)*	0.0443
		Vigor	Mean (SD)	0.7 (7.5)	3.6 (9.0)*	0.0023
		Confusion	Mean (SD)	0.6 (2.9)	3.8 (5.2)*	0.2139
		Total Mood Disturbance	Mean (SD)	-5.6 (24.9)	10.4 (30.8)*	0.1862
* represents significant difference ($p < 0.05$) between groups after controlling for covariates						

Hemoglobin Responders vs. Non-Responders Analyses

Analysis of Variance (ANOVA/ANCOVA)

An analysis of variance was done in order to understand if any difference existed between the responders and non-responders, determined by changes in Hb, on any of the questionnaire subscales. No variables were significantly different at baseline or endline between the responders and non-responders as defined by Hb. The summary value for the Profile of Moods questionnaire at endline, however, was close to significant at $p < 0.1$. Responders had a much lower Total Mood Disturbance score compared to non-responders (Table 12). After controlling for covariates, the difference between responders and non-responders, on Total Mood Disturbance became significant at $p=0.0001$.

A number of other variables became significant at baseline after controlling for covariates. From the SF36v2 questionnaire, Bodily Pain scores were significantly different ($p=0.0102$) between groups with non-responders having higher scores for Bodily Pain compared to responders. SF36v2 Physical Component Score (PCS) also showed significant ($p=0.0560$) differences between groups after controlling covariates with non-responders having a higher average PCS compared to responders. From the WHOQOL questionnaire, Physical Health ($p=0.0912$) and from the POMS questionnaire, Depression ($p=0.0836$) showed almost significant differences between groups. Responders had a higher score compared to non-responders for Physical Health and responders had higher Depression scores compared to non-responders after controlling for covariates (Table 12).

At endline, many variables became significant or almost significant after the addition of covariates. Two variables from the SF36v2 questionnaire were almost significant between

groups: General Health (p=0.0815) and Role Emotional (p=0.0815). The responder groups for both Role Emotional and General Health had higher scores compared to their respective non-responder averages (Table 12).

From the WHOQOL questionnaire, two variables presented significant differences, and one an almost significant difference after the control of covariates. There were significant differences (p=0.0405) between responders and non-responders for Physical with responders having a higher Physical Health Score at endline compared to non-responders. After controlling for covariates, there were significant differences (p=0.0561) between groups for Psychological Health. Responders had a higher average score for Psychological Health compared to non-responders. Social Health also showed an almost significant (p=0.0900) difference between groups with responders having a higher average Social Health score compared to non-responders at endline (Table 12).

The Perceived Stress variable at endline also became significantly different (p=0.0521) between groups after controlling for covariates. Non-responders had a higher average score for Perceived Stress compared to Responders. Tension, Anger, Confusion, and Depression from the POMS questionnaire all presented significant differences after controlling for covariates. For all of those variables, non-responders had higher scores compared to responders (Table 12).

Table 12: Responder vs. Non-Responder (according to Hb) Analysis of Variance (ANOVA)

			Baseline			Endline		
	Variable		Responder (Hb) n=12	Non-Responder (Hb) n=21	p	Responder (Hb) n=12	Non-Responder (Hb) n=11	p
SF36v2	Physical Functioning	Mean (SD)	94.2 (15.8)	95.2 (6.4)	0.785	93.8 (12.1)	97.4 (4.6)	0.225
	Role Physical	Mean (SD)	87.5 (24.9)	89.9 (15.5)	0.736	88.0 (20.0)	93.8 (14.0)	0.341
	Bodily Pain	Mean (SD)	76.2 (25.7)	85.2 (18.1)*	0.247	85.7 (23.2)	86.8 (18.9)	0.884
	General Health	Mean (SD)	75.6 (14.9)	72.1 (15.8)	0.539	72.3 (15.4)	69.4 (16.1)*	0.621
	Vitality	Mean (SD)	53.1 (18.2)	53.3 (16.6)	0.981	63.5 (10.9)	53.9 (20.9)	0.147
	Social Functioning	Mean (SD)	75.0 (22.6)	86.9 (16.5)	0.092	86.5 (14.6)	86.9 (14.5)	0.933
	Role Emotional	Mean (SD)	77.8 (21.1)	80.6 (19.1)	0.701	87.5 (12.6)	81.3 (18.6)*	0.317
	Mental Health	Mean (SD)	72.1 (19.5)	73.1 (11.6)	0.852	75.0 (10.2)	69.0 (18.1)	0.306
	Physical Component Score	Mean (SD)	55.9 (7.7)	56.7 (5.7)	0.723	55.7 (8.2)	57.8 (4.4)	0.327
	Mental Component Score	Mean (SD)	45.3 (10.9)	46.8 (7.7)	0.639	49.9 (4.4)	45.6 (9.8)	0.167
WHO Quality of Life	Physical Health	Mean (SD)	13.1 (1.7)	12.6 (2.3)	0.457	13.4 (1.6)	12.5 (1.7)*	0.145
	Psychological Health	Mean (SD)	13.8 (1.6)	13.9 (2.2)	0.861	14.4 (2.0)	13.8 (1.7)*	0.416
	Social Health	Mean (SD)	14.9 (3.3)	16.1 (2.8)	0.287	17.1 (2.3)	15.9 (3.1)*	0.236
	Environmental Health	Mean (SD)	15.0 (2.5)	15.5 (2.3)	0.635	15.8 (2.1)	15.4 (2.5)	0.601
PSS	Perceived Stress Scale	Mean (SD)	17.3 (5.1)	16.7 (6.3)	0.788	12.8 (4.5)	15.7 (6.5)*	0.178
Profile of Moods (POMS)	Tension	Mean (SD)	9.4 (5.5)	8.9 (6.4)	0.802	7.3 (4.6)	10.2 (6.3)*	0.168
	Anger	Mean (SD)	7.8 (5.1)	7.9 (8.7)	0.98	3.4 (1.9)	6.6 (7.9)*	0.18
	Fatigue	Mean (SD)	7.5 (7.2)	7.1 (5.6)	0.875	6.2 (6.2)	8.8 (6.3)	0.258
	Depression	Mean (SD)	13 (7.3)	11.7 (10.0)*	0.69	9.9 (7.3)	12.0 (9.8)*	0.517
	Vigor	Mean (SD)	10.3 (7.5)	9.6 (6.2)	0.771	12.1 (7.8)	12.1 (6.3)	0.996
	Confusion	Mean (SD)	6.5 (4.3)	7.0 (3.9)	0.713	6.7 (1.9)	8.3 (5.7)*	0.349
	Total Mood Disturbance	Mean (SD)	22.6 (28.1)	26.5 (34.4)	0.739	11.3 (16.1)	28.9 (32.8)*	0.094
* represents significant differences ($p<0.05$) between groups within a given time point after controlling for covariates								

Analysis of Variance (ANOVA/ANCOVA): Evaluating the Difference in Scores between Time Points

This analysis again includes the difference values obtained from subtracting the baseline scores from the endline scores for each participant. An ANOVA using these difference values aims to understand if there is a significant difference between responders and non-responders (according to hemoglobin) in their changes over time. Table 13 represents the average changes over time for each subscale in each group as well as the p-value determining whether the difference was significant. Baseline values were controlled for in this analysis.

The SF36v2 subscales Physical Functioning ($p=0.0005$), Role Physical ($p=0.0268$), Bodily Pain ($p=0.0267$), General Health ($p=0.0054$), Social Functioning ($p<0.0001$), and Role Emotional ($p=0.0004$) score changes over time were significantly different between responders and non-responders with and without controlling for covariates. Responders had an average decrease in their Physical Functioning scores over time while the non-responders had an average increase of Physical Functioning scores. Non-responders had a larger average increase in scores for Role Physical compared to responders. Responders had a larger average increase in scores for Social Functioning, Role Emotional, and Bodily Pain compared to the non-responders. Both responders' and non-responders' scores for General Health decreased, but the responders' scores decreased more (Table 13). After controlling for covariates, the differences between changes over time for the summary values Physical Component Score ($p=0.0843$) and Mental Component Score ($p=0.0698$) were almost significant ($p<0.1$). The responders had an average decrease in Physical Component Score while the non-responders had an average increase. The responders had an average increase in Mental Component Score, however, while the non-responders had an average decrease. Vitality score differences were no longer significant in the ANCOVA.

Change in scores on WHOQOL subscales Physical Health (p=0.0013) and Social Health (p=0.0004) were significantly different both with and without controlling for covariates. Responders had an average increase for Physical Health and Social Health scores while the non-responders had an average decrease. After controlling for covariates, differences between changes in Psychological Health scores became almost significant (p= 0.0674) and differences between changes in Environmental Health (p=0.0620) remained almost significant. Like the other two subscales, responders had an average increase for Psychological Health and Environmental Health while the non-responders had an average decrease (Table 13).

After controlling for covariates, there still remained significant differences (p=0.0123) in change over time for the Perceived Stress Scale. Both responders and non-responders had an average decrease in scores for Stress, but the responders had a larger average decrease than the non-responders (Table 13).

All of the POMS subscale scores: Tension (p<0.0001), Anger (p<0.0001), Fatigue (p=0.0073), Depression (p=0.0001), Vigor (p=0.0305), Confusion (p=0.0047), and Total Mood Disturbance (p<0.0001) had significantly different changes over time between responders and non-responders with and without controlling for covariates. Responders had an average decrease while non-responders had an average increase in scores for Tension, Fatigue, Depression, and Total Mood Disturbance. Both responders and non-responders had average decreases in scores for Anger, but the responders had a larger average decrease. Non-responders had a larger increase in scores for Vigor compared to responders. Both responders and non-responders had an average increase in scores for Confusion, but the responders increased less than the non-responders (Table 13).

Table 13: Difference (score at endline minus score at baseline) Analysis of Variance (ANOVA) (Hb R vs. NR)

		Variable		Responder (Hb) n=12	Non-Responder (Hb) n=21	p
Difference	SF36v2	Physical Functioning	Mean (SD)	-0.4 (8.1)	2.1 (6.0)*	0.0001
		Role Physical	Mean (SD)	0.5 (17.6)	3.9 (17.6)*	0.0008
		Bodily Pain	Mean (SD)	9.5 (10.0)	1.6 (23.9)*	0.0068
		General Health	Mean (SD)	-3.3 (17.4)	-2.6 (15.4)*	0.0139
		Vitality	Mean (SD)	10.4 (13.9)	0.6 (14.4)	0.0247
		Social Functioning	Mean (SD)	11.5 (22.3)	0 (21.3)*	<0.0001
		Role Emotional	Mean (SD)	9.7 (20.7)	0.8 (16.0)*	0.0004
		Mental Health	Mean (SD)	2.9 (19.5)	-4.0 (11.4)	0.0204
		Physical Component Score	Mean (SD)	-0.2 (3.6)	1.1 (6.5)	0.007
		Mental Component Score	Mean (SD)	4.6 (8.8)	-1.2 (5.3)	0.002
	WHO Quality of Life	Physical Health	Mean (SD)	0.3 (1.7)	-0.02 (1.6)*	0.0003
		Psychological Health	Mean (SD)	0.6 (1.4)	-0.06 (1.3)*	0.014
		Social Health	Mean (SD)	2.2 (4.0)	-0.2 (2.4)*	0.0004
		Environmental Health	Mean (SD)	0.8 (2.1)	-0.1 (1.1)	0.062
	PSS	Perceived Stress Scale	Mean (SD)	-4.5 (6.7)	-0.3 (3.9)*	0.0102
	Profile of Moods (POMS)	Tension	Mean (SD)	-2.2 (4.2)	1.3 (8.7)*	0.0001
		Anger	Mean (SD)	-4.4 (5.0)	-1.3 (10.9)*	<0.0001
		Fatigue	Mean (SD)	-1.3 (4.7)	1.6 (6.8)*	0.0073
		Depression	Mean (SD)	-3.1 (6.1)	0.4 (12.4)*	0.001
		Vigor	Mean (SD)	1.8 (7.9)	2.5 (7.3)*	0.005
		Confusion	Mean (SD)	0.2 (3.4)	1.2 (5.5)*	0.0241
		Total Mood Disturbance	Mean (SD)	-11.3 (21.9)	2.3 (33.3)*	0.0007
* represents significant difference ($p < 0.05$) between groups after controlling for covariates						

Chapter 6

Discussion

This was the first study, to our knowledge, to simultaneously look at the relationship between iron status in WRA and stress, mood, and quality of life. We found that iron had a significant role in improving feelings of stress, mood, and quality of life. Specifically, iron treatment in iron deficient WRA improved feelings of Physical Functioning and Social Functioning and decreased feelings of Bodily Pain, Stress, Tension, Anger, Depression, Confusion and overall Mood Disturbance compared to placebo given to iron sufficient women. Participants whose Hb status improved over time (irrespective of treatment) experienced improvements in Social Functioning, Physical Health, Social Health and decreases in Bodily Pain, Role limitations due to Emotional health, Stress, Tension, Anger, Fatigue, Depression, Confusion and overall Mood Disturbance compared to placebo. Participants whose Ft status improved (irrespective of treatment) experienced improvements in Social Functioning, Physical Health, Psychological Health, and Social Health and decreases in Bodily Pain, Role limitations due to Emotional health, Tension, Anger, Depression, and overall Mood Disturbance compared to placebo. Ft and body iron were significant predictors for Tension at both baseline and endline. They were negatively associated indicating that as Ft levels decrease (indicating poorer iron status), scores for and therefore feelings of Tension increase. Body Iron was also a significant predictor of and negatively associated with Depression, as Body Iron decreases (indicating poorer iron status), scores for Depression increase, indicating more depression.

Stress

Very few, if any studies have directly looked for an association between iron and stress in a population of non-pregnant WRA. In a study published by Beard et al. in 2005, iron status was determined to be related to stress (and cognitive function and depression) in African mothers postpartum. Despite the populations being different, the results of the present study agree with the findings from the Beard et al. 2005 study. In this study iron deficient participants, receiving iron treatment, decreased feelings of stress more than iron sufficient participants receiving placebo. More significantly, participants whose Hb status improved (responders) saw a significantly larger decrease in feelings of Stress compared to non-responders according to Hb so much so that at endline, the responders had significantly less feelings of stress compared to non-responders. This finding is particularly interesting in comparison with the Beard et al. study in that the South African mothers were categorized as Iron Deficient Anemic, which is directly related to Hb. Both studies saw significant improvements related to Hb status.

Mood

The present study found iron treatment to improve some indicators of mood. Iron deficient participants treated with iron experienced changes that were significantly different from those of the iron sufficient group treated with placebo for Tension, Anger, Depression, Confusion, and overall Mood Disturbance. In fact, for all of those measures, the ID group decreased while the IS group increased scores so much so that there was a significant difference between the scores at endline with the ID group having significantly lower scores compared to the IS group. Interestingly, Vigor increased for both groups after treatment, but increased

significantly more for the IS group compared to the ID group. There were improvements in Vigor after treatment, but they cannot necessarily be attributed to treatment with iron. There were no significant effects on Fatigue in the main analysis. The results were the same for the responders vs. non-responders according to Hb. Those whose Hb status improved saw the same decreases in mood related indicators (and an increase in Vigor) but the Fatigue scores decreased significantly more in the responder group compared to the non-responders. Results were similar for the responders vs. non-responders according to Ft. Those whose Ft status improved saw decreases in mood indicators but only Tension, Anger, and Total Mood Disturbance showed significant differences between scores at endline with the responders having significantly lower scores compared to non-responders. Fatigue was not significant in this model and the groups both increased feelings of Confusion in this model but the non-responders showed a larger increase than the responders. At endline, the responders had a significantly lower score for Confusion compared to the non-responders but both scores still increased over time.

The available literature comparing iron and mood indicators in non-pregnant WRA shows mixed results. Studies by Benton and Donohue 1999, Rangan et al. 1998, Fordy and Benton 1994, Hunt and Penland 1999, Beck et al. 2012, and Richardson et al. 2015 all showed no association between iron status and indicators related to mood. However, studies by Patterson et al. 2000, Verdon et al. 2003, Vahdat Shariatpanaahi et al. 2007, McClung et al. 2009, and Vaucher et al. 2012, showed significant effects of iron on indicators related to mood: treatment with iron led to improvements on mood related indicators. With the exception of Vahdat Shariatpanaahi et al. 2007, all of the studies presenting a significant relationship between iron and mood (where iron helped improve mood) were intervention studies while the studies

showing no relationship were not. The present study agrees with the findings of the intervention studies: that iron has a significant effect on improving indicators related to mood.

Quality of Life

Quality of Life indicators showed a larger variation in results. Iron treatment improved Physical Functioning, Social Functioning and overall Physical Health (PCS) and decreased feelings of Bodily Pain significantly more than the placebo treatment. Treatment also seemed to decrease the perception of role limitations from physical health and emotional health for both groups, but the IS group showed significantly more improvements compared to the ID group. General Health also decreased for both groups with the ID group decreasing significantly more than the IS group. Physical Health and Environmental Health scores decreased over time in the ID group while they increased for the IS group so much so that the ID group had significantly lower scores at endline after treatment compared to the IS Group. Both groups improved Social Health, but the IS group improved significantly more.

When the groups were recategorized, those whose Hb status improved showed different significant improvements in quality of life. Social Functioning, Physical Health and Social Health increased significantly more for responders compared to non-responders and responders saw a significant decrease in Bodily Pain as well as Role limitations due to Emotional health. Scores for Physical and Social Health were significantly higher for the responder group compared to the non-responder group at endline. While both responders and non-responders saw significant decreases in Role limitations due to Physical health, the non-responder group had more significant results compared to the responder group. General Health decreased for both

groups, but unfortunately decreased more for the responder group. Physical Functioning decreased for the responder group while it increased for the non-responder group.

As far as the ferritin responders, those whose Ft status improved experienced improvements in Social Functioning, Physical Health, Psychological Health, and Social Health which were significantly greater than non-responders. They also experienced significant decreases in feelings of Bodily Pain and Role limitations according to Emotional health compared to non-responders. For Physical Functioning and General Health, both groups experienced improvements, but the non-responders improved significantly more than responders. Similarly, Role limitations due to Physical Health decreased for both groups but the non-responders saw a more significant improvement.

There were many improvements in quality of life due to iron, but there also were a few quality of life indicators that did not improve after iron treatment. The literature also seems to be fairly inconsistent in relating quality of life and iron. Studies by Duport et al. 2003, Beck et al. 2012, Vaucher et al. 2012, and Rigas et al. 2015 did not identify a relationship between iron and indicators of quality of life. Patterson et al. 2000, Ando et al. 2006, Grondin et al. 2008, and Comin-Colet et al. 2013, however, did find a relationship between iron and indicators of quality of life, where iron improved quality of life or higher iron status was associated with higher quality of life. The studies varied in type, but there were not clear differences in findings based on whether the study was an intervention or an association study. The present study, on the whole agrees with the studies by Patterson et al. 2000, Ando et al. 2006, Grondin et al. 2008, and Comin-Colet et al. 2013 finding iron to improve feelings of quality of life. More studies are needed to confirm this however.

Strengths and Limitations

Some strengths of the present study include the examination of a variation of affective outcomes: stress, mood, and quality of life. Additionally, choosing scales that broke down the affective outcomes into subscales allowed for a more specific look at the effect that iron has. Another strength is including analyses with a variety of group categorizations (IS vs. ID, R vs. NR (Ft), and R vs. NR (Hb)). Using multiple group categorizations makes results that transcend the group categorizations particularly significant, as well as providing the opportunity to see important significant results in one group categorization that may not have shown through in the other group categorizations (due to small sample size or other elements.)

A limitation of the study is the small sample size. That limitation is partially mitigated, however, by the existence of significance despite a small sample size. Another limitation is the amount of missing data. Because the study was completed in three phases, one of the phases was completed with a different group than the other two and less attention was paid to ensuring that the data was complete. Given that this was an exploratory pilot study, the supplementation was not given randomly, but rather only to iron deficient participants.

Future Directions

Moving forward, it is important to continue studying the effects of iron on this population. While currently, the literature represents inconsistent results, there is promise indicating that iron treatment may improve the affective outcomes of stress, mood, and quality of life. While the small sample size may have precluded us from clearly showing the benefits of iron for affective outcomes, this study identified no detriments to treating iron deficient women

with iron. This population is of particular importance as many WRA will eventually become pregnant. Ensuring that they have adequate iron stores is essential for their future baby, and focusing on intervening before pregnancy, creates less of an urgency or problem during future pregnancies.

BIBLIOGRAPHY

Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci 2014: 19(2):164-174.

Ando K, Morita S, Higashi T, Fukuhara S, Watanabe S, Park J, Kikuchi M, Kawano K, Wasada I, et al. Health-related quality of life among Japanese women with iron-deficiency anemia. Qual Life Res. 2006: 15:1559–63.

Beard JL, Hendricks MK, Perez EM, Murray-Kolb LE, Berg A, Vernon-Feagans L, Tomlinson M. Maternal Iron Deficiency Anemia Affects Postpartum Emotions and Cognition. The Journal of Nutrition 2005: 135(2):267–272.

Beck KL, Conlon CA, Kruger R, Heath A-LM, Matthys C, Coad J, Stonehouse W. Iron Status and Self-Perceived Health, Well-Being, and Fatigue in Female University Students Living in New Zealand. Journal of the American College of Nutrition 2012: 31(1):45–53.

Benton D, Griffiths R, Haller J. Thiamine supplementation mood and cognitive functioning. Psychopharmacology 1997: 129:66–71.

Benton D, Donohoe RT. The effects of nutrients on mood. Public Health Nutrition 1999: 2(3a):403–409.

Beutler E, Gelbart T, Lee P, Trevino R, Fernandez MA, Fairbanks VF. Molecular characterization of a case of atransferrinemia. Blood. 2000: 96:4071–4074.

Blanton CA, Green MW, Kretsch MJ. Body iron is associated with cognitive executive planning function in college women. British Journal of Nutrition 2013: 109(5):906–913.

Borel MJ, Smith SM, Derr J, Beard JL. Day-to-day variation in iron-status indices in healthy men and women. Am J Clin Nutr 1991: 54:729-735.

Camaschella C, Roetto A, Calì A, et al. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat Genet. 2000: 25:14–15.

Cohen S, Kamarck T, Mermelstein R. A Global Measure of Perceived Stress. Journal of Health and Social Behavior 1983: 24(4):385–396.

Comín-Colet J, Enjuanes C, González G, Torrens A, Cladellas M, Meroño O, Ribas N, Ruiz S, Gomez M, Verdu JM, Bruguera J. Iron deficiency is a key determinant of health-related quality of life in patients with chronic heart failure regardless of anaemia status. European Journal of Heart Failure 2013: 15(10):1164–1172.

Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood. 2003: 101:3359-3364.

Corwin EJ, Murray-Kolb LE, Beard JL. Low hemoglobin level is a risk factor for postpartum depression. J Nutr 2003: 133(12):4139-4142.

de Benoist B, McLean E, Egli I, Cogswell M. eds. World prevalence of anaemia 1993-2005. WHO Global Database on Anaemia. Geneva, World Health Organization, 2008.

Dev S, Babitt JL. Overview of iron metabolism in health and disease. Hemodialysis International 2017: 21:S6–S20.

Drakesmith H, Schimanski LM, Ormerod E, et al. Resistance to hepcidin is conferred by hemochromatosis-associated mutations of ferroportin. Blood. 2005: 106:1092–1097.

Drakesmith H, Nemeth E, Ganz T. Ironing out Ferroportin. Cell Metab. 2015: 22:777–787.

Duport N, Preziosi P, Boutron-Ruault MC, Bertrais S, Galan P, Favier A, Lanfond JL, Hercberg S. Consequences of iron depletion on health in menstruating women. European Journal of Clinical Nutrition 2003: 57:1169-1175.

Enjuanes C, Klip IjT, Bruguera J, Cladellas M, Ponikowski P, Banasiak W, van Veldhuisen DJ, van der Meer P, Jankowska EA, Comín-Colet J. Iron deficiency and health-related quality of life in chronic heart failure: Results from a multicenter European study. International Journal of Cardiology 2014: 174(2):268–275.

Evstatiev R, Gasche C. Iron sensing and signalling. Gut. 2012: 61:933–952.

Fabrigar LR, Wegener DT, MacCallum RC, Strahan, EJ. Evaluating the use of exploratory factor analysis in psychological research. Psychological Methods 1999: 4(3):272-299.

Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996: 13:399–408.

Ferrari M, Mistura L, Patterson E, Sjostrom M, Diaz LE, Strehle P, et al. Evaluation of iron status in European adolescents through biochemical iron indicators: the HELENA Study. Eur J Clin Nutr 2011: 65:340-9.

Fordy J, Benton D. Does low iron status influence psychological functioning? Journal of Human Nutrition and Dietetics 1994: 7(2):127–133.

Grondin MA, Ruivard M, Perre`ve A, Derumeaux-Burel H, Perthus I, Roblin J, Thiollie`res F, Gerbaud L. Prevalences of iron deficiency and health-related quality of life among female students. J Am Coll Nutr 2008: 27:337–41.

Harris ZL, Takahashi Y, Miyajima H, Serizawa M, MacGillivray RT, Gitlin JD. Aceruloplasminemia: Molecular characterization of this disorder of iron metabolism. Proc Natl Acad Sci USA. 1995: 92:2539–2543.

Hunt JR, Penland JG. Iron status and depression in premenopausal women: an MMPI study. Behav Med 1999: 25:62–68.

Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr 2010: *91*(5): 1461S–1467S

Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc: a Report of the Panel on Micronutrients. Washington, DC: National Academy Press; 2001.

Kaufert, P. Menopause as Process or Event: The Creation of Definitions in Biomedicine. Biomedicine Examined. 1988: 13:331-349.

Le CHH. The Prevalence of Anemia and Moderate-Severe Anemia in the US Population (NHANES 2003-2012). PLoS ONE 2016:11(11): e0166635.

Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. JAMA 1997: 277(12), 973–976.

Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. Lancet. 2016: 387:907-916.

Loreal O, Cavey T, Bardou-Jacquet E, Guggenbuhl P, Robert M, Brissot P. Iron, hepcidin, and the metal connection. Front Pharmacol. 2014: 5:28.

McClung JP, Karl JP, Cable SJ, Williams KW, Nindl BC, Young AJ, Lieberman HR. Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. The American Journal of Clinical Nutrition 2009: 90(1):124–131.

McDowell LR. 2nd ed. Amsterdam: Elsevier Science; 2003. Minerals in Animal And Human Nutrition; p. 660.

McKie AT, Barrow D, Latunde-Dada GO, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science. 2001: 291:1755–1759.

McNair PM, Lorr M, Droppleman L (1992) POMS Manual: Profile of Mood States. Educational and Industrial Testing Service.

Mims MP, Guan Y, Pospisilova D, et al. Identification of a human mutation of DMT1 in a patient with microcytic anemia and iron overload. Blood. 2005: 105:1337–1342.

Murray-Kolb LE. Iron Status and Neuropsychological Consequences in Women of Reproductive Age: What Do We Know and Where Are We Headed? The Journal of Nutrition 2011: 141(4):747S–755S.

Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004: 306:2090-2093.

Papanikolaou G, Samuels ME, Ludwig EH, et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet. 2004: 36:77–82.

Patterson AJ, Brown WJ, Powers JR, Roberts DC. Iron deficiency, general health and fatigue: results from the Australian Longitudinal Study on Women's Health. Qual Life Res 2000: 9:491-497.

QualityMetric Incorporated (2009). Quick Start Guide for the SF-36v2 Health Survey.

Rangan AM, Blight GD, Binns CW. Iron status and non-specific symptoms of female students. Journal of the American College of Nutrition 1998: 17(4):351–355.

Reddon JR, Marceau R, Holden RR. A confirmatory evaluation of the profile of mood states: Convergent and discriminant item validity. Journal of Psychopathology and Behavioral Assessment 1985: 7(3):243–259.

Richardson AC, Heath A-LM, Haszard JJ, Polak MA, Houghton LA, Conner TS. Higher Body Iron Is Associated with Greater Depression Symptoms among Young Adult Men but not Women: Observational Data from the Daily Life Study. Nutrients 2015: 7(8):6055-6072.

Rigas AS, Pedersen OB, Sorensen CJ, et al. No association between iron status and self-reported health-related quality of life in 16,375 Danish blood donors: results from the Danish Blood Donor Study. Transfusion 2015: 55:1752–1756.

Roetto A, Papanikolaou G, Politou M, et al. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat Genet. 2003: 33:21–22.

Ross SD, Fahrbach K, Frame D, Scheye R, Connelly JE, Glaspy J. The effect of anemia treatment on selected health-related quality-of-life domains: A systematic review. Clinical Therapeutics 2003: 25(6):1786–1805.

Stirling IT: Stirling University. 2012. SF-36. *Framework for Measuring Impact.* http://www.measuringimpact.org/s4-sf-36

The WHOQOL Group. (1996) WHOQOL-BREF: Introduction Administration, Scoring, and Generic Version of the Assessment. Field Trial Version. Programme on Mental Health. World Health Organization. Geneva, Switzerland.

Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc. 2001 Mar; 101(3):294-301.

U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory Home Page. 2013

Vahdat Shariatpanaahi M, Vahdat Shariatpanaahi Z, Moshtaaghi M, Shahbaazi SH, Abadi A. The relationship between depression and serum ferritin level. Eur. J. Clin Nutr 2007: 61:532–535.

Vaucher P, Druais PL, Waldvogel S, Favrat B. Effect of iron supplementation on fatigue in nonanaemic menstruating women with low ferritin: A randomised controlled trial CMAJ 2012: 184:1247-1254.

Verdon F, Burnand B, Stubi CL, Bonard C, Graff M, Michaud A, Bischoff T, de Vevey M, Studer JP, Herzig L, Chapuis C, Tissot J, Pecoud A, Favrat B. Iron supplementation for unexplained fatigue in non-anemic women: double blind randomized placebo controlled trial. BMJ 2003: 326(7399):1124.

Worthen CA, Enns CA. The role of hepatic transferrin receptor 2 in the regulation of iron homeostasis in the body. Front. Pharmacol. 2014: 5, 34.

Yang J, Mori K, Li JY, Barasch J. Iron, lipocalin, and kidney epithelia. Am J Physiol Renal Physiol. 2003: 285:F9–18.

Appendix A: Baseline and Endline Covariates

		Baseline	Endline
SF36v2	Physical Functioning	vit	
	Role Physical	BMI, pdd, med	vit
	Bodily Pain	drink, med	drink
	General Health		BMI
	Vitality		pdd
	Social Functioning		
	Role Emotional		smoke, med
	Mental Health		pdd
	Physical Component Score	pdd, med	med
	Mental Component Score		pdd
WHOQOL	Physical Health	med	drink, med
	Psychological Health	BMI, med	BMI, smoke, med
	Social Health		BMI
	Environmental Health		
PSS	Perceived Stress Scale	pdd	smoke, pdd
POMS	Tension		smoke
	Anger		smoke
	Fatigue		
	Depression	BMI	smoke
	Vigor		pdd, vit
	Confusion	pdd, vit	smoke
	Total Mood Disturbance		smoke

BMI=Body Mass Index, vit=Vitamin Use (0: No 1: Yes), med=Medication Use (0: No 1: Yes), pdd=difference between last menses and examination for study, drink=Drinking (0: No 1: Yes), smoke=Smoking (0: No 1: Yes)

Appendix B: Questionnaires

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.
Thank you for completing this survey!

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. <u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c. Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d. Climbing several flights of stairs	☐ 1	☐ 2	☐ 3
e. Climbing one flight of stairs	☐ 1	☐ 2	☐ 3
f. Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g. Walking more than a mile	☐ 1	☐ 2	☐ 3
h. Walking several hundred yards	☐ 1	☐ 2	☐ 3
i. Walking one hundred yards	☐ 1	☐ 2	☐ 3
j. Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Were limited in the kind of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Had difficulty performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Did work or other activities less carefully than usual	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

6. **During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?**

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

7. **How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?**

None	Very mild	Mild	Moderate	Severe	Very severe
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

8. **During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)?**

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks…

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Have you been very nervous?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Have you felt so down in the dumps that nothing could cheer you up?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e. Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f. Have you felt downhearted and depressed?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g. Did you feel worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
h. Have you been happy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
i. Did you feel tired?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a. I seem to get sick a little easier than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Thank you for completing these questions!

WHOQOL-BREF

The following questions ask how you feel about your quality of life, health, or other areas of your life. I will read out each question to you, along with the response options. **Please choose the answer that appears most appropriate.** If you are unsure about which response to give to a question, the first response you think of is often the best one.

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the last four weeks.**

		Very poor	Poor	Neither poor nor good	Good	Very good
1.	How would you rate your quality of life?	1	2	3	4	5

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
2.	How satisfied are you with your health?	1	2	3	4	5

The following questions ask about **how much** you have experienced certain things in the last four weeks.

		Not at all	A little	A moderate amount	Very much	An extreme amount
3.	To what extent do you feel that physical pain prevents you from doing what you need to do?	5	4	3	2	1
4.	How much do you need any medical treatment to function in your daily life?	5	4	3	2	1
5.	How much do you enjoy life?	1	2	3	4	5
6.	To what extent do you feel your life to be meaningful?	1	2	3	4	5

		Not at all	A little	A moderate amount	Very much	Extremely
7.	How well are you able to concentrate?	1	2	3	4	5
8.	How safe do you feel in your daily life?	1	2	3	4	5
9.	How healthy is your physical environment?	1	2	3	4	5

The following questions ask about how completely you experience or were able to do certain things in the last four weeks.

		Not at all	A little	Moderately	Mostly	Completely
10.	Do you have enough energy for everyday life?	1	2	3	4	5
11.	Are you able to accept your bodily appearance?	1	2	3	4	5
12.	Have you enough money to meet your needs?	1	2	3	4	5
13.	How available to you is the information that you need in your day-to-day life?	1	2	3	4	5
14.	To what extent do you have the opportunity for leisure activities?	1	2	3	4	5

		Very poor	Poor	Neither poor nor good	Good	Very good
15.	How well are you able to get around?	1	2	3	4	5

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
16.	How satisfied are you with your sleep?	1	2	3	4	5
17.	How satisfied are you with your ability to perform your daily living activities?	1	2	3	4	5
18.	How satisfied are you with your capacity for work?	1	2	3	4	5
19.	How satisfied are you with yourself?	1	2	3	4	5

20.	How satisfied are you with your personal relationships?	1	2	3	4	5
21.	How satisfied are you with your sex life?	1	2	3	4	5
22.	How satisfied are you with the support you get from your friends?	1	2	3	4	5
23.	How satisfied are you with the conditions of your living place?	1	2	3	4	5
24.	How satisfied are you with your access to health services?	1	2	3	4	5
25.	How satisfied are you with your transport?	1	2	3	4	5

The following question refers to how often you have felt or experienced certain things in the last four weeks.

		Never	Seldom	Quite often	Very often	Always
26.	How often do you have negative feelings such as blue mood, despair, anxiety, depression?	5	4	3	2	1

Do you have any comments about the assessment?

_____

_____

[The following table should be completed after the interview is finished]

		Equations for computing domain scores	Raw score	Transformed scores*	
				4-20	0-100
27.	**Domain 1**	(6-Q3) + (6-Q4) + Q10 + Q15 + Q16 + Q17 + Q18 ☐ + ☐ + ☐ + ☐ + ☐ + ☐ + ☐	a. =	b:	c:
28.	**Domain 2**	Q5 + Q6 + Q7 + Q11 + Q19 + (6-Q26) ☐ + ☐ + ☐ + ☐ + ☐ + ☐	a. =	b:	c:
29.	**Domain 3**	Q20 + Q21 + Q22 ☐ + ☐ + ☐	a. =	b:	c:
30.	**Domain 4**	Q8 + Q9 + Q12 + Q13 + Q14 + Q23 + Q24 + Q25 ☐ + ☐ + ☐ + ☐ + ☐ + ☐ + ☐ + ☐	a. =	b:	c:

* See Procedures Manual, pages 13-15

ID _____ Date _____

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?..... **0 1 2 3 4**
2. In the last month, how often have you felt that you were unable to control the important things in your life? **0 1 2 3 4**
3. In the last month, how often have you felt nervous and "stressed"? **0 1 2 3 4**
4. In the last month, how often have you felt confident about your ability to handle your personal problems? **0 1 2 3 4**
5. In the last month, how often have you felt that things were going your way?..... **0 1 2 3 4**
6. In the last month, how often have you found that you could not cope with all the things that you had to do?..... **0 1 2 3 4**
7. In the last month, how often have you been able to control irritations in your life?..... **0 1 2 3 4**
8. In the last month, how often have you felt that you were on top of things?... **0 1 2 3 4**
9. In the last month, how often have you been angered because of things that were outside of your control?..... **0 1 2 3 4**
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them? **0 1 2 3 4**

POM180

POMS™ Standard Form

BY DOUGLAS M. McNAIR, Ph.D., MAURICE LORR, Ph.D., JW P. HEUCHERT, Ph.D., & LEO F. DROPPLEMAN, Ph.D.

Client ID: _____ **Age:** _____ **Gender:** Male Female (Circle one)

Birth Date: ___ / ___ / ___ (Month / Day / Year) **Today's Date:** ___ / ___ / ___ (Month / Day / Year)

To the Administrator:

Place a checkmark ✔ in one box to specify the time period of interest.

To the Respondent:

Below is a list of words that describe feelings that people have. Please read each word carefully. Then circle the number that best describes

- ☐ how you have been feeling during the PAST WEEK, INCLUDING TODAY.
- ☐ how you feel RIGHT NOW.
- ☐ other: _____

If no box is marked, please follow the instructions for the first box.

POMS™

		Not at all	A little	Moderately	Quite a bit	Extremely
1.	Friendly	0	1	2	3	4
2.	Tense	0	1	2	3	4
3.	Angry	0	1	2	3	4
4.	Worn out	0	1	2	3	4
5.	Unhappy	0	1	2	3	4
6.	Clear-headed	0	1	2	3	4
7.	Lively	0	1	2	3	4
8.	Confused	0	1	2	3	4
9.	Sorry for things done	0	1	2	3	4
10.	Shaky	0	1	2	3	4
11.	Listless	0	1	2	3	4
12.	Peeved	0	1	2	3	4
13.	Considerate	0	1	2	3	4
14.	Sad	0	1	2	3	4
15.	Active	0	1	2	3	4
16.	On edge	0	1	2	3	4
17.	Grouchy	0	1	2	3	4
18.	Blue	0	1	2	3	4
19.	Energetic	0	1	2	3	4
20.	Panicky	0	1	2	3	4
21.	Hopeless	0	1	2	3	4
22.	Relaxed	0	1	2	3	4
23.	Unworthy	0	1	2	3	4
24.	Spiteful	0	1	2	3	4
25.	Sympathetic	0	1	2	3	4
26.	Uneasy	0	1	2	3	4
27.	Restless	0	1	2	3	4
28.	Unable to concentrate	0	1	2	3	4
29.	Fatigued	0	1	2	3	4
30.	Helpful	0	1	2	3	4

Please flip over.
Items continue on the back page...

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		Not at all	A little	Moderately	Quite a bit	Extremely
31.	Annoyed	0	1	2	3	4
32.	Discouraged	0	1	2	3	4
33.	Resentful	0	1	2	3	4
34.	Nervous	0	1	2	3	4
35.	Lonely	0	1	2	3	4
36.	Miserable	0	1	2	3	4
37.	Muddled	0	1	2	3	4
38.	Cheerful	0	1	2	3	4
39.	Bitter	0	1	2	3	4
40.	Exhausted	0	1	2	3	4
41.	Anxious	0	1	2	3	4
42.	Ready to fight	0	1	2	3	4
43.	Good natured	0	1	2	3	4
44.	Gloomy	0	1	2	3	4
45.	Desperate	0	1	2	3	4
46.	Sluggish	0	1	2	3	4
47.	Rebellious	0	1	2	3	4
48.	Helpless	0	1	2	3	4
49.	Weary	0	1	2	3	4
50.	Bewildered	0	1	2	3	4
51.	Alert	0	1	2	3	4
52.	Deceived	0	1	2	3	4
53.	Furious	0	1	2	3	4
54.	Efficient	0	1	2	3	4
55.	Trusting	0	1	2	3	4
56.	Full of pep	0	1	2	3	4
57.	Bad-tempered	0	1	2	3	4
58.	Worthless	0	1	2	3	4
59.	Forgetful	0	1	2	3	4
60.	Carefree	0	1	2	3	4
61.	Terrified	0	1	2	3	4
62.	Guilty	0	1	2	3	4
63.	Vigorous	0	1	2	3	4
64.	Uncertain about things	0	1	2	3	4
65.	Bushed	0	1	2	3	4

Please ensure you have answered every item.
Thank you for completing this questionnaire.

Academic Vitae

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EDUCATION

The Pennsylvania State University

Schreyer Honors College **Class of 2018**

Honors in Nutritional Sciences

Bachelor of Science in Community, Environment, and Development

Option: International Development

Specialization: Nutrition

College of Agricultural Sciences

Minor in Nutritional Sciences

College of Health and Human Development

Minor in International Agriculture

College of Agricultural Sciences

RELEVANT COURSEWORK

Nutritional Science Courses

Elementary Biochemistry
Fundamentals of Organic Chemistry
Nutrient Metabolism I
Nutrient Metabolism II
Assessment of Nutritional Status
Global Nutrition Problems: Health, Science, and Ethics

Community, Environment, and Development Courses

Introductory Environmental and Resource Economics
Land Use Dynamics
Community, Local Knowledge, and Democracy
Comparative Community Development
Women in Developing Countries
International Development, Renewable Resources, and the Environment

EXPERIENCE

Research Intern June 2017-July 2017
International Center for Diarrheal Disease Research, Bangladesh ***Dhaka, Bangladesh***

- In partnership with the Maternal and Childhood Malnutrition department, conducted research on food hygiene/food safety practices in a cohort of women whose children had been a part of the ongoing Malnutrition and Enteric Disease (MAL-ED) study over the past 5 years.
- Wrote and received grants to fund the travel.
- Wrote proposal for Penn State's Institutional Review Board (IRB)
- Data analysis/presentation is ongoing.

Undergraduate Research August 2014 - Present
The Pennsylvania State University ***University Park, PA***

- Conducting research alongside Dr. Laura Murray-Kolb studying the effects of iron nutrition and iron status on cognitive and physical development of women of reproductive age and children in the United States and internationally.
- Focus on data analysis using SAS statistical software and synthesizing literature.

Study Abroad and Research Shadowing July 2016-August 2016
Christian Medical College ***Vellore, India***

- Spent 4 weeks travelling throughout India visiting universities in New Delhi, Jaipur, and Pune and an elementary school in Dahanu learning about education, culture, and social and economic structures in India.
- Spent an additional week shadowing MAL-ED Research Team at Christian Medical College in Vellore.

ACTIVITIES

President August 2016-December 2017
International Justice Mission Penn State Chapter ***Human Rights Organization***

- Oversee meetings and event planning to raise awareness and funds to help fight human trafficking
    - Examples: Documentary Screenings, Gala/Art Gallery, Informational Tables, Petition Signing, Congress Calling

Student Manager December 2016-April 2017
State College Crop Mobs ***Community Organization***

- Connect local farmers with students to volunteer on farms.

Secretary and Member August 2015 – December 2016
Global Environmental Brigades Penn State ***Holistic/Sustainable Development Organization***

- Email coordination based on notes taken during meetings.
- 10 day trip to Panama to implement "model farm" in partnership with local community.

PROJECTS

The Effects of Nutritional Iron Status on Mood, Stress, and Quality of Life in Women of Reproductive Age
Undergraduate Honors Thesis

I am working on a thesis that studies how iron status and an iron supplementation program effects stress, assessed by the Perceived Stress Scale (PSS), mood, assessed by the Profile of Moods questionnaire (POMS), overall quality of life, assessed by the WHO Quality of Life questionnaire (WHOQOL), and functional physical and mental health as assessed by the SF36v2 questionnaire. The first hypothesis is that women who are categorized as iron sufficient will perform better on previously mentioned tasks compared to women categorized as iron deficient. The second hypothesis is that women who receive iron supplementation will perform better on the previously mentioned tasks after taking iron supplements.

Environmental and Health Factors Contributing to Unusual Inverse Relationship between Child Cognitive Development and Protein Intake in Bangladesh: An Analysis from the MAL-ED Study

When analyzing data from a multi-site study examining the effects of repeated enteric infections and nutrient deficiencies on child growth and development, an unexpected relationship was discovered between child development and protein intake for children aged 0-24 months in Bangladesh. Given the importance of protein for brain development, one would expect to find a positive relationship between protein intake and child development. However, a negative relationship was found between protein intake from meat, fish, and poultry sources and child development in Bangladesh. The hypothesis is that unsafe food hygiene practices during preparation and storage of protein from meat, fish, and poultry sources may have increased incidence of illness in these children which, in turn, negatively impacted child development.

AWARDS

World Food Prize Student Scholarship October 2017
Birth Kermit Kenyon Memorial Scholarship August 2017-Present
Student Engagement Network Grant May 2017-August 2017
Undergraduate International Research Competitive Grant May 2017-August 2017
Academic Excellence Scholarship: Schreyer Honors College August 2014-Present
Wodock Scholarship: College of Health and Human Development August 2014-December 2016
Lewis E Young Memorial Scholarship August 2014-December 2016