Research Proposal: Plant-Based Nutrition Intervention Providing the Recommended Dietary

Allowance for Iron in Menstruating, Female Endurance Athletes

by

Abigail Fenske, RD, CD

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Dietetics Mount Mary University

Dana Scheunemann PhD, MS, RD, CD Assistant Professor, Coordinated Program in Dietetics

Pat Kempen, MS, RDN, CD Dietetics Department Chair, Coordinated Program Director and Faculty

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#### Abstract

Nutrition has become an increasingly popular topic within athletics, however, there is a general lack of scientific evidence that utilizes a plant-based nutrition intervention in providing the increased nutritional needs required by athletes. The purpose of this research proposal is to recommend a study design that will analyze the impact a plant-based diet has on providing the recommended dietary allowance of iron within menstruating, female endurance athletes. A total of 40 female cross-country athletes aged 19-25 years old will participate within this study. Data collected will include anthropometrics, iron labs indicative of iron deficiency anemia, past eating patterns collected with the use of the National Health and Nutrition Examination Survey Food Frequency Questionnaire, and three unannounced 24-hour dietary recalls. The anticipated results from this study indicate that the plant-based diet intervention can provide the recommended dietary allowance of iron in menstruating female endurance athletes while maintaining iron labs indicative of iron deficiency anemia within normal range. It will be anticipated that iron labs indicative of iron deficiency anemia will improve as the study progresses, secondary to increased calories focusing on non-heme iron food sources. More research is warranted with the use of a plant-based nutrition intervention.

Keywords: iron deficiency anemia, endurance athletes, plant-based diet

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## **Chapter One: Introduction of the Study**

Iron is an essential mineral vital for the transportation of oxygen to cells throughout the body (Johnson-Wimbley & Graham, 2011). Johnson-Wimbley and Graham (2011) stated, when inadequate consumption of iron-rich foods, chronic blood loss, or complications in the absorption, metabolism and physiological process of iron occurs, iron deficiency anemia may develop. Signs of iron deficiency include weakness, fatigue, decreased physical performance, shortness of breath, headaches, dizziness and pica (Mayo Clinic, 2019). When iron deficiency is left untreated, "Mayo Clinic" (2019) mentioned this deficiency can lead to iron deficiency anemia, causing more serious health issues related to heart problems, growth problems, and problems with pregnancy. Iron deficiency anemia is the most common nutritional deficiency worldwide (Johnson-Wimbley & Graham, 2011).

Athletes are at a higher risk of developing iron deficiency anemia when compared to sedentary individuals as red blood cells break down more quickly during physical activity (Gaudiani, 2018). As stated by Gaudiani (2018), it is estimated that nearly half of all female athletes develop iron deficiency anemia, while an estimated 11% of male athletes develop this deficiency. Female athletes are at an increased risk of developing iron deficiency anemia due to frequent blood loss through menstruation (Gaudiani, 2018). Due to this increased risk, previous research studies have examined the effects of an oral iron supplement and animal protein in providing the recommended dietary allowance of iron within female athletes. The study conducted by Gaudiani (2018) indicated that an oral iron supplement and animal protein does assist in providing the recommended dietary allowance and does treat iron deficiency anemia. Yet, consequences specific to the use of an oral iron supplement may include constipation and potential trace mineral deficiencies (Snyder, Dvorak & Roepke, 1989). Little is known on the

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topic of a plant-based nutrition intervention providing the recommended dietary allowance of iron for menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range.

# Background

Iron is a mineral that has many roles in promoting optimal health. This micronutrient is vital for supporting a healthy metabolism, growth and immunity as well as providing oxygen to the body's tissues and muscles (Zelmean, 2019). As explained by Zelman (2019), there are two forms of dietary iron. One form is found primarily in meat as heme iron, and the second form is primarily found in plant and iron-rich foods as non-heme iron. Heme iron is more bioavailable than non-heme iron, with a 15-35 percent absorption rate compared to non-heme iron that has a 1-23 percent absorption rate (Zelmean, 2019). Zelmean (2019) further explained that it is estimated approximately 14-18 percent of iron consumed is absorbed when following a diet that consists of a variety of foods including meat and seafood, and approximately 5-12 percent of iron consumed is absorbed when following a.

Non-heme iron is generally consumed in larger quantities when compared to heme iron due to the abundant amount of non-heme iron found in a variety of plant-based foods. When non-heme iron is consumed with vitamin C rich foods, absorption rates are enhanced. The amount of vitamin C consumed is directly correlated with the amount of non-heme iron absorbed (Beck, 2014). Beck (2014) mentioned approximately 25-100 mg of vitamin C has been shown to enhance the absorption of non-heme iron, four times as great when compared to non-heme iron consumption alone. However, when paired with calcium rich foods such as tofu, cheese or milk, absorption rates of non-heme iron will be inhibited (Zelmean, 2019). When inadequate consumption of iron occurs, the risk of developing iron deficiency anemia increases (Zelmean, 2019). As stated by Zelmean (2019), about half of all anemia diagnoses are due to iron deficiency. If iron deficiency is not reversed, serious medical conditions may occur, including death. Zelmean (2019) mentioned, it has been estimated that about 50 percent of preschooled children, menstruating females, and pregnant women in developing countries (30-40% in developed countries) may become deficient in this nutrient. If individuals are unable to consume adequate amounts of dietary iron, an oral iron supplement will be recommended, especially for individuals at high risk for developing iron deficiency anemia. However, side effects from an oral iron supplement may occur such as constipation, stomach pain, nausea and trace mineral deficiencies (Zelmean, 2019).

There is limited research regarding the ability of a plant-based nutrition intervention in providing the recommended dietary allowance of iron in menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range. This research proposal will explore the topic of iron deficiency anemia and outline a possible study that utilizes a plantbased nutrition intervention within a high-risk population. Iron, a micronutrient, will be reviewed significantly as it relates to the function, absorption, food sources high in iron, requirements, iron deficiency anemia stages, and current research regarding iron deficiency within female athletes in the following chapters.

#### **Problem Statement**

Current research related to treating and preventing iron deficiency within menstruating, female endurance athletes focuses on the use of oral iron supplements and/or animal protein consumption. Research is lacking as it relates to a nutrition intervention following a plant-based

diet to provide the recommended dietary allowance of iron while maintaining iron labs indicative of anemia within normal range, for this specified, high-risk population.

# **Purpose of the Study**

The purpose of this 17-week randomized, controlled trial is to investigate if a plant-based diet is effective in providing the recommended dietary allowance of iron in menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range.

### **Research Question and Hypotheses**

- The research questions is, does a plant-based diet provide the recommended dietary allowance of iron for menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range?
- The null hypothesis is that a plant-based diet will not provide the recommended dietary allowance of iron for menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range.
- Alternatively, it is hypothesized that a plant-based diet will provide the recommended dietary allowance of iron for menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range.
- Table 1, page 42 indicates both the independent and dependent variables within this study.

# Nature of the Study

This study will be a randomized, controlled trial that will last a total of 17 weeks. The study will include subjects from the University of Wisconsin-Madison and the University of Wisconsin-Milwaukee women's cross-country teams during the months of July through September of 2021.

A normal distribution will be used to ensure data is normally distributed, so no wrong conclusions are made. A central tendency will be applied to collect the mean, mode and median to determine the typical value in the dataset. Data collected will include the mean age of the participants, body mass index, training habits, and duration of the diet consumed for individuals participating within the intervention group.

A chi-square test will be utilized in this study as the dependent variables are categorical. The results from this study will be compared between the intervention and control group to determine if the intervention provides the recommended dietary allowance of iron in menstruating, female endurance athletes and maintains iron labs indicative of anemia within normal range.

# Definitions

<u>Iron deficiency</u>: A common nutrition deficiency caused by inadequate amount of iron consumption (Mayo Clinic, 2019).

<u>Recommended Dietary Allowance (RDA) for iron:</u> a basis for the percent daily values; RDA for females aged 19-50 is 18 mg iron per day (Stöppler, 2018).

<u>Iron deficiency anemia</u>: inadequate amount of healthy red blood cells present in the blood, a common type of anemia. This is diagnosed by having red blood cells that are smaller and more pale than normal, low hematocrit, hemoglobin and ferritin levels (Mayo Clinic, 2019). <u>Endurance athletes</u>: An individual who participates in a sport of prolonged stamina, typically 14-21 hours of exercise per week ("Endurance", 2020).

<u>Plant-based diet</u>: An eating pattern where foods consumed are primarily from plants, such as vegetables, fruits, nuts, seeds, whole grains, legumes, and oils (McManus, 2018). In this study, a plant-based diet will include a minimum of 4 cups of vegetables, 3 cups of fruit, 6 ounces of

whole grains, 3 cups of dairy including soy products, 1 serving of iron-fortified foods, and 5.5 ounces of protein with more than half of the protein coming from plant-based food sources, focusing on pairing non-heme iron with enhancers.

<u>Iron</u>: An essential mineral with the purpose of carrying oxygen in the hemoglobin of the red blood cells throughout the body to produce energy (Kohn, J, n.d.).

<u>Non-heme iron</u>: Iron found in plant food sources such as whole grains, nuts, seeds, legumes, and leafy greens as well as animal flesh and fortified foods (Harvard, 2019).

Heme iron: Iron found only in meat flesh such as meat, poultry and seafood (Harvard, 2019).

<u>Inhibitor</u>: Substance that suppresses the activity of another substance. In this study the inhibitors are black tea and calcium rich foods including yogurt and cheese ("Inhibitor," 2020).

Enhancer: A substance that improves or increases the quality of another substance. In this study

the enhancers are foods rich in vitamin C including peppers, oranges, and strawberries

(Cambridge Dictionary, 2020).

<u>Ferritin</u>: A protein containing iron. Ferritin is the stored form of iron that is closely regulated in the body (American Association for Clinical Chemistry, 2020).

<u>Recommended Dietary Allowance</u>: Average daily level of intake to meet the nutrient requirements of 97%–98% healthy individuals (National Institutes of Health – Office of Dietary Supplements, 2020).

<u>Hematocrit levels</u>: Expressed as percentage by volume, determines percentage of red blood cells in the blood (Davis, 2019).

Serum Iron: Circulating iron bound to transferrin and serum ferritin (Sullivan, 2020).

<u>Transferrin Saturation</u>: Value of serum iron divided by total iron-binding capacity of available transferrin, measured as a percentage (Sullivan, 2020).

# Assumptions

It will be assumed that the inclusion criteria for this study is appropriate, and it will be assumed all subjects included within the intervention group will have similar experiences. It is assumed the subjects involved within the intervention group will remain compliant with the plant-based diet, and subjects involved within the control group will remain compliant with their normal eating patterns throughout the duration of the study. It is assumed all subjects will be menstruating throughout the duration of the study as this will allow the research dietitian to understand how feasible the intervention is in providing the required iron needs to maintain normal iron labs indicative of iron deficiency anemia within this population. It will also be assumed subjects will complete the National Health and Nutrition Examination Survey (NHANES) Food Frequency Questionnaire and 24-hour dietary recalls honestly for accurate results. It is assumed the statistical tests chosen to decipher the results between the two groups will provide normality of data with minimal impact made from unknown variables. These listed assumptions are assumed as these factors do not have the ability to be controlled by the research dietitian.

#### Limitations

Limitations to this study include the small sample size of 40 female endurance athletes. This small sample size is associated with low statistical power, inflated false discovery rate, and inflated effect size estimation which may compromise the conclusions drawn from the study. This research proposal does not assess total blood loss via monthly menstruation, which was found by Malczewska et al. (2000) to be a principal cause of iron deficiency in female athletes whose iron intake was sufficient. The short duration of the study involving 17-weeks is also a limitation as the lifespan of red blood cells is around 120 days. To determine if a plant-based nutrition intervention can provide the recommended dietary allowance of iron while maintaining iron labs indicative of iron deficiency anemia, a study longer than three months should be conducted.

Weaver and Rajaram (1992) discussed that the estimated average requirement of iron for female athletes may be 70% higher than the recommended dietary allowance for females aged 19-50. This is due to their increased sweat production, blood loss in the urine, menstrual cycle, and GI tract, and the continuous mechanical force of foot pounding leading to a shorter life span of erythrocytes. It is estimated that 2.3 mg of iron is lost per day in female athletes (Weaver & Rajaram, 1992). This is another limitation in this study as the research question is asking if the menstruating female endurance athletes are meeting the recommended dietary allowance versus their estimated average requirement.

# **Delimitations**

To create boundaries, a set of inclusion and exclusion criteria have been created. Inclusion delimitations include female endurance athletes aged 19-25 years old, female subjects who are menstruating, and females participating on the women's cross-country teams for either the University of Wisconsin-Madison or the University of Wisconsin-Milwaukee. Exclusion delimitations include females who are not menstruating, are pregnant, are vegan or who have dietary limitations that include the elimination of animal food sources, are using an oral iron supplement, have a history of chronic blood loss, have a history of an intestinal disorder such as celiac disease, or other diseases such as hemochromatosis that impacts iron levels. These exclusion delimitations have been created to prevent any variables that may alter results related to the absorption rates of iron.

# Significance

This study has high significance as there is a lack of conclusive research studies utilizing a plant-based nutrition intervention to provide the recommended dietary allowance of iron for menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range. Current nutrition interventions involve the recommendations of consuming an oral iron supplement that may come with unwanted side effects, or consuming animal protein to provide the recommended dietary allowance of iron. This research proposal will provide important information on whether a plant-based nutrition intervention within this high-risk population provides the recommended dietary allowance of iron while maintaining iron labs indicative of anemia within normal range.

## **Summary**

There is limited research related to a plant-based diet providing the recommended dietary allowance of iron within menstruating, female endurance athletes. Therefore, this randomized, controlled trial with subjects from two college cross country teams will provide valuable information related to whether a plant-based diet provides the recommended dietary allowance for iron. The findings from this study will provide as a foundation for future studies to conduct research related on this topic. Future studies will have the ability to find evidence-based results for a plant-based nutrition intervention for menstruating, female endurance athletes in the prevention of iron deficiency anemia by strengthening the weaknesses present in this study.

Within the next chapter, iron will be reviewed and examined to understand the role this micronutrient has within the human body. Current research discussing this micronutrient will be analyzed to understand the association this deficiency has within this high-risk population. Chapter two will also indicate the gap in the research, requiring future studies to be conducted on

this topic to provide a stronger, evidence-based nutrition intervention. Chapter three is the methodology for this research proposal. A randomized, control trial will be described to provide an evidence-based nutrition intervention related to a plant-based diet for menstruating, female endurance athletes. Chapter four will provide the anticipated results collected from this study, and chapter five will discuss the findings from this study and the importance of this topic as it relates to the field of dietetics.

#### **Chapter Two: Review of Literature**

Iron is an essential micronutrient important for several necessary metabolic processes within the body (University of California San Francisco Health, 2019). Athletes are at a higher risk of developing iron deficiency when compared to sedentary individuals as red blood cells break down more quickly during physical activity (Gaudiani, 2018). Gaudiani (2018) discussed how female athletes are at an even higher risk of developing this deficiency due to frequent blood loss through menstruation.

Chapter two will review important aspects in understanding iron deficiency anemia. The function of iron will be examined along with iron absorption, and food sources high in heme and non-heme iron. Risk factors and stages for developing this nutritional deficiency will be thoroughly discussed along with signs and symptoms and current practices. Existing research will review four research studies that have focused on the purpose of determining whether a plant-based nutrition intervention is effective in providing adequate iron within female athletes. A summary will conclude this chapter to compare the research articles analyzed and determine what improvements future research studies may do to create a stronger conclusion that will provide an evidence-based nutrition intervention for this specified group.

### Literature Research Strategy

Original research studies were compiled using several databases including PubMed Central, Google Scholar, and EBSCOhost. Several verbiages including "iron deficiency anemia," "female endurance athletes and iron status," "vegetarian female endurance athletes and iron status," "plant-based female athletes and iron status," and "vegetarian female endurance athletes with iron deficiency" were investigated to conduct a detailed literature search. During the quest for reputable sources, abstracts were reviewed to determine if the study would be included within this literature review based on the specified inclusion criteria.

### Background

**Iron Function.** Iron plays many important roles within the body (University of California San Francisco Health, 2019). The University of California San Francisco Health (2019) stated iron is crucial in blood production with 70% of the body's iron found in heme proteins called hemoglobin, which are located within erythrocytes and myoglobin. The University of California San Francisco Health (2019) continued to explain, hemoglobin transports oxygen throughout the body, while myoglobin carries and stores oxygen in muscle cells. Proteins required to produce DNA synthesis, cell division, neurotransmitters, and connective tissues require iron for obtaining oxygen, making iron crucial in many metabolic processes (Traverso, 2004). Traverso (2004) continued to mention that iron plays an important role in immune system maintenance. Iron assists in cell proliferation and maturation in lymphocytes specifically, which are white blood cells that protect the body from infection ("NCI Dictionary of Cancer Terms," n.d.).

**Iron Absorption.** Iron is highly regulated to ensure adequate storage in the body (National Center for Biotechnology Information, 2001). According to the National Center for Biotechnology Information (2001), only a small amount of iron is lost per day, in the absence of menstruation or pregnancy, to minimize the risk for deficiency and to ensure adequate iron stores. Men require approximately one milligram of iron absorption per day to maintain storage. Females require a higher amount, approximately 1.5 milligrams per day, with variations based on individual needs due to bleeding via menstruation or pregnancy. Iron is a micronutrient found in a range of foods. Depending on the type of iron form consumed, absorption rates differ slightly. The two forms of iron include heme or non-heme iron. Heme iron is more easily absorbed while non-heme iron has several absorption inhibitors, making it less bioavailable. Heme iron is found primarily in the hemoglobin and myoglobin cells present within meats. Non-heme iron is primarily derived from plant and dairy foods. Both forms are absorbed in the lumen of stomach and duodenum (National Center for Biotechnology Information, 2001).

Heme iron is degraded by pancreatic enzymes, then absorbed by the intestinal cells to be further reduced by heme oxygenase-1, releasing the non-heme iron (Johnson-Wimbley & Graham, 2011). Unlike heme iron, which can be immediately absorbed, non-heme iron requires a reduction reaction before absorption, Johnson-Wimbley and Graham (2011) explained. Nonheme iron has two forms known as either ferric (Fe+2) or ferrous (Fe+3) iron, the difference between ferric and ferrous is their oxidation state of iron; ferric is the +3 oxidation and ferrous the +2 (Johnson-Wimbley & Graham, 2011). Once ferric iron enters the duodenum, the duodenal cytochrome b reduces ferric to the ferrous form, which is then transported into the apical membrane of the enterocyte (Zhang & Enns, 2009). Once reduced, specific foods may enhance or inhibit the absorption of non-heme iron (Johnson-Wimbley & Graham, 2011). According to Johnson-Wimbley and Graham (2011), ascorbic acid, citrate, and amino acids from meat and fish enhance the absorption of non-heme iron. Plant phytates found in legumes, rice and grains, tannins found in berries, tea and wine, calcium found in dairy products, tofu and leafy greens, polyphenols found in fruits, vegetables, dry legumes and chocolate, iron overload, and antacids (which decrease stomach acidity) inhibit the absorption of non-heme iron (Johnson-Wimbley & Graham, 2011).

Heme iron is exceedingly bioavailable and not highly impacted by various factors (Bridges, 2001). However, Bridges (2001) stated that lead, cobalt, strontium, manganese, and

zinc are micronutrients that compete for the same intestinal receptors required for heme iron absorption. This may ultimately impede overall iron absorption if foods high in these micronutrients are consumed at the same time.

**Transport, Metabolism, Storage and Excretion of Iron.** Iron can transfer easily between cells due to the reversible binding to transferrin, a transport protein (National Center for Biotechnology Information, 2001). Per the National Center for Biotechnology Information (2001), when iron is bound to transferrin, it can then be transported and bound to a specific transferrin receptor located on the receiving cell requiring iron. Iron will then be stored as ferritin or hemosiderin, a water-insoluble product of ferritin, within the cells, or may be used to balance future iron metabolism. The intracellular iron storage will determine future iron storage, transport and metabolism. Primary iron stores are found in the cells of the liver, spleen and bone marrow (National Center for Biotechnology Information, 2001).

Iron may be lost through bleeding via menstruation or pregnancy; basal loss in women not menstruating; or lost in the urine, gastrointestinal tract, sweat, and feces as stated by Bothwell, Charlton, Cook and Finch (1979). Bothwell et al. (1979) continued to state that only a small amount of iron is lost per day due to the high regulation of iron status. The range of iron lost can vary from 0.5 mg/day, when the body is experiencing iron deficiency anemia, or may be as high as 2 mg/day when an individual is experiencing iron overload.

**Iron Food Sources.** As Johnson-Wimbley and Graham (2011) explained, heme iron is found primarily in meat products such as beef, pork, poultry, and seafood and is better absorbed than non-heme iron, which is the iron form found primarily in fortified cereals, beans, meat, some vegetables such as spinach and peas, and dried fruits including raisins and apricots (Mayo Clinic, 2019). It is important to note that meat contains about 55-60% of non-heme and 40-45% of heme iron, while plant foods contain a very small amount of heme iron with the majority found as non-heme iron ("Iron We Consume," n.d.). Individuals consuming a plant-based diet are at higher risk of developing iron deficiency as non-heme iron is less bioavailable than heme iron, with multiple factors that may inhibit absorption (Mayo Clinic, 2019).

**Iron Requirements.** The recommended dietary allowance for iron was determined by estimating iron losses to meet 97.5% of the healthy general population with an 18% upper limit of iron absorbed ("Dietary Reference," 2001). According to "Dietary Reference" (2001), the requirement for menstruating females was calculated utilizing a simple equation; iron requirement = basal losses + menstrual losses. It is noted that oral contraceptives may lower menstrual blood loss, which in turn reduces the amount of iron loss, lowering the requirement of iron consumed. When individuals consume a plant-based diet, the main source of iron is found in the non-heme form. Non-heme iron is less bioavailable than heme iron, making the recommended dietary allowance for iron 1.8 times higher for individuals consuming a plant-based diet due to the lower bioavailability ("Dietary Reference," 2001).

Approximately 1.75 mg of iron is lost per day in male athletes, and 2.3 mg of iron is lost per day in female athletes (Weaver & Rajaram, 1992). It has also been explained by Ehn and colleagues (1980) that long distance runners have a significantly lower amount of half-life of body iron than sedentary individuals due to the consistent foot pounding that occurs during distant running. According to Weaver and Rajaram (1992), the estimated average requirement for female athletes may be even 70% higher than sedentary females. This increase in iron requirement is caused by the increased sweat production, blood loss in the urine and GI tract, and the continuous mechanical force of the foot pounding leading to a shorter life span of erythrocytes (Gaudiani, 2018). **Risk Factors of Iron Deficiency Anemia.** There are specific populations that are at a higher risk of developing iron deficiency anemia. These groups include athletes, women, infants, children, vegetarians, frequent blood donors, individuals with colon cancer, certain gastrointestinal disorders, individuals with a history of gastrointestinal surgery, and/or diagnosed with heart failure (Office of Dietary Supplements, n.d.).

Women are at an increased risk for developing this nutritional deficiency due to frequent blood loss via menstruation (Mayo Clinic, 2019). According to "Mayo Clinic" (2019), infants born prematurely or with a low birth rate who may not be consuming enough breast milk or formula are at an increased risk. If children are not consuming a healthy, varied diet with adequate iron rich foods, their risk increases due to rapid growth. Vegetarians are likely to develop iron deficiency due to inadequate amounts of iron-rich foods consumed.

Individuals who frequently donate blood are at an increased risk for this deficiency as hemoglobin levels are greatly reduce after the donation (Mayo Clinic, 2019). For example, 2,425 blood donors remained iron deficient for up to 24-weeks post blood donation if they were not consuming a daily oral iron supplement (Cable et al., 2011). The study conducted by Cable et al. (2011) discussed that men who had donated blood for a minimum of at least three times and women for a minimum of at least two times in the previous year, were more than five times more likely to show signs of iron deficiency anemia.

Up to 60% of individuals diagnosed with colon cancer have also been diagnosed with secondary iron deficiency anemia, which is likely caused by either chronic blood loss, cancerinduced anorexia or chemotherapy-induced anemia (Office of Dietary Supplements, n.d.). As the Office of Dietary Supplements (n.d.) explained, individuals with gastrointestinal disorders including celiac disease, ulcerative colitis, and Crohn's disease, or who have a history of gastrointestinal surgery including gastrectomy or intestinal resection, are at increased risk of iron deficiency anemia secondary to problems with iron absorption. Individuals diagnosed with heart failure are at high risk of developing this deficiency caused by poor diet, malabsorption, cardiac cachexia and the use of medications such as aspirin, which may impede the absorption of iron.

Athletes are at risk of developing iron deficiency anemia as iron is lost through sweat, the skin and urine (Gaudiani, 2018). Gaudiani (2018) states that when athletes participate in high intensity and endurance activities, up to 70% of circulating iron can be lost when compared to sedentary individuals. In athletes who compete in sports with high amounts of running, the mechanical force of the continuous foot pounding breaks down erythrocytes, leading to a shorter life span and thus less iron is found circulating within the blood.

**Female Endurance Athletes.** Endurance is defined as the ability to perform a high physical activity for a prolonged duration, whether it involves running, cycling, swimming, rowing, dancing, or playing sports such as basketball, tennis, volleyball, or soccer ("Endurance," n.d.). Multiple factors may impact an individual's endurance ability such as aerobic capacity, VO2max, muscle strength, cardiovascular endurance, and lactate threshold ("Lactate Threshold Training," 2014). Continuous endurance training allows an athlete to improve their ability to tolerate fatigue, the main factor that negatively impacts athletic performance ("Endurance Definition: What is Endurance?," n.d.).

**Iron Deficiency Anemia Stages.** Iron deficiency is characterized into five stages. Stage one is indicated by the decreased levels of ferritin which fall below 20 ng/mL (Braunstein, 2018). Braunstein (2018) explained this is caused by a decrease in bone marrow iron stores, causing an increase level of transferrin, a protein that binds and transports iron throughout the body. In stage two, transferrin levels remain increased. However, the level of serum iron

decreases. Erythropoiesis is diminished when serum iron levels fall below  $< 50 \ \mu g/dL$ , transferrin saturation is < 16%, and the serum transferrin receptor rises to  $> 8.5 \ mg/L$ . In stage three, erythrocytes remain to appear normal, but red blood cell indices begin to evolve. The red blood cell indices are part of the complete blood count and involves the average size of red blood cells, hemoglobin per red blood cell ratio, and the concentration of hemoglobin per red blood cell. Stage four of iron deficiency involves the microcytosis, defined as smaller red blood cells than normal, and hypochromia, decreased hemoglobin causing a paler looking red blood cell. Stage five of iron deficiency will begin to impact tissues, causing signs and symptoms such as exhaustion, pale skin, headaches, inflamed tongue, brittle nails and pica (Braunstein, 2018).

Symptoms of Iron Deficiency Anemia. Symptoms vary from individual to individual and worsen as iron deficiency becomes more severe (Gaudiani, 2018). Per Gaudiani (2018), when athletes experience iron deficiency, they may experience symptoms more noticeably when compared to sedentary individuals. As iron is essential for oxygen transportation and energy metabolism, symptoms may be more prominent in athletes as iron is an essential component for fueling aerobic exercise, increasing exercise capacity and VO2 max. Symptoms of iron deficiency may include weakness, fatigue, exhaustion, decreased athletic performance, increased heart rate, headaches, dizziness, and pica (Gaudiani, 2018). Signs specific to iron deficiency anemia may include the symptoms previously listed, as well as pale skin, cold extremities, inflammation or soreness of the tongue, poor appetite, and brittle nails (Mayo Clinic, 2019). If iron deficiency anemia is left untreated, Mayo Clinic (2019) stated it can create more serious health issues including heart problems, problems with pregnancy and growth problems in children and infants.

**Iron Deficiency Treatment and Current Practice.** As mentioned by "Mayo Clinic" (2019), iron deficiency may be caused by a lack of iron consumed within the diet, frequent blood donations, impaired gastrointestinal absorption caused by an underlying health condition, increased need requirements due to higher physical activity, or increased iron loss due to heavy menstrual bleeding, pregnancy, or intestinal bleeding. Labs including a complete blood count, hemoglobin and hematocrit levels, and a mean corpuscular volume will provide the necessary information to determine if an individual has iron deficiency anemia (National Heart, Lung and Blood Institute, n.d.). The National Heart, Lung and Blood Institute (n.d.) stated if more information is required, a ferritin lab value will be drawn to determine iron stores, and a peripheral smear may be conducted to determine if red blood cells look smaller and paler when compared to normal red blood cells.

Several treatment options exist for treating iron deficiency, including the use of an oral iron supplementation, diet changes to include more iron-rich food sources, procedures, and surgery (National Heart, Lung and Blood Institute, n.d.). The National Heart, Lung and Blood Institute (n.d.) mentioned, when utilizing oral iron supplements, individuals may be prescribed to consume an iron pill several times per day over a time span of three to six months to replenish the body's iron stores. However, several side effects may occur from taking oral iron supplements including vomiting, diarrhea, constipation, upset stomach or cause a trace mineral deficiency. A diet change nutrition intervention may be prescribed for individuals to consume more iron-rich foods within their diet. These foods include red meats, fish, poultry, dried beans, eggs, iron-fortified breads and cereals, and dark green leafy vegetables. Foods rich in vitamin C including peppers, oranges, and strawberries can help with the absorption of non-heme iron. Black tea and calcium rich foods including yogurt and cheese should be avoided when consuming iron-rich foods, as they are shown to inhibit iron absorption due to their calcium content (National Heart, Lung, and Blood Institute, n.d.).

If oral iron supplements or dietary changes do not replenish iron stores, several other treatments may be provided. Per the National Heart, Lung, and Blood Institute (n.d.), the use of parenteral IV iron may be infused when severe iron deficiency anemia is diagnosed, or if an individual has chronic kidney disease or celiac disease that is impeding their ability for adequate iron absorption. A red blood cell transfusion may be recommended if an individual is having lifethreatening side effects, as red blood cells rich in iron will directly enter within the bloodstream to provide the oxygen required throughout the body. Surgery of the upper endoscopy or colonoscopy may be a means of intervention to stop internal bleeding if that is the etiology of the deficiency (National Heart, Lung, and Blood Institute, n.d.).

**Plant-Based Diet.** A plant-based diet is commonly used interchangeably with a plantforward or vegetarian diet (McManus, 2018). As mentioned by McManus (2018), the focus of a plant-based diet revolves around the consumption of fruits, vegetables, nuts, seeds, oils, whole grains, and legumes. The plant-based diet does allow meat and dairy in small amounts. However, the primary focus of calories and nutrients consumed are obtained from plant food sources. The plant-based diet provides all the nutrients our body require. This includes the necessary macronutrients of protein, carbohydrates and fats; the micronutrients, including vitamins, minerals, phytochemicals, antioxidants, and fiber, that our bodies require for longevity and increased quality of life (McManus, 2018).

A vegetarian diet is an umbrella term used to define numerous plant-based dietary patterns including vegan, lacto-vegetarian, ovo-vegetarian, lacto-ovo vegetarian, pescatarian and the flexitarian diet ("Vegetarian Diet: How to Get the Best Nutrition," 2019). "Vegetarian Diet:

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How to Get the Best Nutrition" (2019) explained that the vegan diet excludes all animal-based products, while the lacto-, ovo-, lacto-ovo, pescatarian and flexitarian diets only partially exclude animal products from the diet. In general, the vegetarian diets focus on a plant-based diet approach while partially or completely limiting the consumption of animal food products.

The vegetarian diets are similar to the plant-based diet as both focus on the high consumption of fiber rich foods and lean proteins. However, the difference between the two is that the plant-based diet does not eliminate animal foods, it just limits the consumption. This reasoning is due to animal foods containing certain micronutrients that are found to be more bioavailable when compared to plant-based foods. With the incorporation of animal products within the plant-based diet, nutrients including vitamin B12, zinc and heme iron will continue to be absorbed at optimal rates (McManus, 2018). McManus (2018) explained that a plant-based diet is high in nutrient content yet low in empty calories. This is due to the plant-based diet having a low consumption of pre-packaged foods that are commonly high in sodium, added sugars and saturated fats.

A plant-based diet has been shown in many previous research studies to play a key role in overall health status ("Plant-Based Diets", 2019). As mentioned by "Plant-Based Diets" (2019), it has been shown that this type of eating pattern may improve plasma lipid concentrations, blood pressure, promote weight loss, improve blood glucose control, and help decrease the risk of developing chronic diseases such as diabetes, obesity, heart disease and certain cancers. "Plant-Based Diets" (2019) continued to mention that these health benefits are due to the high consumption of vegetables, fruits, whole grains, and leans proteins along with the vast number of vitamins, minerals, antioxidants, phytonutrients, and fiber found in these food sources. Foods within this eating pattern are low in cholesterol, saturated fat, trans fat, sodium, and calories, dietary factors that have been shown to have a detrimental impact on overall health. Eating a variety of nutrient dense foods included within this diet will provide the macronutrients and micronutrients essential to health, while sustaining a healthy lifestyle that promotes optimal quality of life ("Plant-Based Diets", 2019).

The plant-based eating pattern has been around for as long as history dates back (Hultin, 2019). Hultin (2019) mentions that the trend of plant-based eating has increased in the past several years but have not always been trendy. With the plant-based eating pattern becoming more popular, it will be crucial for dietitians to educate on the importance of a well-planned plant-based eating pattern to ensure individuals are consuming a reliable source of iron, vitamin B12, omega-3 fatty acids, iron, zinc, and calcium (Hultin, 2019).

#### **Current Research**

Influence of dietary iron on measures of iron status among female runners. Snyder et al. (1989) conducted a randomized experimental study to determine whether female runners consuming a vegetarian diet have different measures of blood iron than female runners consuming a diet that included red meat. Snyder et al. (1989) hypothesized inadequate dietary intake, low bioavailability of non-heme iron, and high rates of iron loss as the multiple factors implicating iron deficiency within this population. The purpose of this study was to investigate whether female vegetarian runners are at an increased risk for developing iron deficiency, when compared to female runners consuming a diet including red meat.

A questionnaire conducted by Snyder et al. (1989) was completed by 18 local female, menstruating, distance runners. Based on their answers, subjects were separated into two groups with a total of nine subjects informed to consume a diet including red meat, and the other nine subjects informed to consume a modified vegetarian diet defined as one that included milk, eggs, fish, poultry, and < 100 grams to no consumption of red meat. Blood samples were obtained in a fasted state at the midpoint of the subject's menstrual cycles, then classified into three stages of iron deficiency based on their lab results. Stage one was indicated as a depletion of iron storage with a serum ferritin value of less than 12 ng/100 mL. Stage two was indicated by iron deficiency erythropoiesis with a serum iron value below 60 ug/100 mL, total iron binding capacity above 400 ug/100 mL, and percent saturation below 16%. Stage three was indicated by hemoglobin levels below 12 gm/dL to determine iron deficiency anemia. A nutritional breakdown involving the number of calories, macronutrient composition, percentage of the recommended dietary allowances for vitamins and minerals, and bioavailability of iron consumed within subject's meals was calculated (Snyder et al. 1989).

Results indicated no significant differences between the two groups as stated by Snyder et al. (1989). Three subjects from the modified vegetarian group and one subject from the group consuming red meat had stage two iron deficiency. Both groups had an average consumption of less than 1,800 calories per day and consumed mean values of iron below the recommended dietary allowance of 18 mg that is recommended for menstruating females (Snyder et al. 1989). Snyder et al. (1989) discussed that iron deficiency within this population may impede physical performance due to excess lactate production and reduced endurance capacity, making it essential for female athletes to be consuming adequate calories and iron. Future research is required to determine what nutritional interventions may work for improving iron status within this population.

The small sample size, short duration of the study, and collection of only three dietary records were several limitations within this study. The use of multiple iron labs to determine iron status, assessing for multiple factors that may impede iron status, and determined aerobic

capacity strengthened this study. By utilizing multiple iron labs, subjects were grouped into the stages of iron deficiency, providing more thorough details on how several factors may influence this nutritional deficiency. The results from this study indicate both groups were not consuming adequate amounts of iron. This is crucial for dietitians to understand as higher amounts of iron may be recommended for this high-risk group of female runners, whether they follow a plant-based diet or not.

**Dietary iron intervention for improvement of iron in female runners.** A prospective, experimental study designed by Alaunyte, Stojceska, Plunkett, & Derbyshire (2014) utilized a nutrition intervention to determine if a staple food product improved iron status in female runners. The purpose of this study was to determine whether Teff bread, a whole grain high in non-heme iron, provided adequate iron to prevent iron deficiency in recreational female endurance runners during a six-week intervention (Alaunyte et al., 2014).

A total of 15 females volunteered from local running clubs and athletic centers, however, based on the inclusion criteria, a total of 11 female subjects participated within the study (Alaunyte et al., 2014). Alaunyte et al. (2014) informed subjects to continue their daily diet and exercise habits but to include Teff bread in place of their usual bread. Diet, exercise regimens, anthropometric measurements, iron labs and exercise performance were assessed at baseline (week 0), midpoint (week 3), and end (week 6). Subjects completed a Teff bread consumption log to monitor compliance. Multiple 24-hour dietary recalls were completed at baseline, midpoint and end, collecting data on dietary intakes and monitored for use of oral supplements (Alaunyte et al., 2014).

Prior to the beginning of the study conducted by Alaunyte et al. (2014), all subjects consumed diets low in iron. During the intervention, total dietary iron increased from 10.7 to

18.5 mg/day (p < 0.05). Consuming Teff bread versus their usual bread,  $7.0 \pm 3.3$  mg iron contributed to 45% of daily recommendations. This increase in iron consumption improved hematological values. However, no statistically significant differences were observed in the lab values including serum transferrin, serum transferrin receptor, serum ferritin, and total iron binding capacity when comparing pre- and post-intervention. A significant positive relation was observed post-intervention between increased dietary iron and increase in iron body stores (Alaunyte et al., 2014).

As stated by Alaunyte et al. (2014), research continues to indicate that female athletes are not consuming a diet that is meeting their nutritional needs. Regardless, the results from this study indicate positive outcomes from the intervention. The subjects with depleted iron stores showed the greatest improvements in hematological indices. An increase in dietary iron can positively impact female athletes in relation to iron stores (Alaunyte et al., 2014).

Alaunyte et al. (2014) thoroughly discussed external and internal variables that may have altered their results, assessed for under-reporting during the 24-hour diet recalls with the use of the Goldberg cut-off limits, and informed subjects to complete a bread consumption log to monitor compliance of consuming the Teff bread which are all strengths to this study. However, many limitations did exist within this study. The small sample and short duration of the study did not indicate a recommendation for the female athlete endurance population. The selection of the subjects was bias as the researchers found them through local running clubs and physical centers. The researchers did not account for non-heme iron enhancers and inhibitors of absorption nor did they account for heme iron subjects consumed in their daily diets. Potential bias may have existed within this study as the researchers were the ones who created and baked the Teff bread used as the nutrition intervention (Alaunyte et al., 2014).

This study provides a base for current treatment research studies to understand that a dietary iron intervention does improve iron status in female endurance athletes. Future research is necessary to better understand this topic.

**Iron status in female endurance athletes and in non-athletes**. The study conducted by Malczewska, Raczynski, & Stupnicki (2000) focused on iron status in female endurance athletes and non-athletes. The purpose of this study was to identify factors that promote iron deficiency within these populations accounting for nutritional status and menstruation. This study included 126 female endurance athletes and 52 female non-athletes in a prospective, cohort, observational study design (Malczewska et al., 2000).

The study conducted by Malczewska et al. (2000) included 126 female endurance athletes that participated in various sports including rowing, swimming, running, cross-country skiing, and modern pentathlon, with data collected in the autumn-winter and spring seasons from 1992-1995. The total amount of physical activity per week was 15 hours, about two and a half hours per day. The control group included general collegiate females participating in recreational swimming and low or moderate physical activity. Multiple 24-hour dietary recalls were conducted including both weekdays and weekends to determine the typical diets of the subjects, accounting for iron coming from heme iron and non-heme iron food sources as well as foods containing non-heme iron enhancers or inhibitors. Variables accounted for by the researchers included iron stores, hematological values, iron intake from heme and non-heme food sources, iron metabolism, menstrual bleeding, and training factor specific to the athletic group (Malczewska et al., 2000).

Iron deficiency was shown to be as high as 50% in the control group, and 26% in the athletic group, as mentioned by Malczewska et al. (2000). Iron stores and iron deficiency did not

differ significantly regarding age or somatic variables in both groups, nor did training volume and athletic experience differ in the athletic group. Both iron deficient subgroups were shown to have lower iron stores, overall iron consumption, and iron metabolism. Within the iron deficient athletic group, these subjects were shown to have a higher menstrual bleeding index than the normal iron stores subgroup (Malczewska et al., 2000).

Research has been variable on the impact physical activity has on iron stores. Several research studies have concluded lower iron stores in athletes when compared to sedentary individuals. However, the study conducted by Malczewska et al. (2000) indicated the opposite – it was found that iron deficiency symptoms were more common in control subjects, with higher iron stores found in athletes. According to the results, low iron intake, dietary imbalance, and menstruation were the factors responsible for the development of iron deficiency in women. Within the control group, lack of iron intake was the dominant factor indicating iron deficiency. Within the athletic group, the higher intake of calcium, a known non-heme iron inhibitor, and menstrual bleeding were the dominate factors promoting iron deficiency (Malczewska et al., 2000).

Malczewska et al. (2000) defined and explained the variables thoroughly and separated the subjects within different subgroups to conclude a more concise conclusion. The adequate use of multiple iron lab values, the 24-hour dietary recalls assessing for both heme and non-heme iron and non-heme iron enhancers and inhibitors, calculating the differences in menstrual length and intensity, sample size, and the length of time strengthened this study design. A limitation to this study included not providing the iron deficient subgroups with an intervention to indicate whether non-heme iron may positively impact iron levels when compared to heme iron. The conclusions from this study provides insight on the adequacy a plant-based diet nutrition intervention would be for this high-risk population.

**Comparison of vegetarian and non-vegetarian diet in Indian female athletes**. Khanna et al. (2006) designed a prospective, observational study focusing on the affect a vegetarian and non-vegetarian diet have on nutritional status and exercise performance amongst Indian female athletes. When nutritional deficiencies occur, athletic performance declines. This study examined a vegetarian diet to determine whether nutritional deficiencies are more common in a vegetarian or non-vegetarian diet and whether it relates to performance (Khanna et al., 2006).

A total of 64 female athletes aged 16-25 years were divided by Khanna et al. (2006) into three groups based on their dietary consumption. Group one included non-vegetarians who consumed both plant and animal-based foods, group two included lacto-vegetarians who included plant and dairy products, and group three included ovo-lacto vegetarians who consumed plant food along with dairy products and eggs. Anthropometrics, body density calculated from skin fold measurements, percentage body fat, lean body mass, and nutrient intakes via 24-hour recalls were collected. Subjects were informed to continue consuming their normal dietary habits. Aerobic capacity was calculated by a treadmill test to determine maximal endurance performance. A venous blood test was completed to determine hemoglobin concentrations (Khanna et al., 2006).

Results indicated 39 subjects were non-vegetarians, 14 subjects were lacto-vegetarians, and 11 were ovo-lacto vegetarians, as stated by Khanna et al. (2006). Between the three groups, no significant differences were found between the height, weight and lean body mass, but body fat was significantly higher in lacto-vegetarians when compared to non-vegetarians and ovolacto vegetarians. All subjects within this study consumed a range of 3,030 to 3,449 calories with
most of the calories coming from carbohydrates, fat and then protein. Iron was found to be significantly higher in non-vegetarians ranging from 22.7 +1.88 mg, 21.8 +1.67 in lacto vegetarians, and 20.8 +2.33 in ovo-lacto vegetarians. Non-vegetarians had the highest hemoglobin concentration when compared to ovo-lacto and lacto vegetarians. When hemoglobin is present in higher amounts, the body has more iron available which provides the body with required oxygen needed for optimal physical performance (Khanna et al., 2006).

Strengths included the use of multiple 24-hour dietary recalls to gather information related to diet and a total of 64 female athletes within the study. However, many limitations existed within this study. More labs should have been collected to determine nutrient biomarkers. Menstrual cycles for the female athletes were not taken into consideration, nor was the use of a possible nutritional supplement or oral contraceptives that may have altered the results. The findings from this study will allow future studies to understand the affect different vegetarian diets may have on the iron status in female athletes (Khanna et al., 2006).

### Summary

Iron is an essential micronutrient crucial in blood production, oxygen transportation, and cell proliferation and maturation ("The Crucial Role of Iron in the Body," n.d.). The recommended dietary allowance is 18 mg of iron per day for premenopausal women, which is a higher recommendation than any other population group due to monthly menstruation and pregnancy ("Dietary Reference", 2001). Two forms of iron exist including heme and non-heme iron (Johnson-Wimbley & Graham, 2011). As Johnson-Wimbley and Graham (2011) discussed, heme iron is found primarily in meat products and is more bioavailable than non-heme iron, which is found primarily in plant products. Due to the lower bioavailability of non-heme iron,

individuals consuming a plant-based diet require 1.8 times the recommended dietary allowance to ensure adequate iron stores to prevent deficiency ("Dietary Reference," 2001).

As mentioned by Weaver and Rajaram (1992), the estimated average requirement for female athletes may be 70% higher than the recommended dietary allowance for females aged 19-50. This is due to their increased sweat production, blood loss in the urine and GI tract, and the continuous mechanical force of foot pounding leading to a shorter life span of erythrocytes (Gaudiani, 2018). Gaudiani (2018) explained signs and symptoms may be more noticeable in athletes due to less oxygen to fuel aerobic exercise, decreased exercise capacity and VO2 max with iron deficiency symptoms ranging from exhaustion to dizziness. As deficiency progresses, symptoms may worsen to include poor appetite, brittle nails and potential cardiovascular and/or pregnancy problems if the deficiency is not reversed (Mayo Clinic, 2019).

The several research articles summarized within this literature review included menstruating female athletes as the subjects. All studies had a small sample size for a short duration of time, yet surprisingly, all studies concluded upon a similar finding. Within the studies, most subjects were consuming diets that consisted of a lower amount of iron than what is recommended for this population. A low iron intake, dietary imbalance, and menstruation were the factors responsible for the development of iron deficiency in menstruating females, independent of whether they consumed a plant-based or animal-based diet. The study conducted by Alaunyte et al. (2014) was the only study to utilize a non-heme iron source as an intervention to determine if this served as an acceptable recommendation to prevent deficiencies within menstruating athletes. This indicates a gap within the literature. There is a lack of conclusive research studies utilizing a plant-based nutrition intervention to provide the recommended dietary allowance of iron within female endurance athletes who are menstruating while maintaining iron labs indicative of anemia within normal range. The current research available indicates that registered dietitians should be providing nutritional tips to all menstruating females on the importance of iron consumption, as current research indicates most females are lacking sufficient amounts within their diets indifferent from what type of diet they consume and whether they participate in an endurance sport. Future research studies are essential within this area to determine if a plant-based diet provides the recommended dietary allowance of iron in menstruating female endurance athletes while maintaining iron labs indicative of anemia within normal range.

### **Chapter Three: Methodology**

Iron deficiency is a common nutritional concern for menstruating female endurance athletes, however, this deficiency is not regularly assessed and monitored for (Mayo Clinic, 2019). The purpose of this research proposal is to investigate if a plant-based diet provides the recommended dietary allowance of iron for menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range. This chapter will review the research design of this 17-week randomized, controlled trial, discussing the intricate details developed to create a strong research study. The inclusion and exclusion criteria set to recruit subjects for this study will be discussed, as well as the reasoning behind why this criterion was chosen. The protocol will be explained to review the forms used to collect dietary information consumed by both groups with the use of the NHANES Food Frequency Questionnaire and three unannounced 24-hour dietary recalls; these forms will be used to assess and monitor for diet compliance within the intervention and control group, and analyzed to discuss comparisons that may be shown by the data collected between the two groups. The descriptive and inferential statistical tests that will be used to assess and analyze the collected data will be evaluated, as will the description of threats that may alter the validity. Lastly, the ethical procedures that will be taken to protect the health and safety of the participating subjects will be reviewed.

#### **Research Design**

This research proposal will be a 17-week randomized, controlled trial to investigate if a plant-based diet provides the recommended dietary allowance of iron in menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range. The research questions is, Does a plant-based diet provide the recommended dietary allowance of iron for menstruating, female endurance athletes while maintaining iron labs indicative of

anemia within normal range? The null hypothesis is that a plant-based diet will not provide the recommended dietary allowance of iron for menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range. Alternatively, it is hypothesized that a plant-based diet will provide the recommended dietary allowance of iron for menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range.

The variables in this study are shown in Table 1. The independent variable within this study is the plant-based diet which will be consumed by the intervention group and the normal diet which will be consumed by the control group. Dependent variables include the iron labs indicative of iron deficiency anemia that will be collected during week zero and week 17. It is noted that week zero is the week prior to the start date for both team's first practice of the season. This study was purposefully curated to begin the week prior to the team's practices, as the first week of the study will be when the research dietitian spends the most time collecting data; this will ensure no time is taken from practice. A nominal scale will be used to assess this information to determine whether each lab value collected is within normal range. If iron labs are below normal range, iron deficiency anemia may be suspected. When suspected, the research dietitian will provide the necessary information to the subject and recommend a visit to their physician for further evaluation. The research dietitian will then analyze the data collected by the physician, if subjects are willing to share this information.

Diet assessment will be measured with the use of three, unannounced 24-hour dietary recalls completed during week three, week nine, and week 15, shown in table 4. These 24-hour dietary recalls will be used to evaluate the consumption of both heme and non-heme iron food sources for both groups, and to measure compliance of the intervention group to determine if they are consuming the plant-based diet designed to provide enough iron. Compliance from the

control group will also be monitored to determine if they are continuing to consume their normal diet throughout this study. A nominal scale will be used to measure the results depicting whether subjects within both the intervention and control group are consuming the recommended dietary allowance of iron, of 18 mg/day.

Table 1

Description of Variables

Variable Type	Variable Name	Variable Source	Potential Responses	Level of Measurement
Independent	Plant-based diet, normal diet	Assigned Group	Yes or No	Nominal Scale
Dependent	Hemoglobin, hematocrit, ferritin, serum iron, transferrin saturation, and total iron binding capacity	Labs collected before and after the study	Yes - Within Normal Limits No – Not Within Normal Limits	Nominal Scale
Dependent	Diet assessment	Three 24-hour dietary recalls	Yes 18 mg or No 18 mg	Nominal Scale

### **Setting and Sample**

**Sample Size.** Sample size will be determined by the number of participants on the University of Wisconsin-Madison and the University of Wisconsin-Milwaukee cross-country teams who are willing to participate within this study. It is estimated that approximately 40 cross-country female runners will participate for both university teams combined.

Before subjects will be included within this study, females will be screened based on questions related to the inclusion criteria: if they are between the ages of 19 and 25, are menstruating, and if they are a part of either the University of Wisconsin-Madison or the University of Wisconsin-Milwaukee women's cross country teams who are expected to be running 55 +/- 14 miles per week. Females will also be screened based on questions related to the exclusion criteria: if they are not menstruating, are pregnant, are vegan or have any dietary limitations, are using an oral iron supplement recommended by their physician, or have a history of a disease that may impair their iron absorption such as celiac disease or hemochromatosis that is known to impact iron levels. Females that pass this criterion will be included within this study and randomly placed into either the intervention or control group as designed by the computer numbering system. All participating subjects will sign an informed consent (see appendix A) before they may begin participating within the study, and all subjects will be informed they may drop-out of this study at any time without any ramifications.

**Recruitment.** The research dietitian will begin this recruitment process during the 2020 women's cross-country season, to begin the conversation with the team's coaches to discuss the study and the importance it holds. Speaking with both coaches the year prior will help determine the interest of the study. The research dietitian will remain in contact with the coaches throughout the year, from the initial conversation until the start of the next season, when the study is scheduled to begin. During this time, the research dietitian will answer any questions or concerns the coaches may have regarding the study and will provide information related to the methods. The amount of time to collect data for the duration of this study will be discussed, as it is anticipated to be less than 15 hours total with no hinder on the time allocated for team practices. During these conversations with the coaches, the research dietitian will also thoroughly

explain the importance of iron status within this high-risk population and the benefits each team may acquire from their participation in this study, such as peak performance rates.

The purpose of the previous conversations with the coaches will be to provide detailed information of the study and to influence their participation. Once both teams commit to their involvement, the research dietitian will begin to recruit subjects at the beginning of the season in July 2021. An IRB approval form will be completed and submitted to begin the start of this study (see appendix B). Inclusion criteria will consist of menstruating females aged 19-25 years of old, and subjects participating on either the University of Wisconsin-Madison and the University of Wisconsin-Milwaukee women's cross-country teams who are expected to be running 55 +/- 14 miles per week. This criterion has been set specifically to include females that are losing additional iron during their monthly menstrual cycle. The age range is to compare iron consumption to the recommended dietary allowance set specifically for females aged 19-50 years old. Including female subjects from the both University of Wisconsin-Madison and the University of Wisconsin-Milwaukee is due to the convenience of the research dietitian living within this area, the geographical location involving minimal elevation as high elevation has been shown to promote iron deficiency, and to include a number of subjects to participate within this study to strengthen the design.

Exclusion criteria includes females who are not menstruating, are pregnant, are vegan or who have dietary limitations that include the elimination of animal food sources, are using an oral iron supplement, have a history of chronic blood loss from a peptic ulcer, a hiatal hernia, colorectal cancer, or who have a history of an intestinal disorder such as celiac disease, or other diseases such as hemochromatosis that impacts iron levels.. This criterion was set due to the possible effects these variables may have on the collected results. Subjects who have eliminated animal food sources prior to the start of this study will not be able to participate within the intervention group when randomly placed, due to the requirement of consuming some amounts of animal proteins. Subjects consuming an oral iron supplement will not be included in this study as their labs may impact overall collected results, showing false readings. By setting these inclusion and exclusion criterions, the results from this study will provide generalized data that will be valuable for future research studies related to this topic.

### Instrumentation

All subjects will complete the NHANES Food Frequency Questionnaire (see appendix C) before the beginning of the study during week zero, to collect semi-quantitative data related to previous dietary intakes from the past 12 months. The data collected by the NHANES Food Frequency Questionnaire will provide important information as it relates to previous calorie and iron consumption within the subjects. The data collected from the NHANES Food Frequency Questionnaire will provide insight as to why labs indicative of iron deficiency anemia may be low for the lab analysis completed during week zero, while also being used to compare to the 24-hour dietary recalls to determine how diet may improve for all subjects over the duration of this study.

The research dietitian will meet face-to-face with all subjects in both the intervention and control group unannounced during week three, week nine and week 15 to assess subject diet compliance with the use of a 24-hour dietary recalls (see appendix D) and answer any personal questions subjects may have while also providing individualized nutrition education as needed. If subjects are unable to attend an in-person 24-hour dietary recall session during the scheduled times, a phone interview will be completed as necessary, within reason. The purpose of the 24-hour dietary recalls is to measure the compliance of the individuals within the intervention and

control group, while also measuring if an improvement in calorie consumption is seen in the control group as the study progresses from the nutrition education provided by the research dietitian (see appendix F).

Labs indicative of iron deficiency anemia will be drawn during week zero and week 17 to determine any lab value changes for both groups. These labs include hemoglobin, hematocrit, ferritin, serum iron, transferrin saturation, and total iron-binding capacity, and will be drawn and analyzed at a local hospital, for convenience.

#### Protocol

During week zero, a week before the start of the team's seasonal practice begins, the research dietitian will obtain all informed consents from each participating subject (see appendix A). Once forms are collected, each subject will be assigned a random number that will be generated by a computer to place them within either the control or the intervention group. Once groups are compiled, the research dietitian will educate all subjects of the design and layout of the study. The research dietitian will also educate participants on the accuracy needed to be obtained during the study and will encourage compliance and honesty for each subject as they participate within this controlled trial to produce accurate results. Compliance of the intervention, and compliance of the control group will be indicated as consuming normal eating patterns as shown from the NHANES Food Frequency Questionnaire results. If a subject in the intervention group continuously does not consume the plant-based diet as they are educated by the third collected 24-hour dietary recall, their results will not be included within the results for this study.

The NHANES Food Frequency Questionnaire (see appendix C) will be completed by all subjects during week zero to collect information on previous eating patterns consumed for the

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past 12 months prior to the start of the study. The NHANES Food Frequency Questionnaire will be completed during week zero in a classroom setting for both teams to complete, one week before the start of their team's practice. The research dietitian will be present when both teams are completing this questionnaire to answer questions any of the subjects may have related to completing the form. Depending on the team's schedules, the research dietitian will meet with the University of Wisconsin-Milwaukee on the first day of the study in the morning to complete the form. The research dietitian will then commute to Madison, in which these subjects will complete the questionnaire in the early afternoon.

The research dietitian will provide directions to both teams before they fill-out the food frequency questionnaire. The research dietitian will define a food frequency questionnaire as a finite list of foods and beverages frequently consumed. The research dietitian will review the directions to take the questionnaire, such as using a no. 2 pencil, circling answers that seem the most fit, and stating that the test should take about 45 minutes to complete. After the NHANES Food Frequency Questionnaire is completed, the research dietitian will spend about a half hour educating both teams on the importance of consuming adequate calories, protein and fluids based on their high activity levels, and answering any general questions or concerns that may arise (see appendix F). The results from the NHANES Food Frequency Questionnaire will be compared to the 24-hour dietary recalls near the end of the study, to determine the change in overall eating patterns for all subjects, from before the start to the end of the study.

All subjects will have labs drawn during week zero to assess the prior eating patterns, as also indicated by the NHANES Food Frequency Question, and current iron status. At this time, subjects will also provide their age, information related to their training habits including training frequency per week, running distance per week in miles, and running time per week in hours, which will be confirmed by each team's coach. Body mass index (BMI) of each subject will be calculated to determine if they are underweight, normal weight or overweight, keeping in consideration high muscle mass. Labs will be drawn at the local hospital in the morning during week zero for all subjects. The purpose of drawing labs before the beginning of the study is to determine the iron status of the subjects before they begin consuming the plant-based diet. This will be important to know, to prevent any disruption in results collected.

During week zero, the research dietitian will provide a team-based approach for all subjects, providing generalized nutrition education specifically related to increased portion sizes to meet the increased physical activity demands of these individuals (see appendix F). However, education regarding the plant-based diet will only be provided to the subjects on each team that were randomly placed within the intervention group, in a group setting (see appendix E). This education for the intervention group will include plant-based food options they may consume for their breakfast, lunch, and dinner meals, weekend meals, and snacks. The research dietitian will also exemplify a list of foods the subjects within the intervention group may consume on the plant-based diet including iron-fortified foods, what foods to pair together for the best non-heme absorption rates, what foods to limit consuming together to decrease the inhibition of non-heme iron absorption, and recipe links to assist in creating a variety of plant-based meals. The intervention group will consume a plant-based diet including a minimum of 4 cups of vegetables, 3 cups of fruit, 6 ounces of whole grains, 3 cups of dairy including soy products, 1 serving of iron-fortified foods, and 5.5 ounces of protein with more than half of the protein coming from plant-based food sources, which is based on a 2,000 calorie intake. Individuals within this group will be educated on the importance of focusing on pairing non-heme iron with absorption enhancers including food sources high in vitamin C. It will be noted that some portions may be

above or below the minimum requirement for subjects based on their weight, height, and personal needs that may influence caloric needs.

Team-based educational sessions will be completed during week zero for all subjects, discussing the importance of calories due to their high physical demands. The research dietitian will meet face-to-face with all subjects in both the intervention and control group unannounced during week three, week nine and week 15 to measure subject compliance with the use of a 24-hour dietary recalls (see appendix D) and answer any personal questions subjects may have regarding their group's specified nutrition education, providing individualized nutrition education as needed. The time scheduled to complete these 24-hour dietary recalls will be confirmed by both coaches for the University of Wisconsin-Madison and the University of Wisconsin-Milwaukee prior to the start of their practice. If subjects are not able to participate within the scheduled 24-hour dietary recalls, the research dietitian will set-up a time that works best, within reason, for a phone call to complete the task.

During week three and week nine, the unannounced 24-hour dietary recalls conducted by the research dietitian will focus specifically on subject compliance in both the intervention and control group. The 24-hour dietary recalls will be assessed with the use of ESHA, a diet analysis software. If a subject does not consume the required number of portions within each food group for one or all three of the 24-hour dietary recalls, further education will be completed. If their results for the 24-hour dietary recall during week 15 indicates non-compliance, their data will not be included within the results for this study.

During week 17 of the study, all subjects will have their labs drawn one last time to determine if their iron labs indicative of anemia are within normal range. The control group's

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labs will also be drawn during this week to determine if there is a comparison between the two groups.

#### **Data Analysis Plan**

**Descriptive Statistics.** A normal distribution will be used to ensure data is normally distributed, so no wrong conclusions are made. A central tendency will be applied to collect the mean, mode and median to determine the typical value in the dataset. The data collected from the NHANES Food Frequency Questionnaire will be useful in comparing the eating patterns for the subjects within the intervention group and the control group prior to the start of the study. Data collected will include the mean age of the participants, body mass index, and training habits. This data will be collected during week zero and analyzed with a central tendency.

**Inferential Statistics.** A chi-square test will be utilized for all analysis in this study as the dependent variable is categorical. The results from this study will be compared between the intervention and control group to determine if the intervention provides the recommended dietary allowance of iron in menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range. Data collected from the NHANES Food Frequency Questionnaire and the three, 24-hour dietary recalls will be evaluated with the use of ESHA, a dietary analyzer. The final 24-hour dietary recall conducted during week 15 will provide the p-values that will determine if the hypothesis will be accepted or rejected. A p-value less than 0.05 will show a statistically significant change that will reject the null hypothesis. Table 2 provides an overview of the variables including name, source, potential response, and level of measurement.

### **Threats to Validity**

To ensure clarity, comprehensibility, and appropriateness, the NHANES Food Frequency Questionnaire will be used to collect semi-quantitative data on all subject's previous dietary consumption. Threats to the validity of this design include the memory, compliance and honesty of the subjects, lack of blinding that may cause negative social interactions between the two groups, and generalizability. Due to the small sample size, the results from this study will not be able to be generalized for the entire population of menstruating, female endurance athletes. Future research studies are needed on this topic.

### **Ethical Procedures**

Before the start of the study, the Institutional Review Board (IRB) approval will be received (see appendix B). Subjects will not be put at risk for harm throughout the duration of this study. However, discomfort may occur during and a short time after the lab draws are completed during week zero and week 17. An informed consent (see appendix A) will be obtained from each subject prior to the start of this study. The informed consent form will explain the procedures, risks, safeguards, freedom to withdraw, and the confidentiality that will be taken within this study. All data collected will be stored in a confidential web-based folder requiring a password to enter. Data will be entered per team using initials, age and their number assigned to each subject during the randomization process to code each subject's information. Subjects will be aware that they may withdraw from the study at any time with no consequence. **Summary** 

This 17-week randomized, controlled trial will provide insight related to providing a nutrition intervention using a plant-based diet, to determine if it provides the recommended dietary allowance of iron while maintaining iron labs indicative of anemia within normal range

for menstruating, female endurance athletes. All subjects will be provided with generalized nutrition education (see appendix F) specifically related to their increased calorie range due to their high physical activity status during week zero, three, nine, and 15. Subjects within the intervention group will be provided with additional nutrition education specific to a plant-based diet, and will be provided with plant-based recipes they may follow to ensure highest compliance (see appendix E).

Central tendency and a chi-square test will be used to analyze the collected data to compare the intervention and control group to demonstrate if they are similar, to determine if the intervention is the reason for the outcome. Labs indicative of iron deficiency will be drawn during weeks zero and 17 to determine if they are within normal range or not within normal range. Descriptive statistics will be used to describe the population, while inferential statistics will look at an association between the nutrition intervention and if it provides the recommended dietary allowance while maintaining labs indicative of iron deficiency anemia within normal limits. Chapter four will review the anticipated results for this study.

### **Chapter 4: Anticipated Results**

A total of 40 female endurance runners from both the University of Wisconsin-Madison and the University of Wisconsin-Milwaukee cross country teams will participate within this 17week randomized controlled trial. All 40 female members of both cross-country teams will be included within this study. Twenty female participants will be randomly selected to participate within the intervention group consuming the plant-based diet, while the other 20 female participants will be placed within the control group consuming their normal eating patterns.

### **Characterization of Study Population**

Measures of central tendency will be computed to summarize the data for the age, body mass index, training habits and duration of the diet consumed within this study for both the intervention and control group. It is expected that two dropouts from the intervention and one dropout from the control group will occur due to the possible transferring of schools, inability to stay compliant with the plant-based diet for the intervention subjects, and other personal reasons. Measures of dispersion will be computed to understand the variability of score. Anticipated results are shown in Table 2.

### Table 2

### Characterization of the study population

	Intervention	Control
Age, years	22 +/-3	22 +/-3
BMI, kg/m^2	19.5 +/-1	19.5 +/- 1
Training Habits		
Training Frequency per week	$5.19\pm0.90$	$5.04\pm0.98$
Running distance per week, mi	55.41 ± 14.53	$55.03 \pm 14.66$
Running time per week, h	$19.38 \pm 1.43$	$19.72 \pm 1.11$
Dropout Rate	17 weeks +/-2	17 weeks +/-1

### **NHANES Food Frequency Questionnaire**

The NHANES Food Frequency Questionnaire will be used to collect information on previous eating patterns consumed by all participants before the start of the study. Table 3 below indicates the averaged results for both the intervention group with the control group, the p-value will be anticipated to indicate no significant difference. A central tendency test will be used to assess the data compiled from all completed NHANES Food Frequency Questionnaires within each group. This data will be useful in determining the comparisons of eating patterns of the subjects within the intervention group versus the control group, while also assessing for changes that may occur from the beginning to the end of the study when comparing the NHANES Food Frequency Questionnaire to the 24-hour dietary recalls.

# Table 3

# NHANES Food Frequency Questionnaire Averaged Results

Group	Intervention Group	Control Group	P-value
Energy intake	1800 calories	1800 calories	P = 1.0
Vegetable Intake	2 cups	2 cups	P = 1.0
Fruit Intake	1 cup	1 cup	P = 1.0
Whole Grain Intake	5 ounces	5 ounces	P = 1.0
Animal Protein Intake	4 ounces	4 ounces	P = 1.0
Iron Intake	14 mg	14 mg	P = 1.0
Dairy Intake	2 cups	2 cups	P = 1.0
Processed Foods Intake	4 servings	4 servings	P = 1.0

### **24-Hour Dietary Recall**

Throughout the duration of the study, diet compliance will be assessed with the use of three unannounced 24-hour dietary recalls conducted by the research dietitian, as shown in table 4. The 24-hour dietary recalls will be collected during week three, nine and 15 for both the intervention and control group to assess the amount of energy, vegetable, fruit, whole grain, animal protein, plant protein, iron, dairy, and processed foods consumed. It is anticipated that 75% of the subjects in the intervention group will be compliant with the plant-based diet as shown by the averaged 24-hour diet recalls collected during week three, and the control group will continue to consume their normal eating patterns with a gradual increase in calorie consumption.

The anticipated results for weeks nine and 15 are anticipated to indicate significant differences between the intervention and control groups for the animal protein, plant protein and processed foods. It is noted that energy consumption will improve as the study progresses for the control group. The results collected for the 24-hour dietary recalls will be compared with the results from the NHANES Food Frequency Questionnaire as shown in table 3, to understand the overall dietary changes that are anticipated to occur throughout the duration of this study for both groups involved.

# Table 4

Average Nutrient Intake of	f the Study Population	on Determined via 24-hour	Dietary Recall, Weeks 3, 9, and 15

	Week 3		Week 9		Week 15		P - Value		
Group	Intervention	Control	Intervention	Control	Intervention	Control	Week 3	Week 9	Week 15
Energy	2100 cal	1850 cal	2200 cal	2100 cal	2300 cal	2200 cal	0.05	0.30	0.30
Vegetable	4 c	3 c	4 c	3 c	4 c	4 c	0.80	0.80	1
Fruit	3 c	2 c	3 c	2 c	3 c	2 c	0.80	0.80	0.80
Whole Grain	6 oz	5 oz	6 oz	7 oz	6 oz	7 oz	0.90	1	0.90
Animal Protein	1 oz	5 oz	1 oz	бoz	1 oz	7 oz	0.04	0.04	0.03
Plant Protein	5 oz	1 oz	5 oz	1 oz	5 oz	1 oz	004	0.04	0.03
Iron	18 mg	16 mg	18 mg	18 mg	18 mg	19 mg	0.28	0.70	0.3
Dairy	3 c	2 c	3 c	2 c	3 c	4 c	1	1	0.28
Processed Foods	1 serv	4 serv	1 serv	4 serv	1 serv	4 serv	0.05	0.05	0.05

### Lab Analysis

As shown in table 5, it is anticipated that there will be no significant difference between the intervention or control group for the lab analysis collected during week zero. This is anticipated to be caused by similar eating patterns consumed prior to the start of the study as shown by the NHANES Food Frequency Questionnaire. It will also be anticipated that lab values for hemoglobin, hematocrit, ferritin, serum iron, transferrin saturation, and total iron-binding capacity will all be borderline low for both the intervention and control group during week zero's lab analysis.

The anticipated results from the lab analysis for both the intervention and control group during week 17's lab draw is also displayed in table 5. The lab results collected during week 17 are anticipated to indicate no statistically significant differences between the intervention and control group. This is expected as both groups will be educated by the research dietitian on the topic of consuming a higher number of calories to meet nutritional needs due to high physical activity; the energy increase for the control group can be found in table 4 when comparing week three to week 15. Table 5 presents the finding that all iron labs indicative of iron deficiency anemia will improve for both groups when comparing week zero with week 17.

# Table 5

# Average Lab Analysis of the Study Population Determine via Lab Draws Week Zero and Seventeen

Iron Labs	Week 0 Intervention Control		Week 17 Intervention Control		P - Value Week 0 Week 17	
Hemoglobin	10.4 g/dl +/- 2.04	11.2 g/dl +/- 3.83	13.2 g/dl +/- 2.04	15.3 g/dL +/- 3.83	P = 0.06	P = 0.07
Hematocrit	38%	39%	40%	46%	P = 0.07	P = 0.09
Ferritin	<30 ng/mL	<33 ng/mL	<38 ng/mL	<43 ng/mL	P = 0.07	P = 0.06
Serum Iron	75 mcg/dL	80 mcg/dL	80 mcg/dL	87 mcg/dL	P = 0.07	P = 0.07
Transferrin Saturation	<21%	<24%	<25%	<29%	P = 0.07	P = 0.07
Total Iron Binding Capacity	455 mcg/dL	459 mcg/dL	440 mcg/dL	435 mcg/dL	P = 0.06	P = 0.06

## **Diagnosis of Iron Deficiency Anemia**

The labs drawn and analyzed during week zero and 17 are iron labs indicative of iron deficiency anemia. These labs are anticipated to improve for both groups by the end of the study, likely related to the increase in calorie and iron consumption to better meet nutritional needs due to the high activity level for all subjects participating in this study. It is anticipated that no subjects from the intervention or control group will have labs indicative of iron deficiency anemia during week zero or week 17.

#### **Chapter 5: Discussion**

A plant-based diet plays a key role in optimal health status ("Plant-Based Diets", 2019). Despite knowing this, research has been lacking as it relates to a nutrition intervention of plantbased eating in endurance athletes. A gap in the literature exists with the use of a plant-based nutrition intervention to provide the recommended dietary allowance of iron in menstruating, female endurance athletes while maintaining iron labs indicative of anemia within normal range. This chapter will discuss the anticipated results from this study, compare these anticipated results to the research studies conducted by Alaunyte et al. (2014), Khanna et al. (2006), Malczewska et al. (2000), and Snyder et al, (1989), and conclude with suggestions recommended for future research studies.

### **Interpretation of Results**

This 17-week randomized controlled trial will investigate if a plant-based diet provides the recommended dietary allowance of iron in menstruating, female endurance athletes. It is anticipated that the null hypothesis will be rejected, and the alternative hypothesis will be accepted as the plant-based diet will provide the recommended dietary allowance of iron in menstruating female endurance athletes while maintaining labs indicative of iron deficiency anemia within normal range. This will be indicated by comparing the NHANES Food Frequency Questionnaire to the 24-hour dietary recalls, showing an increase in iron consumption from eating habits prior to the start to the end of the study for the intervention group. The iron labs indicative of iron deficiency anemia will support the alternative hypothesis as iron labs are anticipated to increase from week zero to week 17, for the intervention group specifically. The use of the NHANES Food Frequency Questionnaire and three unannounced 24-hour dietary recalls will be used to compare both assessment tools within the intervention and control group. This will allow the research dietitian to analyze results between the two groups, confident that both groups will display lab results portraying the true amount of heme and non-heme iron consumed within their specified diet.

**Characterization of Study Population.** Subjects participating within this study are expected to be menstruating females aged 22 +/- 3 years old. The body mass index for both the intervention and control group is expected to be within the normal range. Training habits for both groups are expected to be similar and indicate no significant differences. Similar characteristics between the two groups will be important as differences could alter the end results, indicating a false conclusion.

#### NHANES Food Frequency Questionnaire. The NHANES Food Frequency

Questionnaire will be used to collect data during week zero to gather information on previous eating patterns consumed by all participants. The data collected from this questionnaire is located on Table 3, page 55. The anticipated results will indicate that both groups will consume on average, 1,800 calories per day, which is less calories than what is recommended based on the high physical activity levels within this population. This low-calorie consumption will also signify a low consumption of vegetables, fruits, whole grains, and food sources high in iron within the studied population. A low-calorie and iron consumption has previously been shown in the study conducted by Snyder et al. (1989) whose findings indicated that their female running subjects were consuming calorie ranges of 1,600-1,800 calories and inadequate intakes of food sources high in iron. It was discussed in the study conducted by Snyder et al. (1989) that the low-

calorie consumption was one of the factors influencing a low iron status as subjects were not consuming enough nutrients based on their high activity levels.

A nutritional breakdown of previous eating patterns as shown by the NHANES Food Frequency Questionnaire will be analyzed to determine the number of calories, macronutrient composition and amount of iron consumed within subject's diet with the use of ESHA. It is anticipated that female subjects in both groups will not consume the recommended dietary allowance of iron prior to the start of this study. This finding will also be supported by the 24hour dietary recalls indicating less than or equal to 18 mg/day for the first two 24-hour dietary recalls within the control group. This research proposal's findings are similar to Snyder et al. (1989) who found that subjects were consuming 14.7 +/- 2.0 mg.d-1 for the modified vegetarian group and 14.0 +/- 2.2 mg.d-1 for the group consuming red meat, which is less than the recommended dietary allowance of 18 mg per day.

**24-Hour Dietary Recall.** The results from the three unannounced 24-hour dietary recalls will reveal some differences in nutrient intake between the two groups as the study progresses. This information can be viewed on Table 4, page 57. These 24-hour dietary recalls will assess total energy, vegetable, fruit, whole grain, animal protein, plant-protein, iron, dairy, and processed food intake. The control group will gradually improve their eating patterns as the study progresses, indicating the importance of nutrition education provided by the research dietitian. The study conducted by Snyder et al. (1989) found that calorie consumption was initially low within their subjects, which was a factor that influenced iron deficiency. However, a finding from this study indicated that as calories increased by 1,000 calories, so did iron intake by 6 mg, signifying the importance of subjects consuming their total energy nutritional needs (Snyder et al., 1989).

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The research dietitian will continue to provide nutrition education for subjects within the intervention group throughout the duration of the study. However, this education will be most important during week zero of the study where some information may be unknown knowledge for some subjects (see appendix E). It will be expected that all subjects within the intervention group will be 75% percent compliant with the intervention by week three and throughout the duration of the study as shown by the unannounced 24-hour dietary averaged recalls.

The control group will be provided with generalized nutrition education throughout the duration of the study, focusing on increasing calories to better meet nutritional needs (see appendix F). It is expected that calorie amounts will increase for the control group, as the research dietitian will be providing several team education sessions on the importance of increased nutrition based on the subject's high physical activity levels.

Due to the intervention group consuming more plant-based meals compared to the control group, it is expected that the dietary pattern consumption will be higher in several areas due to consuming a plant-based diet. Although not measured, the intervention group is expected to be consuming higher amounts of fiber, iron, folate, vitamin C, B-vitamins, calcium and zinc when compared the control group. Consumption of total fat and saturated fat is expected to be higher in the control group versus the intervention group due to the higher consumption of animal food products, although this will not be measured either.

The studies conducted by Alaunyte et al. (2014) and Khanna et al. (2006) utilized 24hour dietary recalls to collect dietary assessment from their subjects. Alaunyte et al. (2014) discovered a higher amount of fiber, vitamin C, B-vitamins, calcium, iron and zinc, and lower levels of saturated fat and total fat post-intervention of utilizing the Teff bread. Khanna et al. (2006) analyzed data from the 24-hour dietary recalls to assess macronutrient percentages. The findings indicated that carbohydrate and energy was not significantly different between the two groups, yet protein percentages were higher for the non-vegetarian group and fat percentage was higher for the lacto-ovo vegetarian group. Other variations from this study included calcium intake found to be significantly higher in lacto-vegetarians, iron and niacin intake higher in nonvegetarians, and no significant difference in vitamin C intake between the groups (Khanna et al., 2006). These studies correlate with the several findings from this study in the perspective that non-vegetarians typically have higher iron levels due to the consumption of heme iron, and that plant-based diets do offer the recommended dietary allowance for iron when calories needs are met.

Lab Analysis. Labs will be drawn during week zero and week 17 for all 40 subjects participating within both the intervention and control group. The anticipated results collected in week zero will indicate that half of the subjects participating in this study will report inadequate iron intake as shown by the NHANES Food Frequency Questionnaire. Alaunyte et al. (2014) discovered similar results, with over a third of their subjects showing depleted bodily iron stores due to inadequate dietary iron intake at the start of their study.

As previously shown on Table 5 page 59, it is anticipated that all hematological lab values will be borderline low during week zero for both the intervention and control group. As the study progresses to week 17, it is anticipated that both groups will have an improvement in all labs including hemoglobin, hematocrit, ferritin, serum iron, transferrin saturation, and total iron-binding capacity. This increase in iron labs indicative of iron deficiency anemia is likely be an effect from the nutrition education provided.

**Diagnosis of Iron Deficiency Anemia.** It is indicated by the anticipated results that iron levels will increase for the intervention group, as it coincides with the nutrition education for

increased calorie amounts (see appendix F) and the pairing of non-heme iron with non-heme absorption enhancers (see appendix E). However, it is expected that the control group will have slightly higher iron labs when comparing week zero with week 17 due to the consumption of animal food products and increased calorie consumption. This finding is similar to Khanna et al. (2006) whose study found that iron levels were higher in the non-vegetarian group which they also contributed to the consumption of heme iron found in animal food products.

### **Comparison to Other Studies**

The anticipated results from this current study are consistent with previous research conducted by Alaunyte et al. (2014), Khanna et al. (2006), Malczewska et al. (2000), and Snyder et al, (1989). These research studies indicated that female athletes may have various ranges of iron deficiency due to low calorie consumption, low bioavailability of non-heme iron, and loss of iron from monthly menstruation. Yet, research indicates a gap in the literature. Many studies related to athletes and iron status involved an intervention including an oral iron supplement or increasing animal protein, not a plant-based intervention as this study proposes.

The research study conducted by Malczewska et al. (2000) showed most menstruating female endurance athletes were not consuming adequate calories or iron consumption to meet their higher estimated nutritional needs based on their increased physical activity. The study also indicated that the amount of blood loss during monthly menstruation impacts iron status; the more blood loss during menstruation increases the individual's risk of developing iron deficiency due to their higher amount of iron lost on a monthly basis. Due to the complicated nature of estimating blood loss during menstruation, this factor was not included within this proposal.

Other differences and similarities between this research proposal with the studies conducted by Alaunyte et al. (2014), Khanna et al. (2006), Malczewska et a. (2000), and Snyder

et al, (1989) include focusing on a plant-based diet to increase optimal health within female athletes. However, only one study conducted by Alaunyte et al. (2014) utilizes a plant-based nutrition intervention. In this study subjects were instructed to replace their current bread with teff bread, a whole grain that is high in iron. The other studies conducted by Khanna et al. (2006), Malczewska et al. (2000), and Snyder et al, (1989) only compared a plant-based diet to a diet high in animal meat consumption.

The use of a food frequency questionnaire and 24-hour dietary recalls were used to collect dietary information within the studies conducted by Alaunyte (2014), Khanna et al. (2006), and Malczewska et al (2000). The study conducted by Malczewska et al. (2000) ensured that the 24-hour dietary recalls were conducted to include both weekdays and weekends to determine typical diets of the subjects, accounting for both non-heme and heme iron sources as well as food containing non-heme iron enhancers or inhibitors. By collecting data on diet assessment, these studies indicated an improvement in iron labs as diets improved, like this research proposal. Multiple iron labs were assessed to determine the level of iron status within participating subjects to determine whether there was a statistically significant difference in iron levels over-time, as was also assessed within this proposal.

Variables accounted for within these several studies by Alaunyte (2014), Khanna et al. (2006), Malczewska et al (2000), and Snyder et al. (1989) included iron stores, hematological values, iron intake from both heme and non-heme iron sources, menstrual bleeding and training levels. These variables, other than the menstrual bleeding, were accounted for within this research proposal as they are factors that may have an impact on iron status. Accounting for these numerous factors will be useful in drawing a strong conclusion.

### **Strengths and Limitations**

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Strengths of this study include the use of several iron labs indicative of iron deficiency anemia to determine iron status, utilizing a research dietitian to offer group and individualized nutrition education for subjects participating within the intervention and control group, collecting dietary information with the use of NHANES Food Frequency Questionnaire before the start of the study to understand prior eating habits, conducting three unannounced 24-hour dietary recalls, and assessing the training habits of the female subjects.

Limitations to this study include the small sample size of 40 female endurance athletes. This small sample size is associated with low statistical power, inflated false discovery rate, and inflated effect size estimation which may compromise the conclusions drawn from the study. This research proposal does not assess total blood loss via monthly menstruation, which was found by Malczewska et al. (2000) to be a principal cause of iron deficiency in female athletes whose iron intake was sufficient. The short duration of the study involving 17-weeks is also a limitation as the lifespan of red blood cells is around 120 days. To understand if a plant-based nutrition intervention can provide the recommended dietary allowance of iron while maintaining iron labs indicative or iron deficiency anemia, a study longer than three months is warranted.

As previously mentioned by Weaver and Rajaram (1992), the estimated average requirement for iron for female athletes may be 70% higher than the recommended dietary allowance for females aged 19-50. This is due to their increased sweat production, blood loss in the urine, monthly menstruation and GI tract, and the continuous mechanical force of foot pounding leading to a shorter life span of erythrocytes. It is estimated that 2.3 mg of iron is lost per day in female athletes (Weaver & Rajaram, 1992). This is another limitation in this study as the research question was asking if the menstruating female endurance athletes are meeting the

recommended dietary allowance versus the estimated average requirement or each individual's estimated amount.

### **Suggestions for Future Studies**

The anticipated results from this study demonstrate that a plant-based nutrition intervention is beneficial within this high-risk population. The anticipated results also indicate the importance of providing nutrition education for menstruating, female endurance athletes to ensure they are consuming their estimated nutrition needs. Future studies should continue to utilize a plant-based nutrition intervention within a larger sample size to limit low statistical power, inflated false discovery rate and inflated effect size estimation. Future research should conduct long-term studies to understand the impact a plant-based nutrition intervention has on iron status, while considering the lifespan of red blood cells that is around 120 days. When strengthening the limitations within this study design, future studies have the potential to provide general plant-based nutrition interventions to all high-risk population groups as it relates to iron deficiency anemia. More research is warranted.

### References

- Absorption definition and examples biology online dictionary. (2020, January 28). Retrieved from <a href="https://www.biologyonline.com/dictionary/absorption">https://www.biologyonline.com/dictionary/absorption</a>
- Alaunyte, I. Stojceska, V. Plunkett, A., & Derbyshire, E. (2014). Dietary iron intervention using a staple food product for improvement of iron status in female runners. *Journal of the International Society of Sports Nutrition*, 11(1). doi: 10.1186/s12970-014-0050-y.
- Arnarson, A. (2018, June 28). Phytic acid 101: Everything you need to know. Retrieved from https://www.healthline.com/nutrition/phytic-acid-101

Beck, J (2014). ICP - ICP: Articles. Retrieved from <u>www.icppharm.com/News-</u> Resources/Articles/Effects-of-Vitamin-C-on-Iron-Absorption.aspx.

- Bothwell, T.H. Charlton, R.W. Cook, J.D. & Finch, C.A. (1979). *Iron metabolism in man*. Oxford: Blackwell Scientific.
- Braunstein, E. M. (n.d.). Iron deficiency anemia hematology and oncology. Retrieved from <u>https://www.merckmanuals.com/professional/hematology-and-oncology/anemias-caused-</u> <u>by-deficient-erythropoiesis/iron-deficiency-anemia#v969037</u>
- Bridges, K. R. (n.d.). Iron absorption. Retrieved from https://sickle.bwh.harvard.edu/iron\_absorption.html
- Cable, R. G., Glynn, S. A., Kiss, J. E., Mast, A. E., Steele, W. R., Murphy, E. L., & Simon, T. L.
  (2011). Iron deficiency in blood donors: The REDS-II Donor Iron Status Evaluation
  (RISE) Study. *Transfusion*, 52(4), 702–711. doi: 10.1111/j.1537-2995.2011.03401.x

Citrate. (n.d.). Retrieved from https://www.merriam-webster.com/dictionary/citrate

Collings, R., Harvey, L. J. Hooper, L. Hurst, R. Brown, T. J. Ansett, J. Fairweather-Tait, S. J.
(2013). The absorption of iron from whole diets: A systematic review. *The American Journal of Clinical Nutrition*, 98(1), 65–81. doi: 10.3945/ajcn.112.050609

Davis, C. P. (2019). Hematocrit blood test: Normal, high, low ranges & results.

Retrieved from <u>https://www.emedicinehealth.com/hematocrit\_blood\_test/article\_em.htm</u>

Descriptive and inferential statistics. (n.d.). Retrieved from <u>https://statistics.laerd.com/statistical-</u> guides/descriptive-inferential-statistics.php

Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. (2001). *Institute of Medicine (US) Panel on Micronutrients*. Retrieved from

https://www.ncbi.nlm.nih.gov/books/NBK222309/.

- Ehn, L. Carlmark, B. & Hoglund, S. (1980). Iron status in athletes involved in intense physical activity. *Med Sci Sports Exerc* 12:61–64.
- Eldridge, L. (2019). What is MCV (Mean Corpuscular Volume) on your blood count? Retrieved from <u>https://www.verywellhealth.com/mean-corpuscular-volume-overview-4583160</u>

Endurance. (n.d.). Retrieved from https://www.merriam-webster.com/dictionary/endurance

Endurance Definition: What is Endurance? (n.d.). Retrieved from

https://training4endurance.co.uk/physiology-of-endurance/what-is-endurance/

Endurance exercise (aerobic). (2020). Retrieved from https://www.heart.org/en/healthy-

living/fitness/fitness-basics/endurance-exercise-aerobic

Enhance: Meaning in the Cambridge English Dictionary. (n.d.). Retrieved from

https://dictionary.cambridge.org/dictionary/english/enhance

Etiology. (n.d.). Retrieved from https://www.merriam-webster.com/dictionary/etiology

FastStats - leading causes of death. (2017). Retrieved from

https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm

Ferritin. (n.d.). Retrieved from https://labtestsonline.org/tests/ferritin

Gaudiani, J. (2018). Iron deficiency in athletes. Retrieved from
<a href="http://www.gaudianiclinic.com/gaudiani-clinic-blog/2018/8/29/iron-deficiency-in-athletes">http://www.gaudianiclinic.com/gaudiani-clinic-blog/2018/8/29/iron-deficiency-in-athletes</a>

- Goldstein, R. E. (1990). Exercise capacity. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK404/
- Gotter, A. (2018). Everything you need to know about microcytic anemia.

Retrieved from https://www.healthline.com/health/microcytic-anemia

Hemoglobin and functions of iron. (2019). Retrieved from

https://www.ucsfhealth.org/education/hemoglobin-and-functions-of-iron

- Hemosiderin. (n.d.). Retrieved from <u>https://www.sciencedirect.com/topics/medicine-and-</u> dentistry/hemosiderin
- Hultin, Ginger (2019). "Ginger Hultin." *Food & Nutrition Magazine*. Retrieved from foodandnutrition.org/from-the-magazine/the-history-of-vegetarian-diets-explore-the-progression-of-plant-based-eating/.

Hypochromia. (n.d.). Retrieved from https://www.ucsfbenioffchildrens.org/tests/003455.html

Inhibitor. (n.d.). Retrieved from https://www.merriam-webster.com/dictionary/inhibitor

Iron. (2019). Retrieved from https://www.hsph.harvard.edu/nutritionsource/iron/
Iron-deficiency anemia. (n.d.). Retrieved from <u>https://www.nhlbi.nih.gov/health-topics/iron-</u> deficiency-anemia

Iron deficiency anemia. (2019). Retrieved from <u>https://www.mayoclinic.org/diseases-</u> <u>conditions/iron-deficiency-anemia/symptoms-causes/syc-20355034</u>

Iron We consume. (n.d.). Retrieved from http://www.irondisorders.org/iron-we-consume/

- Johnson-Wimbley, T. D. & Graham, D. Y. (2011). Diagnosis and management of iron deficiency anemia in the 21st century. *Therapeutic Advances in Gastroenterology*, 4(3), 177–184. doi: 10.1177/1756283x11398736
- Kay, J. (2015). The female athlete triad. Retrieved from <u>https://www.mirror-</u> mirror.org/female-athlete-triad.htm
- Khanna, G. L. Lal, P. R. Kommi,, K. & Chakraborty, T. (2006). A comparison of a vegetarian and non-vegetarian diet in indian female athletes in relation to exercise performance. *Journal of Exercise Science and Physiotherapy*, 2, 27–34.
- Kohn, J. (n.d.). Iron. Retrieved from <u>https://www.eatright.org/food/vitamins-and-supplements/types-</u> of-vitamins-and-nutrients/iron
- Lactate Threshold Training. (2014). Retrieved from <u>https://runnersconnect.net/what-is-</u>lactate-threshold/
- Lau, F. (2016). Methods for survey studies. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK481602/
- Malczewska, J. Raczynski, G. & Stupnicki, R. (2000). Iron status in female endurance athletes and in non-athletes. *Internationa l Journal of Sport Nutrition and Exercise Metabolism*, 10(3), 260–276. doi: 10.1123/ijsnem.10.3.260

McManus, K. D. (2018). What is a plant-based diet and why should you try it?

Retrieved from <u>https://www.health.harvard.edu/blog/what-is-a-plant-based-diet-and-why-should-you-try-it-2018092614760</u>

Menstruation. (n.d.). Retrieved from https://www.dictionary.com/browse/menstruation

Metabolic process - dictionary definition. (n.d.). Retrieved from

https://www.vocabulary.com/dictionary/metabolicprocess

Micronutrient facts. (2020). Retrieved from https://www.cdc.gov/nutrition/micronutrient-

malnutrition/micronutrients/index.html

Myoglobin. (n.d.). Retrieved from https://www.sciencedirect.com/topics/neuroscience/myoglobin

NCI dictionary of cancer terms. (n.d.). Retrieved from

https://www.cancer.gov/publications/dictionaries/cancer-terms/def/lymphocyte

NCI drug dictionary. (n.d.). Retrieved from

https://www.cancer.gov/publications/dictionaries/cancer-drug/def/ascorbic-acid

Office of dietary supplements - iron. (n.d.). Retrieved from https://ods.od.nih.gov/factsheets/Iron-

HealthProfessional/

Pancreatic enzymes and supplements. (2018). Retrieved from

https://www.pancan.org/facing-pancreatic-cancer/living-with-pancreatic-cancer/diet-andnutrition/pancreatic-enzymes/

Pica. (n.d.). Retrieved from https://www.merriam-webster.com/dictionary/pica

"Plant-Based Diets" (2019). Physicians Committee for Responsible Medicine. Retrieved from

www.pcrm.org/good-nutrition/plant-based-diets.

Shiel, W. (2018). Definition of transferrin. Retrieved from

https://www.medicinenet.com/script/main/art.asp?articlekey=15591

Snyder, A. C. Dvorak, L. L. & Roepke, J. B. (1989). Influence of dietary iron source on measures

of iron status among female runners. Medicine & Science in Sports & Exercise, 21(1), 7-

10. doi: 10.1249/00005768-198902000-00002

Stöppler, M. C. (2018). Definition of deficiency, iron. Retrieved from

https://www.medicinenet.com/script/main/art.asp?articlekey=2924

Sullivan, D. (2020). Serum Iron Test: Purpose, Procedure, And Results. Healthline. Available at:

https://www.healthline.com/health/serum-iron.

The Editors of Encyclopedia Britannica. (2016). Tannin. Retrieved from

https://www.britannica.com/science/tannin

The nutrition care process (NCP). (n.d.). Retrieved from https://www.ncpro.org/nutrition-care-

process

Transferrin receptor. (n.d.). Retrieved from

https://www.sciencedirect.com/topics/neuroscience/transferrin-receptor

Traverso, M. (2004). The Crucial Role of Iron in the Body. Retrieved from

http://www.chemistry.wustl.edu/~edudev/LabTutorials/CourseTutorials/Tutorials/Ferritin/IronB

#### ody.htm

Vegetarian Diet: How to Get the Best Nutrition. (2019). Retrieved from

https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-

depth/vegetarian-diet/art-20046446

Vo2 max. (n.d.). Retrieved from https://www.merriam-webster.com/dictionary/VO2max

Weaver, C. & Rajaram, S. (1992). Exercise and Iron Status.

Zelmean, K. (2019). Micronutrients: Iron. Retrieved from foodannutrition.org/from-themagazine/micronutrients-iron/.

#### Appendix A

**Consent Form** 



# Research Participant Information and Consent Form Mount Mary University

**Title of Study:** Research Proposal: Plant-Based Nutrition Intervention Providing the Recommended Dietary Allowance for Iron in Menstruating, Female Endurance Athletes

**Invitation to Participate and Purpose of the Research** You are invited to participate in a research study that seeks female athletes aged 19-24 years old participating on the University of Wisconsin-Madison and the University of Wisconsin-Milwaukee cross country teams. Participants will be asked to participate in a 17-week randomized, controlled trial to investigate if a plant-based diet provides the recommended dietary allowance of iron in menstruating, female endurance athletes while maintaining labs indicative of iron deficiency within normal range. Data will be de-identified and analyzed by researchers. Participants will be randomized into the control or intervention group. All subjects will complete the NHANES Food Frequency Questionnaire, three unannounced 24-hour dietary recalls, and labs will be drawn for all subjects.

**Benefits and Risks** This research is designed to benefit the dietetics profession to investigate if a plantbased diet provides the recommended dietary allowance of iron in menstruating, female endurance athletes. Participants may or may not benefit personally from being in this research study. Participants within the intervention group will be provided with education related to a plant-based diet to provide adequate calorie and iron amounts based on their individualized needs. The findings generated by this research will provide a better understanding if a plant-based diet provides the recommended dietary allowance for menstruating, female endurance athletes, and information for future study designs to further knowledge on this topic. There will be no monetary compensation. Discomfort may occur during and a short time after the lab draws completed during week zero and week 17. Please address any questions or issues of concern to the researchers using the contact information provided above.

**Confidentiality** All information obtained will be kept confidential by the researchers who will be the only people with access to the data. The anonymity and confidentiality will be kept private, all data collected will be stored in a confidential web-based folder requiring a password to enter. Data will be entered per team using initials, age and their number assigned to each subject during randomization to code subject's information. Per the U.S. Office of Human Research Protections (code §46.115), all data will be destroyed 3 years after the end of data collection. Paper files will be shredded, and electronic files will be deleted. Individual participants will not be identified in any report or publication about this study.

**Contact Information** If you have questions about this research study, your rights as a research subject, or would like to know the outcome of the research, please contact Abby Fenske, 715-651-9228, <u>fenskea@mtmary.edu</u> or Dr. Dana Scheunemann, 414-930-3502, <u>scheuned@mtmary.edu</u>. If you have any questions regarding your rights or privacy as a participant in this study, please contact Dr. Tammy Scheidegger, Mount Mary University Institutional Review Board Chair, 2900 North Menomonee River Parkway, Milwaukee, Wisconsin, 53222-4597, telephone (414) 930-3434 or email <u>schediet@mtmary.edu</u>.

**Consent** By signing below, you are indicating that you have read this consent form, have been given the opportunity to ask questions, and have agreed to voluntarily participate. You may withdraw from participation at any time, or refuse to answer any question herein, without penalty or loss of benefits to which other participants are entitled.

You may request a copy of this page for your records. Thank you for your participation.

Signature of participant\_\_\_\_\_

Date \_\_\_\_\_

**Appendix B** 



Mount Mary University Institutional Review Board (IRB) for the Protection of Human Subjects

**Application for IRB Review** 

## **<u>I. Required Documentation</u>** - No action will be taken without these attachments.

Are the following attached to the IRB application?

Informed Consent Document	√Yes	Informed Consent Documents should include an explanation of procedures, risk, safeguards, freedom to withdraw, confidentiality, offer to answer inquiries, third party referral for concerns, signature and date. See Appendix.A and use the <b>MMU Informed Consent Template</b> to avoid delays in the process.
Questionnaire/Survey Instrument(s)	√ Yes	If a survey is being administered in any written format (e.g., survey monkey, qualtrics), a copy of that survey must accompany this application. If a survey is being conducted verbally, a copy of the introductory comments and survey questions being asked must be attached to this application. If survey includes focus group questions, a complete list of the question must be attached. For research using a published/purchased instrument, a photocopy of the instrument will suffice.
Verification of Human Subjects Training	√ Yes	Copy of transcript, certificate or other evidence that ALL members of the research team have completed the required training.

Copy of cooperating institution's IRB approval. <b>II. Investigator(s):</b>	N/A Yes	Not required if there is no cooperating institution.	
Name: Abigail Fenske Affiliation with Mount Mary Unistudent, etc.): Dr. Dana Scheune Email: <u>scheuned@mtmary.edu</u>	iversity (e mann	Phone: 715-651-9228 .g. faculty,	
Signature: <u>Abigail Fenske</u>		Date: 5/8/20	
If student, list Research Advisor and c	omplete t	the application. Research Advisor must provide	
requested information and verify.			
		Department:	
Research Advisor's Name:			
Email:	il: Phone:		
Research Advisor: Have you completed	Human Sı	ubject's Training? 🗌 Yes 🗌 No	
Research advisor's signature indicates student compliance with all IRB requi	responsi rements.	bility for	
Signature:		Date:	
Research Advisor			

### **III. Project Description – Required by all applicants**

# 1) Objectives (purpose of project):

The purpose of this randomized, controlled trial is to investigate if a plant-based diet provides the recommended dietary allowance of iron in menstruating, female endurance athletes while maintaining labs indicative of iron deficiency within normal range.

#### 2) Relevance to practice/body of knowledge:

This study has high significance as there is a lack of conclusive research studies utilizing a plantbased diet nutrition intervention to provide the recommended dietary allowance for menstruating, female endurance athletes. This research proposal will provide important information on whether a plant-based diet provides the recommended dietary allowance of iron within this high-risk group.

### 3) Describe the research design:

This research proposal will be a 17-week randomized, controlled trial to investigate if a plantbased diet provides the recommended dietary allowance of iron in menstruating, female endurance athletes. Sample size will be determined using previous research sample sizes and specified inclusion and exclusion criteria. Subjects will be recruited from the Women's Cross-Country teams from both the University of Wisconsin-Madison and the University of Wisconsin-Milwaukee during the cross-country season occurring in the fall of 2021. Each subject will be assigned a random number that will be generated by a computer to place them within either the control or intervention group. Both groups will complete the NHANES Food Frequency Questionnaire, have labs drawn, and complete three unannounced 24-hour dietary recalls throughout the study. Nutrition education will be provided to both groups on portion sizes to meet individualized nutrition needs in the beginning of the study. However, only the intervention group will be provided with nutrition education on plant-based food options to consume for meals and snacks, what foods to pair together for the best non-heme absorption rates, what foods to limit, and recipes to assist in creating a variety of plant-based meals and snacks. A normal distribution will be used to ensure data is normally distributed, so no wrong conclusions are made. A central tendency will be applied to collect the mean, mode and median to determine the typical value in the dataset. A chi-square test will be utilized in this study as the dependent variables are categorical.

### 4) What measurement/data collection tools are being used?

NHANES Food Frequency Questionnaire and three unannounced 24-hour dietary recalls will be collected throughout the study. Several labs will be drawn at the beginning and end of the study to determine iron status.

### IV. Additional Project Information – Required by all applicants

1) What human subjects training has the researcher completed (e.g. course work, online certification)?

Researcher has completed CITI and HIPPA training.

2) What process is used for obtaining informed consent (attach the informed consent application)? See Appendix for consent application.Note Appendix A – Consent Form.

3) Does the research include special populations?

Minors under 18 years of age?	Yes	√No	
Persons legally incompetent?	Yes	√No	
Prisoners?	Yes	√No	
Pregnant women, if affected by research?	Yes	√No	
Persons institutionalized?		Yes	√No
Persons mentally incapacitated?		Yes	√No

4) If <u>**YES**</u>, describe additional precautions included in the research procedures. N/A.

5) Does the research involve any of the following procedures?

False or misleading information to subjects?	Yes	√No
Withholds information such that their informed consent might be	Yes	√No
questioned?		
Uses procedures designed to modify the thinking, attitudes, feelings,	Yes	√No
or other aspects of the behavior of the subjects?		

6) If <u>YES</u>, describe the rationale for using procedures, how the human subjects will be protected and what debriefing procedures are used. N/A.

7) Does the research involve measurement in any of the following areas?

Sexual behaviors?	Yes	√No
Drug use?	Yes	√No
Illegal conduct?	Yes	√No
Use of alcohol?	Yes	√No

8) If  $\underline{\mathbf{YES}}$ , describe additional precautions included in the research procedures. N/A.

9) Are any portions of the research being conducted online?

Survey posted on a website? $\sqrt{Yes}$	🗌 No	If yes, assure anonymity
--	------	--------------------------

URL for survey includes information that	√Yes	🗌 No	If yes, assure anonymity
could identify participants?			
Invitation to participate sent by email?	√Yes	🗌 No	If yes, assure anonymity
Items use drop-down box?	√Yes	🗌 No	If yes, assure that items
			allow choice of "no

# 10) If <u>YES</u>, describe additional procedures.

The anonymity and confidentiality will be kept private, all data collected will be stored in a confidential web-based folder requiring a password to enter. Data will be entered per team using initials, age and their number assigned to each subject during randomization to code subject's information. Subjects will be aware that they may withdraw from the study at any time with no consequence.

11) Describe the methods used to ensure confidentiality of data obtained.

The anonymity and confidentiality will be kept private, all data collected will be stored in a confidential web-based folder requiring a password to enter. Data will be entered per team using initials, age and their number assigned to each subject during randomization to code subject's information.

# **Risks and Benefits**

1) Describe risks to the subjects and the precautions that will be taken to minimize them. (Risk includes any potential or actual physical risk of discomfort, harassment, invasion of privacy, risk of physical activity, risk to dignity and self-respect, and psychological, emotional or behavioral risk.)

Multiple steps will be taken during this study to ensure research ethics. Subjects will not be put at risk for harm throughout the duration of this study.

2) Describe the benefits to subjects and/or society. (These will be balanced against risk.) This randomized, controlled trial will provide a better understanding if a plant-based diet provides the recommended dietary allowance of iron for menstruating, female endurance athletes, and information for future study designs to further knowledge on this topic.

# V. <u>Is the proposed project "research" as defined by Institutional Review Board</u> <u>requirements? - Required by all applicants</u>

- Research is defined as a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge.
- A human subject is defined as a living individual about whom an investigator obtains either 1) data through intervention or interaction with the individual; or 2) identifiable private information.

Does the research involve human subjects or official records about human subjects?

response"

√Yes □ No

If the results will be available in the library, presented at a professional conference (includes any presentation to group(s) outside of the classroom), or published, please check the Yes box:

 $\Box$  Yes  $\sqrt{No}$ 

Appendix C

# **NHANES Food Questionnaire**



Public reporting burden of this collection of information is estimated to be 45 minutes per response for total participation, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. An agency may not conduct or sponsor, and a person is not required to respond to collection of information unless it displays a currently valid OMB control number. Send comments regarding this burden estimate or any other aspects of this collection of information, including suggestions for reducing burden to: CDC/ATSDR Reports Clearance Officer, 1800 Clifton Road, MS D-24, Atlanta, GA 30333, Attention: PRA (0920-0237).

1. Over the past 12 months, how often did you drink How often did you drink other fruit drinks (such as 6. tomato juice or vegetable juice? cranberry cocktail, Hi-C, lemonade, or Kool-Aid, diet or regular)? NEVER O NEVER (GO TO QUESTION 7) 1 time per month or less O 2–3 times per month O 2–3 times per day 1 time per month or less 1 time per day O 2–3 times per day 2–3 times per month 1–2 times per week 0 4–5 times per day 3–4 times per week 6 or more times per day 1–2 times per week 0 4–5 times per day 5-6 times per week 3–4 times per week O 6 or more times per day 5-6 times per week 2. How often did you drink orange juice or grapefruit 6a. How often were your fruit drinks diet or juice? sugar-free drinks? NEVER Almost never or never About <sup>1</sup>/<sub>4</sub> of the time 1 time per month or less About 1/2 of the time. O 2–3 times per month O 2–3 times per day About <sup>3</sup>/<sub>4</sub> of the time 1–2 times per week 0 4–5 times per day O Almost always or always O 3–4 times per week O 6 or more times per day 5-6 times per week 7. How often did you drink milk as a beverage (NOT in coffee, NOT in cereal)? (Please include chocolate 3. How often did you drink apple juice? milk and hot chocolate.) O NEVER O NEVER (GO TO QUESTION 8) 1 time per month or less 1 time per month or less 2–3 times per month 2–3 times per day 2–3 times per day 2–3 times per month 1–2 times per week 0 4–5 times per day 1–2 times per week 0 4–5 times per day O 6 or more times per day O 6 or more times per day O 3–4 times per week O 3–4 times per week 5-6 times per week ○ 5–6 times per week 7a. What kind of milk did you usually drink? 4. How often did you drink grape juice? Whole milk O NEVER 2% fat milk 1% fat milk 1 time per month or less O Skim, nonfat, or 1/2% fat milk O 2–3 times per day 2-3 times per month O Soy milk 1–2 times per week 0 4–5 times per day Rice milk O 6 or more times per day Raw, unpasteurized milk O 3–4 times per week Other 5–6 times per week 5. How often did you drink other 100% fruit juice or 100% fruit juice mixtures (such as pineapple, prune, or others)? NEVER BAR 1 time per month or less CODE O 2–3 times per month 2–3 times per day 1–2 times per week 0 4–5 times per day LABEL O 3–4 times per week O 6 or more times per day HFRF 5–6 times per week г. 1 Question 8 appears on the next page.

#### PLANT-BASED INTERVENTION AND IRON STATUS

```
10.
                                                                     Over the past 12 months, did you drink beer?
    Over the past 12 months...
_
          How often did you drink meal replacement, energy,
    8.
                                                                     O NO (GO TO QUESTION 11)
_
          or high-protein beverages such as Instant
-
          Breakfast, Ensure, Slimfast, Sustacal or others?
-
                                                                     O YES
-
          O NEVER
_
=
          1 time per month or less 0 1 time per day
                                                                     10a. How often did you drink beer IN THE SUMMER?
          2-3 times per month
                                    2–3 times per day
=
          1-2 times per week
                                    4-5 times per day
                                                                          O NEVER
          O 3–4 times per week
                                    6 or more times per day
=
                                                                          1 time per month or less 0 1 time per day
          ○ 5–6 times per week
                                                                                                    2-3 times per day
                                                                           2–3 times per month
-
                                                                          1-2 times per week
                                                                                                    4-5 times per day
=
    9.
          Over the past 12 months, did you drink soft drinks,
                                                                           O 3–4 times per week
                                                                                                    O 6 or more times
          soda, or pop?
                                                                          5-6 times per week
                                                                                                       per day
_
          O NO (GO TO QUESTION 10)
-
                                                                     10b. How often did you drink beer DURING THE
                                                                           REST OF THE YEAR?
-
          O YES
-
_
                                                                          O NEVER
-
-
               How often did you drink soft drinks, soda, or
                                                                          1 time per month or less
          9a.
               pop IN THE SUMMER?
Ξ
                                                                          2–3 times per month
                                                                                                    2-3 times per day
                                                                          1-2 times per week
                                                                                                    4-5 times per day
               O NEVER
                                                                           3–4 times per week
                                                                                                    O 6 or more times
                                                                          5–6 times per week
                                                                                                       per day
_
               1 time per month or less
                                         1 time per day
               O 2–3 times per month
                                         2–3 times per day
=
               O 1–2 times per week
                                         O 4-5 times per day
                                                               11.
                                                                     How often did you drink wine or wine coolers?
                3–4 times per week
                                         O 6 or more times
-
               ○ 5–8 times per week
                                                                     O NEVER
                                            per day
=
          9b. How often did you drink soft drinks, soda, or
                                                                     1 time per month or less
               pop DURING THE REST OF THE YEAR?
-
                                                                     2–3 times per month
                                                                                               2-3 times per day
-
                                                                     1-2 times per week
                                                                                               4-5 times per day
-
               O NEVER
                                                                     3-4 times per week
                                                                                               O 6 or more times per day
Ξ
                                                                     ○ 5–6 times per week
               1 time per month or less O 1 time per day
                                         2-3 times per day
                2–3 times per month
Ξ
                                         O 4-5 times per day
               1-2 times per week
                                                               12.
                                                                    How often did you drink liquor or mixed drinks?
               3-4 times per week
                                         O 6 or more times
                                                                     O NEVER
               5–6 times per week
                                            per day
=
          9c. How often were these soft drinks, soda, or pop
                                                                     1 time per month or less
                                                                                              1 time per day
=
               diet or sugar-free?
                                                                     2-3 times per month
                                                                                               2-3 times per day
                                                                     1-2 times per week
                                                                                               4-5 times per day
=

    Almost never or never

                                                                     3-4 times per week
                                                                                               O 6 or more times per day
                About <sup>1</sup>/4 of the time
                                                                     ○ 5–6 times per week
Ξ
               About <sup>1</sup>/<sub>2</sub> of the time
               About <sup>3</sup>/4 of the time
               Almost always or always
=
          9d
              How often were these soft drinks, soda, or pop
               caffeine-free?
-
_
_
               Almost never or never
-
                About <sup>1</sup>/<sub>4</sub> of the time
_
               About 1/2 of the time
               About <sup>3</sup>/4 of the time
_
               Almost always or always
    Question 10 appears in the next column.
```

Over the past 12 months ... 14a. How often was the cold cereal you ate a whole grain type (such as shredded wheat, Wheaties, -Did you eat oatmeal, grits, or other cooked Cheerios, Raisin Bran or other bran, oat, or 13. cereal? whole wheat cereal)? O NO (GO TO QUESTION 14) Almost never or never About 1/4 of the time O YES About 1/2 of the time O About <sup>3</sup>/<sub>4</sub> of the time Almost always or always 13a. How often did you eat oatmeal, grits, or other cooked cereal IN THE WINTER? 14b. Was milk added to your cold cereal? **O NEVER** NO (GO TO QUESTION 15) O YES ○ 1–6 times per winter O 2 times per week O 7–11 times per winter O 3-4 times per week 1 time per month 5-6 times per week 14c. What kind of milk was usually added? O 2–3 times per month O 1 time per day 1 time per week 2 or more times O Whole milk per day 2% fat milk 1% fat milk 13b. How often did you eat oatmeal, grits, or other cooked cereal DURING THE REST OF THE Skim, nonfat, or 1/2% fat milk YEAR? O Soy milk Rice milk O NEVER Raw, unpasteurized milk O Other 1-6 times per year 2 times per week ○ 7–11 times per year O 3-4 times per week How often did you eat applesauce? 1 time per month ○ 5–8 times per week 15. 2–3 times per month 1 time per day O NEVER O 2 or more times 1 time per week per day 1-6 times per year 2 times per week 13c. How often was the cooked cereal you ate 7–11 times per year 3-4 times per week oatmeal? 1 time per month ○ 5–8 times per week 2–3 times per month O 1 time per day Almost never or never O 2 or more times per day 1 time per week About 1/4 of the time About 1/2 of the time O About 3/4 of the time How often did you eat apples? 16. Almost always or always O NEVER 14. How often did you eat cold cereal? 1-6 times per year 2 times per week 3-4 times per week 7-11 times per year 1 time per month NEVER (GO TO QUESTION 15) ○ 5–6 times per week 2-3 times per month O 1 time per day 2 times per week 1–6 times per year O 2 or more times per day 1 time per week O 7–11 times per year O 3-4 times per week 1 time per month ○ 5–6 times per week 17. How often did you eat pears (fresh, canned, or 2–3 times per month O 1 time per day frozen)? 1 time per week 2 or more times per day O NEVER 1-6 times per year 2 times per week O 3–4 times per week 7–11 times per year 1 time per month 5-6 times per week 2–3 times per month 1 time per day Question 15 appears in the next column. 1 time per week O 2 or more times per day

```
Over the past 12 months ...
                                                                   21b. How often did you eat peaches, nectarines,
                                                                         or plums (fresh, canned, or frozen) DURING
-
   18. How often did you eat bananas ?
                                                                         THE REST OF THE YEAR?
-
=
                                                                        O NEVER
          O NEVER
-
          1-6 times per year
                                2 times per week
                                                                        1-6 times per year
                                                                                               2 times per week
0 3-4 times per week
          ○ 7–11 times per year
                                O 3-4 times per week
                                                                        O 7-11 times per year
          1 time per month
                                 ○ 5-6 times per week
                                                                        1 time per month
                                                                                               ○ 5–6 times per week
                                                                                               1 time per day
-
          2–3 times per month
                                O 1 time per day
                                                                        O 2-3 times per month
_
                                O 2 or more times per day
                                                                                               O 2 or more times
          O 1 time per week
                                                                        O 1 time per week
                                                                                                 per day
-
How often did you eat pineapple?
    19.
                                                             22. How often did you eat grapes?
          O NEVER
O NEVER
                                O 2 times per week
          ○ 1–6 times per year
          ○ 7–11 times per year
                                ◯ 3-4 times per week
                                                                   ○ 1–6 times per year
                                                                                         O 2 times per week
          O 1 time per month
                                ○ 5–6 times per week
                                                                   ○ 7–11 times per year
                                                                                         O 3–4 times per week
                                                                                          O 5−6 times per week
                                                                   1 time per month
          2–3 times per month
                                O 1 time per day
                                O 2 or more times per day
                                                                    2–3 times per month
                                                                                         1 time per day
          O 1 time per week
                                                                                          2 or more times per day
                                                                   1 time per week
    20.
          How often did you eat dried fruit, such as prunes or
          raisins?
                                                             23.
                                                                   Over the past 12 months, did you eat melons (such
                                                                   as cantaloupe, watermelon, or honeydew)?
          O NEVER
                                                                   O NO (GO TO QUESTION 24)
          ○ 1–6 times per year
                                O 2 times per week
                                O 3−4 times per week
                                                                   O YES
          O 7–11 times per year
          1 time per month
                                O 5−6 times per week
          O 2–3 times per month O 1 time per day
                                                                   23a. How often did you eat fresh melons (such as
          O 1 time per week
                                O 2 or more times per day
-
                                                                         cantaloupe, watermelon, or honeydew) WHEN
-
                                                                         IN SEASON?
          Over the past 12 months, did you eat peaches,
    21.
                                                                        O NEVER
-
          nectarines, or plums?
=
         O NO (GO TO QUESTION 22)
                                                                        1-6 times per season
                                                                                                 O 2 times per week
_
                                                                        O 7-11 times per season
                                                                                                 O 3-4 times per week
                                                                                                 ○ 5–6 times per week
          OYES
                                                                        O 1 time per month
-
                                                                         2-3 times per month
                                                                                                  1 time per day
          V
-
                                                                        O 1 time per week
                                                                                                 O 2 or more times
-
          21a. How often did you eat fresh peaches.
                                                                                                    per day
-
               nectarines, or plums WHEN IN SEASON?
-
                                                                   23b. How often did you eat fresh or frozen melons
O NEVER
                                                                         (such as cantaloupe, watermelon, or honeydew)
                                                                         DURING THE REST OF THE YEAR?
               ○ 1–6 times per season ○ 2 times per week
               7–11 times per season O 3–4 times per week
                                                                        ONEVER
                                     O 5-6 times per week
               1 time per month
               O 2–3 times per month O 1 time per day
                                                                        ○ 1–6 times per year
                                                                                                 O 2 times per week
               1 time per week
                                                                        7–11 times per year
                                                                                                 3-4 times per week
                                     O 2 or more times
                                                                                                 ○ 5–8 times per week
                                        per day
                                                                         1 time per month
                                                                        O 2–3 times per month
                                                                                                 1 time per day
                                                                        O 1 time per week
                                                                                                 O 2 or more times
                                                                                                    per day
=
-
    Question 22 appears in the next column.
                                                             Question 24 appears on the next page.
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25b. How often did you eat oranges, tangerines, Over the past 12 months ... clementines, or tangelos (fresh or canned) -DURING THE REST OF THE YEAR? 24. Did you eat strawberries? -O NO (GO TO QUESTION 25) O NEVER O YES 🔵 1–6 times per year O 2 times per week O 7–11 times per year O 3–4 times per week -1 time per month ○ 5–6 times per week -24a. How often did you eat fresh strawberries 2–3 times per month O 1 time per day WHEN IN SEASON? 1 time per week 2 or more times per day O NEVER 1-8 times per season O 2 times per week Over the past 12 months, did you eat grapefruit? 26. 7–11 times per season O 3–4 times per week 1 time per month O 5–6 times per week NO (GO TO QUESTION 27) O 2–3 times per month YES 1 time per week O 2 or more times per day 24b. How often did you eat fresh or frozen 26a. How often did you eat fresh grapefruit strawberries DURING THE REST OF THE WHEN IN SEASON? YEAR? O NEVER O NEVER 1-6 times per season O 2 times per week 1–8 times per year O 2 times per week O 7–11 times per season O 3–4 times per week 🔿 7–11 times per year 1 time per month 5–6 times per week O 3–4 times per week 1 time per month 5-6 times per week O 2–3 times per month O 1 time per day O 2–3 times per month 1 time per week O 2 or more times 1 time per week O 2 or more times per day per day 26b. How often did vou eat grapefruit (fresh or carded) DURING THE REST OF THE YEAR? 25. Over the past 12 months, did you eat oranges, tangerines, clementines, or tangelos? NEVER O NO (GO TO QUESTION 26) 1–8 times per year O 2 times per week ○ 7–11 times per year O 3–4 times per week O YES 1 time per month 5–6 times per week 2–3 times per month O 1 time per day Ŵ 1 time per week 2 or more times 25a. How often did you eat fresh oranges, per dav tangerines, clementines, or tangelos WHEN IN SEASON? 27. How often did you eat other kinds of fruit? O NEVER O NEVER ○ 1-6 times per season ○ 2 times per week O 7-11 times per season O 3-4 times per week 1–6 times per year O 2 times per week O 3–4 times per week 1 time per month 5–6 times per week 7–11 times per year O 2–3 times per month O 1 time per day 1 time per month O 5–6 times per week 1 time per week O 2 or more times O 2–3 times per month O 1 time per day per day O 1 time per week O 2 or more times per day

\_ 28.

31.

32.

29.

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Over the past 12 months...
                                                          33.
                                                                How often did you eat string beans or green
                                                                beans (fresh, canned, or frozen)?
      How often did you eat COOKED greens (such as
                                                                 O NEVER
      spinach, turnip, collard, mustard, chard, or kale)?
                                                                 1–6 times per year
                                                                                       2 times per week
      O NEVER
                                                                 7–11 times per year
                                                                                       O 3–4 times per week
      1–6 times per year
                             2 times per week
                                                                 1 time per month
                                                                                        🔵 5–6 times per week
      7–11 times per year
                             O 3–4 times per week
                                                                 2–3 times per month
                                                                                       1 time per day
      1 time per month
                             ○ 5–6 times per week
                                                                 1 time per week
                                                                                       O 2 or more times per day
       2–3 times per month
                             1 time per day
                             O 2 or more times per day
      1 time per week
                                                          34.
                                                                How often did you eat peas (fresh, canned, or
                                                                frozen)?
      How often did you eat RAW greens (such as
      spinach, turnip, collard, mustard, chard, or kale)?
                                                                O NEVER
      (We will ask about lettuce later.)
                                                                                       2 times per week
                                                                 1–6 times per year
      O NEVER
                                                                 7–11 times per year
                                                                                       O 3–4 times per week
                                                                 1 time per month
                                                                                        5–6 times per week
      O 1–6 times per year
                             2 times per week
                                                                 2–3 times per month
                                                                                       1 time per day
                             O 3-4 times per week
      7–11 times per year
                                                                 1 time per week
                                                                                       O 2 or more times per day
       1 time per month
                              🔵 5–6 times per week
      2–3 times per month
                             1 time per day
      1 time per week
                             O 2 or more times per day
                                                          35.
                                                                Over the past 12 months, did you eat corn?
                                                                 O NO (GO TO QUESTION 36)
30.
      How often did you eat coleslaw?
                                                                 YES
      O NEVER
      ○ 1-6 times per year
                             2 times per week
                                                                35a. How often did you eat corn (fresh, canned, or
      O 7–11 times per year
                             O 3–4 times per week
                                                                      frozen) WHEN IN SEASON?
      1 time per month
                             5–6 times per week
                                                                      O NEVER
       2–3 times per month
                             1 time per day
                             O 2 or more times per day
      1 time per week
                                                                      ○ 1-6 times per season ○ 2 times per week
                                                                      O 7–11 times per season O 3–4 times per week
      How often did you eat sauerkraut or cabbage
                                                                      1 time per month
                                                                                             5-6 times per week
      (other than coleslaw)?
                                                                      2–3 times per month
                                                                                             1 time per day
                                                                      1 time per week
                                                                                             2 or more times
      O NEVER
                                                                                               per dav
                                                                35b. How often did you eat corn (fresh, canned, or
                             2 times per week
      1-6 times per year
                                                                      frozen) DURING THE REST OF THE YEAR?
      O 7–11 times per year
                             O 3–4 times per week
      1 time per month
                             5-6 times per week
                                                                      O NEVER
       2–3 times per month
                             O 1 time per day
      1 time per week
                             O 2 or more times per day
                                                                      1-6 times per year
                                                                                             2 times per week
                                                                      7–11 times per year
                                                                                             O 3–4 times per week
      How often did you eat carrots (fresh, canned, or
                                                                      1 time per month
                                                                                             5–6 times per week
      frozen)?
                                                                      2–3 times per month
                                                                                             O 1 time per day
                                                                      1 time per week
                                                                                             2 or more times
      O NEVER
                                                                                               per day
      1-6 times per year
                             2 times per week
      O 7–11 times per year
                             O 3–4 times per week
       1 time per month
                             5-6 times per week
      2–3 times per month
                             1 time per day
      O 1 time per week
                             O 2 or more times per day
                                                          Question 36 appears on the next page.
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Over the past 12 months... 41. How often did you eat raw cucumbers (not including pickles)? How often did you eat broccoli (fresh or frozen)? 36. \_ NEVER O NEVER \_ 1–6 times per year 2 times per week 2 times per week 0 7–11 times per year 1–6 times per year O 3–4 times per week ○ 7–11 times per year ○ 3-4 times per week 1 time per month O 5–6 times per week \_ 1 time per month 5–6 times per week 2-3 times per month 1 time per day \_ O 2–3 times per month O 1 time per day 1 time per week O 2 or more times per day -O 1 time per week O 2 or more times per day 42. Over the past 12 months, did you eat fresh tomatoes How often did you eat cauliflower or Brussels 37. (including those in salads)? sprouts (fresh or frozen)? O NO (GO TO QUESTION 43) \_ NEVER YES 1–6 times per year 2 times per week O 7–11 times per year O 3–4 times per week 1 time per month 5–6 times per week 42a. How often did you eat fresh tomatoes 2–3 times per month 1 time per day (including those in salads) WHEN IN SEASON? \_ O 2 or more times per day 1 time per week O NEVER \_ \_ How often did you eat mixed vegetables? 38. 1-6 times per season O 2 times per week \_ O 7–11 times per season O 3–4 times per week \_ NEVER 1 time per month O 5–6 times per week \_ 2–3 times per month O 1 time per day \_ 1–6 times per year 2 times per week 1 time per week O 2 or more times O 7–11 times per year O 3–4 times per week \_ per dav 1 time per month 🔿 5–6 times per week \_ 42b. How often did you eat fresh tomatoes O 2–3 times per month 1 time per day (including those in salads) DURING THE 1 time per week O 2 or more times per day \_ REST OF THE YEAR? \_ 39. How often did you eat onions (including in mixtures)? NEVER \_ \_ O NEVER 1–6 times per year 2 times per week \_ ○ 3–4 times per week 7–11 times per year \_ 1–6 times per year 2 times per week 1 time per month \_ 🔵 5–6 times per week 7–11 times per year O 3-4 times per week 2-3 times per month 1 time per day \_ 1 time per month ○ 5–6 times per week 1 time per week O 2 or more times \_ 2–3 times per month O 1 time per day per day \_ 1 time per week O 2 or more times per day 40. Over the past 12 months, how often did you eat \_ sweet or hot peppers (green, red, or yellow)? -\_ NEVER \_ 2 times per week 1–6 times per year 7–11 times per year O 3–4 times per week 1 time per month 5–6 times per week 2–3 times per month 1 time per day O 2 or more times per day 1 time per week \_ \_ \_ Question 43 appears on the next page.

Over the past 12 months		45.	I5. How often did you eat salad dressing (includi- low-fat) on salads or other vegetables?		
43.	Did you eat summer squash (include yellow and green squash)?		O NEVER	2	
	O YES V 43a. How often did you eat summer squash WHEN		<ul> <li>1-8 times per year</li> <li>7-11 times per year</li> <li>1 time per month</li> <li>2-3 times per month</li> <li>1 time per week</li> </ul>	<ul> <li>2 times per week</li> <li>3-4 times per week</li> <li>5-6 times per week</li> <li>1 time per day</li> <li>2 or more times per day</li> </ul>	
	IN SEASON (include yellow and green squash)? NEVER 1-6 times per season 2 times per week 7-11 times per season 3-4 times per week 1 time per month 5-6 times per week 2-3 times per month 1 time per day 1 time per week 2 or more times per day	46.	How often did you eat sw NEVER 1-6 times per year 7-11 times per year 1 time per month 2-3 times per month 1 time per week	2 times per week 3-4 times per week 5-6 times per week 1 time per day 2 or more times per day	
Ξ	43b. How often did you eat summer squash DURING THE REST OF THE YEAR (include yellow and green squash)?	47.	How often did you eat Fre hash browned potatoes,	ench fries, home fries, , or tater tots?	
31	O NEVER		O NEVER		
	<ul> <li>1−6 times per year</li> <li>7−11 times per year</li> <li>1 time per month</li> <li>2−3 times per month</li> <li>1 time per week</li> <li>2 times per week</li> <li>3−4 times per week</li> <li>5−6 times per week</li> <li>1 time per day</li> <li>2 or more times per day</li> </ul>		<ul> <li>1-6 times per year</li> <li>7-11 times per year</li> <li>1 time per month</li> <li>2-3 times per month</li> <li>1 time per week</li> </ul>	<ul> <li>2 times per week</li> <li>3-4 times per week</li> <li>5-8 times per week</li> <li>1 time per day</li> <li>2 or more times per day</li> </ul>	
44.	How often did you eat lettuce salads (with or without other vegetables)?	48.	How often did you eat pot	tato salad?	
Quest	NEVER (GO TO QUESTION 45)     1-6 times per year 3-4 times per week     7-11 times per year 5-6 times per week     2-3 times per month 1 time per day     1 time per week 2 or more times per day     44a. How often were the lettuce salads you ate made     with dark green leaves?     Almost never or never     About <sup>1</sup> /4 of the time     About <sup>3</sup> /4 of the time     About <sup>3</sup> /4 of the time     About <sup>3</sup> /4 of the time     Almost always or always	49.	<ul> <li>1-8 times per year</li> <li>7-11 times per year</li> <li>1 time per month</li> <li>2-3 times per month</li> <li>1 time per week</li> </ul> How often did you eat ball potatoes? <ul> <li>NEVER</li> <li>1-8 times per year</li> <li>7-11 times per year</li> <li>1-8 times per month</li> <li>2-3 times per month</li> <li>2-3 times per month</li> <li>1 time per week</li> </ul>	2 times per week 3-4 times per week 5-6 times per week 1 time per day 2 or more times per day <b>ked, boiled, or mashed</b> 2 times per week 3-4 times per week 5-6 times per week 1 time per day 2 or more times per day	
quest	In the support of the second method of the second secon	0			

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Over the past 12 months...
                                                         55.
                                                               How often did you eat tortillas or tacos?
                                                                                                                  _
50. How often did you eat salsa?
                                                               NEVER (GO TO QUESTION 56)
                                                                                                                  O NEVER
                                                                                      2 times per week
                                                                🔵 1–6 times per year
                                                                7–11 times per year
                                                                                      O 3-4 times per week
                                                                                                                  -
      1–6 times per year
                            2 times per week
                                                                0 1 time per month
                                                                                      5-6 times per week
                            O 3–4 times per week
      7–11 times per year
                                                                2–3 times per month
                                                                                      1 time per day
      1 time per month
                             5–6 times per week
                                                                1 time per week
                                                                                      O 2 or more times per day
                                                                                                                  2–3 times per month
                            1 time per day
                                                               55a. How often were your tortillas or tacos corn
      1 time per week
                            O 2 or more times per day
                                                                    tortillas or tacos?
51. How often did you eat catsup?
                                                                     Almost never or never
                                                                     About <sup>1</sup>/<sub>4</sub> of the time
      NEVER
                                                                     About <sup>1</sup>/2 of the time
                                                                     O About <sup>3</sup>/4 of the time
      1–6 times per vear

    2 times per week

                                                                     Almost always or always
      O 7–11 times per year
                            O 3–4 times per week
                                                                                                                  0 1 time per month
                            ○ 5–6 times per week
                                                         56.
                                                               How often did you eat cooked dried beans (such as
                                                                                                                 -
      2-3 times per month
                            O 1 time per day
                            O 2 or more times per day
                                                               baked beans, pintos, kidney, blackeyed peas, lima,
                                                                                                                  -
      1 time per week
                                                               lentils, soybeans, or refried beans)? (Please don't
                                                                                                                  -
                                                               include bean soups or chili.)
                                                                                                                  _
    How often did you eat pickles or pickled
52.
                                                                                                                  _
      vegetables?
                                                               NEVER
                                                                                                                  _
                                                                                                                  _
      O NEVER
                                                                                      2 times per week
                                                               1–6 times per year
                                                                                                                  7–11 times per year
                                                                                      O 3-4 times per week
                                                                                                                  _
                                                                                                                  =
      1–6 times per year
                            2 times per week
                                                                1 time per month
                                                                                      5–6 times per week
                            🔘 3-4 times per week
      O 7–11 times per year
                                                                2–3 times per month
                                                                                      1 time per day
      1 time per month
                                                                                                                  _
                             ○ 5–6 times per week
                                                                1 time per week
                                                                                      O 2 or more times per day
      O 2–3 times per month
                           🔘 1 time per day
                                                                                                                  _
                                                                                                                  O 1 time per week
                            O 2 or more times per day
                                                         57. How often did you eat other kinds of vegetables?
                                                                                                                  -
                                                                                                                  53. How often did you eat stuffing, dressing, or
                                                               NEVER
      dumplings?
                                                                                                                  _
                                                                1–6 times per year
                                                                                      2 times per week
                                                                                                                  -
      NEVER
                                                                7–11 times per year 
0 3–4 times per week
                                                                                                                  _
                                                                1 time per month
                                                                                      5–6 times per week
                                                                                                                  _
      1–6 times per year
                            O 2 times per week
                                                                2–3 times per month
                                                                                      1 time per day
                            0 3-4 times per week
      7–11 times per year
                                                                                      O 2 or more times per day
                                                                1 time per week
                                                                                                                  _
      1 time per month
                             5–6 times per week
      O 2–3 times per month
                            1 time per day
                                                                                                                  _
                                                         58.
                                                               How often did you eat rice or other cooked
                            O 2 or more times per day
                                                                                                                  1 time per week
                                                               grains (such as bulgur, cracked wheat, or millet)?
                                                                                                                  -
                                                                                                                  _
                                                                                                                  =
54. How often did you eat chili?
                                                               NEVER (GO TO QUESTION 59)
                                                                                                                  O NEVER
                                                                1–6 times per year
                                                                                      2 times per week
                                                                                                                  7–11 times per year 

O 3–4 times per week
      1–6 times per vear
                            2 times per week
                                                                                      ○ 5–6 times per week
                                                                1 time per month
      7–11 times per year
                            O 3–4 times per week
                                                                O 2–3 times per month
                                                                                      1 time per day
      1 time per month
                            0 5–6 times per week
                                                                O 1 time per week
                                                                                      O 2 or more times per day
      2–3 times per month
                            O 1 time per day
      O 1 time per week
                            O 2 or more times per day
                                                                                                                  _
                                                                                                                  _
                                                                                                                  -
                                                                                                                  -
                                                         Question 59 appears on the next page.
                                                      11
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62.
                                                                       How often did you eat pasta salad or macaroni
Over the past 12 months ...
                                                                        salad?
       58a. How often was the rice or other cooked
                                                                       O NEVER
            grains you ate brown rice, cracked wheat,
            or millet?
                                                                        1–6 times per year
                                                                                                 2 times per week

    Almost never or never

                                                                        7–11 times per year
                                                                                                 O 3–4 times per week
             About <sup>1</sup>/4 of the time
                                                                        1 time per month
                                                                                                 5–6 times per week
             About 1/2 of the time
                                                                        2–3 times per month
                                                                                                 1 time per day
             About <sup>3</sup>/4 of the time
                                                                        1 time per week
                                                                                                 2 or more times per day
             Almost always or always
                                                                       Other than the pastas listed in Questions 60, 61, and
                                                                63.
59.
       How often did you eat pancakes, waffles, or French
                                                                        62, how often did you eat pasta, spaghetti, or other
                                                                        noodles?
       toast?
       O NEVER (GO TO QUESTION 60)
                                                                        NEVER (GO TO QUESTION 64)
       🔘 1–8 times per year
                                O 2 times per week
                                                                        🔵 1–8 times per year
                                                                                                 2 times per week
                                3–4 times per week
       7–11 times per year
                                                                        7–11 times per year
                                                                                                 O 3–4 times per week
                                                                        1 time per month
       1 time per month
                                ○ 5–6 times per week
                                                                                                 5–6 times per week
                                🔘 1 time per day
                                                                                                🔘 1 time per day
       2–3 times per month
                                                                        2–3 times per month
       1 time per week
                                O 2 or more times per day
                                                                        1 time per week
                                                                                                 O 2 or more times per day
       59a. How often was syrup added to your
                                                                        63a. How often did you eat your pasta, spaghetti, or
            pancakes, waffles, or French toast?
                                                                             other noodles with tomato sauce or spaghetti
                                                                             sauce made WITH meat?
             Almost never or never
             About <sup>1</sup>/4 of the time

    Almost never or never

                                                                             About <sup>1</sup>/4 of the time
             O About 1/2 of the time
             About <sup>3</sup>/4 of the time
                                                                             About <sup>1</sup>/<sub>2</sub> of the time

    Almost always or always

                                                                             About <sup>3</sup>/<sub>4</sub> of the time

    Almost always or always

60.
       How often did you eat lasagna, stuffed shells,
                                                                        63b. How often did you eat your pasta, spaghetti, or
       stuffed manicotti, ravioli, or tortellini? (Please do
                                                                             other noodles with tomato sauce or spaghetti
                                                                             sauce made WITHOUT meat?
       not include spaghetti or other pasta.)
       O NEVER
                                                                             Almost never or never
                                                                             About <sup>1</sup>/<sub>4</sub> of the time
       ○ 1–6 times per year
                                2 times per week
                                                                             About <sup>1</sup>/<sub>2</sub> of the time
       7–11 times per year
                                O 3–4 times per week
                                                                                About 3/4 of the time
                                                                                Almost always or always
        1 time per month
                                 🔘 5–6 times per week
       2–3 times per month
                                1 time per day
                                                                       63c. How often did you eat your pasta, spaghetti, or
       O 1 time per week
                                O 2 or more times per day
                                                                             other noodles with margarine, butter, oil, or
                                                                             cream sauce?
61.
       How often did you eat macaroni and cheese?
                                                                             Almost never or never
       NEVER
                                                                             O About <sup>1</sup>/<sub>4</sub> of the time
                                                                                About 1/2 of the time
       O 1–6 times per year
                                O 2 times per week
                                                                             About <sup>3</sup>/<sub>4</sub> of the time
                                O 3–4 times per week
       O 7–11 times per year
                                                                                Almost always or always
       1 time per month
                                5-6 times per week
       2–3 times per month
                                1 time per day
                                O 2 or more times per day
       1 time per week
                                                                 Question 64 appears on the next page.
                                                              12
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67. How often did you eat jam, jelly, or honey on Over the past 12 months ... bagels, muffins, bread, rolls, or crackers? How often did you eat bagels or English muffins? 64. O NEVER O NEVER 1-8 times per year O 2 times per week 1–6 times per year O 2 times per week ○ 7–11 times per year O 3-4 times per week O 3-4 times per week 7–11 times per year 1 time per month O 5−6 times per week ○ 5–6 times per week 1 time per month 2–3 times per month 1 time per day O 2–3 times per month O 1 time per day 1 time per week O 2 or more times per day O 2 or more times per day 1 time per week How often did you eat peanut butter or other 68. The next questions ask about your intake of breads nut butter? other than bagels or English muffins. First, we will ask about bread you ate as part of sandwiches only. Then O NEVER we will ask about all other bread you ate. \_ 1-6 times per year 2 times per week 65. How often did you eat breads or rolls AS PART OF O 3-4 times per week 7–11 times per year SANDWICHES (including burger and hot dog rolls)? 1 time per month ○ 5–6 times per week 1 time per day 2-3 times per month O NEVER (GO TO QUE STION 66) 1 time per week 2 or more times per day 1–6 times per year O 2 times per week How often did you eat roast beef or steak IN O 3-4 times per week 69. ○ 7–11 times per year ○ 5–6 times per week SANDWICHES? 1 time per month 2-3 times per month 1 time per day O NEVER 1 time per week 2 or more times per day 1–6 times per year 2 times per week 65a. How often were the breads or rolls that you used for your sandwiches white bread (including 7–11 times per year O 3-4 times per week burger and hot dog rolls)? 1 time per month ○ 5-6 times per week 2–3 times per month O 1 time per day Almost never or never 1 time per week O 2 or more times per day About 1/4 of the time O About 1/2 of the time 70. How often did you eat turkey or chicken COLD About 3/4 of the time Almost always or always CUTS (such as loaf, luncheon meat, turkey ham, turkey salami, or turkey pastrami)? (We will ask about other turkey or chicken later.) 66. How often did you eat breads or dinner rolls, NOT AS PART OF SANDWICHES? O NEVER O NEVER (GO TO QUESTION 67) 1-6 times per year 2 times per week O 3-4 times per week O 7–11 times per year 1-6 times per year 2 times per week ○ 5–6 times per week 1 time per month 7–11 times per year 3-4 times per week 2–3 times per month 1 time per day 1 time per month ○ 5–6 times per week 1 time per week O 2 or more times per day 2–3 times per month 1 time per day 1 time per week O 2 or more times per day 71. How often did you eat luncheon or deli-style ham? 66a. How often were the breads or rolls you ate (We will ask about other ham later.) white bread? NEVER (GO TO QUESTION 72) \_ Almost never or never About 1/4 of the time 1–8 times per year 2 times per week O 3-4 times per week O About 1/2 of the time 7–11 times per year About 3/4 of the time ○ 5–6 times per week 1 time per month Almost always or always 2–3 times per month O 1 time per day 1 time per week O 2 or more times per day Question 67 appears in the next column. Question 72 appears on the next page. 13

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Over the past 12 months ...
                                                              75.
                                                                     How often did you eat beef hamburgers or
                                                                     cheeseburgers?
       71a. How often was the luncheon or deli-style ham
            you ate light, low-fat, or fat-free?
                                                                      O NEVER (GO TO QUESTION 76)

    Almost never or never

                                                                      1–6 times per year
                                                                                              O 2 times per week
            O About <sup>1</sup>/<sub>4</sub> of the time
                                                                      7–11 times per year
                                                                                              O 3–4 times per week
            O About <sup>1</sup>/<sub>2</sub> of the time
                                                                      1 time per month
                                                                                              5-6 times per week
            O About <sup>3</sup>/<sub>4</sub> of the time
                                                                        2-3 times per month
                                                                                              1 time per day
            Almost always or always
                                                                      1 time per week
                                                                                              O 2 or more times per day
                                                                     75a. How often were the beef hamburgers or
      How often did you eat other cold cuts or luncheon
72.
                                                                           cheeseburgers you ate made with lean ground
       meats (such as bologna, salami, corned beef,
                                                                           beef?
       pastrami, or others, including low-fat)? (Please do not
       include ham, turkey, or chicken cold cuts.)
                                                                           Almost never or never
                                                                           About <sup>1</sup>/4 of the time
      NEVER (GO TO QUESTION 73)
                                                                           About 1/2 of the time
                                                                              About 3/4 of the time
       1–8 times per year
                               2 times per week

    Almost always or always

       🔘 7–11 times per year
                               O 3–4 times per week
       1 time per month
                               5-6 times per week
                                                              76.
                                                                     How often did you eat ground beef in mixtures
        2–3 times per month
                               O 1 time per day
       1 time per week
                               O 2 or more times per day
                                                                     (such as meatballs, casseroles, chili, or meatloaf)?
       72a. How often were the other cold cuts or luncheon
                                                                      O NEVER
            meats you ate light, low-fat, or fat-free?
                                                                                              2 times per week
            (Please do not include ham, turkey, or chicken
                                                                      1-6 times per year
            cold cuts.)
                                                                      7–11 times per year
                                                                                              O 3–4 times per week
                                                                      1 time per month
                                                                                              5-6 times per week

    Almost never or never

                                                                      2–3 times per month
                                                                                              O 1 time per day
             About <sup>1</sup>/<sub>4</sub> of the time
                                                                      1 time per week
                                                                                              O 2 or more times per day
             About 1/2 of the time
             About <sup>3</sup>/4 of the time
                                                              77.
                                                                     How often did you eat hot dogs or frankfurters?
            Almost always or always
                                                                     (Please do not include sausages or vegetarian
                                                                     hot dogs.)
73.
       How often did you eat canned tuna (including in
                                                                     NEVER (GO TO QUESTION 78)
       salads, sandwiches, or casseroles)?
       O NEVER
                                                                      1-6 times per year
                                                                                              O 2 times per week
                                                                      7–11 times per year
                                                                                              O 3–4 times per week
                               2 times per week
       1–8 times per vear
                                                                      1 time per month
                                                                                              🔘 5–8 times per week
       7–11 times per year
                               O 3–4 times per week
                                                                      2–3 times per month
                                                                                              O 1 time per day
       1 time per month
                               ○ 5–6 times per week
                                                                      1 time per week
                                                                                              O 2 or more times per day
       2–3 times per month
                               O 1 time per day
       1 time per week
                               O 2 or more times per day
                                                                     77a. How often were the hot dogs or frankfurters you
                                                                           ate light or low-fat hot dogs?
74.
       How often did you eat GROUND chicken or turkey?

    Almost never or never

       (We will ask about other chicken and turkey later.)
                                                                           O About <sup>1</sup>/4 of the time
                                                                             About 1/2 of the time
       NEVER
                                                                              About 3/4 of the time

    Almost always or always

       1-6 times per year
                               2 times per week
                               3–4 times per week
       🔿 7–11 times per year
       1 time per month
                               ○ 5–6 times per week
       2–3 times per month
                               1 time per day
                               O 2 or more times per day
       1 time per week
                                                               Question 78 appears on the next page.
                                                            14
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82. How often did you eat roast turkey, turkey cutlets, Over the past 12 months... or turkey nuggets (including in sandwiches)? 78 How often did you eat beef mixtures such as beef O NEVER stew, beef pot pie, beef and noodles, or beef and vegetables? 1–6 times per year 2 times per week O NEVER O 7-11 times per year O 3–4 times per week 1 time per month 0 5-6 times per week 1–6 times per year O 2 times per week 2–3 times per month 1 time per day 7–11 times per year O 3–4 times per week 1 time per week 2 or more times per day 1 time per month 5-6 times per week 2–3 times per month 1 time per day 83. How often did you eat chicken as part of O 1 time per week O 2 or more times per day salads, sandwiches, casseroles, stews, or other mixtures? How often did you eat roast beef or pot roast? 79. (Please do not include roast beef or pot roast in NEVER sandwiches.) 1–6 times per year 2 times per week NEVER 7–11 times per year O 3–4 times per week 1 time per month 5-6 times per week 🔿 1–6 times per year 2 times per week 2–3 times per month 1 time per day O 3–4 times per week 7–11 times per year O 2 or more times per day 1 time per week 1 time per month 5-6 times per week 2–3 times per month 🔘 1 time per day How often did you eat baked, broiled, roasted, O 1 time per week O 2 or more times per day 84. stewed, or fried chicken (including nuggets)? (Please do not include chicken in mixtures.) 80. How often did you eat steak (beef)? (Do not include O NEVER (GO TO QUESTION 85) steak in sandwiches) O NEVER (GO TO QUESTION 81) 1–6 times per vear 2 times per week 7–11 times per year O 3-4 times per week 2 times per week 5–6 times per week 1 time per month 1–6 times per year 7–11 times per year O 3–4 times per week 2-3 times per month 1 time per day 🔘 1 time per month 🔿 5–6 times per week 1 time per week 2 or more times per day O 2–3 times per month 1 time per day O 2 or more times per day 84a. How often was the chicken you ate fried 1 time per week chicken (including deep fried) or chicken 80a. How often was the steak you ate lean steak? nuggets? Almost never or never Almost never or never About <sup>1</sup>/4 of the time About <sup>1</sup>/4 of the time O About <sup>1</sup>/<sub>2</sub> of the time O About <sup>1</sup>/2 of the time O About <sup>3</sup>/<sub>4</sub> of the time O About <sup>3</sup>/4 of the time Almost always or always Almost always or always 84b. How often was the chicken you ate WHITE 81. How often did you eat pork or beef spareribs? meat? O NEVER Almost never or never O About <sup>1</sup>/<sub>4</sub> of the time O About <sup>1</sup>/2 of the time 1–6 times per year O 2 times per week 7–11 times per year O 3–4 times per week About <sup>3</sup>/4 of the time 1 time per month ○ 5–6 times per week Almost always or always 2–3 times per month O 1 time per day O 1 time per week O 2 or more times per day Question 85 appears on the next page. 15

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89. How often did you eat bacon (including low-fat)?
Over the past 12 months...
                                                                    O NEVER (GO TO QUE STION 90)
       84c. How often did you eat chicken WITH skin?

    Almost never or never

                                                                    1-6 times per year
                                                                                            2 times per week
             About 1/4 of the time
                                                                    O 7-11 times per year
                                                                                           O 3-4 times per week
             About 1/2 of the time
                                                                    1 time per month
                                                                                            ○ 5–6 times per week
             About <sup>3</sup>/<sub>4</sub> of the time
                                                                    2–3 times per month
                                                                                            1 time per day
                                                                                            O 2 or more times per day
             Almost always or always
                                                                    1 time per week
                                                                    89a. How often was the bacon you ate light, low-fat,
       How often did you eat baked ham or ham steak?
 85.
                                                                         or lean bacon?
       O NEVER
                                                                          Almost never or never
                                                                          About 1/4 of the time
       ○ 1–6 times per year
                               2 times per week
                                                                          About 1/2 of the time
        ○ 7–11 times per year
                               O 3-4 times per week
                                                                          About <sup>3</sup>/<sub>4</sub> of the time
        O 1 time per month
                                  5-6 times per week
                                                                          Almost always or always
        2–3 times per month
                               1 time per day
        1 time per week
                               O 2 or more times per day
                                                             90.
                                                                    How often did you eat sausage (including low-fat)?
                                                                    O NEVER (GO TO QUE STION 91)
 86.
       How often did you eat pork (including chops, roasts,
       and in mixed dishes)? (Please do not include ham,
                                                                    1-6 times per year
                                                                                            2 times per week
       ham steak, or sausage.)
                                                                    ○ 7–11 times per year
                                                                                           O 3-4 times per week
                                                                    1 time per month
                                                                                            🔿 5-6 times per week
       O NEVER
                                                                    2-3 times per month
                                                                                           1 time per day
                                                                                            O 2 or more times per day
                                                                    1 time per week
        🔘 1-0 times per year
                               2 times per week
                                                                    90a. How often was the sausage you ate light,
        O 7-11 times per year
                                  3-4 times per week
                               0
                                  5-6 times per week
                                                                         low-fat, or lean sausage?
        1 time per month
        2–3 times per month
                               1 time per day
                                                                         Almost never or never
        1 time per week
                               O 2 or more times per day
                                                                         O About 1/4 of the time
                                                                          About 1/2 of the time
       How often did you eat gravy on meat, chicken,
87.
                                                                          About <sup>3</sup>/<sub>4</sub> of the time
       potatoes, rice, etc.?
                                                                         Almost always or always
       O NEVER
                                                             91.
                                                                    How often did you eat smoked fish or seafood (such
                                                                    as smoked salmon, lox, or others)?
        1-0 times per year
                                2 times per week
                                                                    O NEVER
        O 7-11 times per year
                                  3-4 times per week
        1 time per month
                                  5-6 times per week
                               1 time per day
        2-3 times per month
                                                                    ○ 1–8 times per year
                                                                                            2 times per week
                                                                                           O 3-4 times per week
        1 time per week
                               O 2 or more times per day
                                                                    7–11 times per year
                                                                                            ○ 5–6 times per week
                                                                    1 time per month
                                                                    2-3 times per month
                                                                                            1 time per day
 88.
       How often did you eat liver (all kinds) or liverwurst?
                                                                    1 time per week
                                                                                            O 2 or more times per day
       O NEVER
                                                             92. How often did you eat sushi?
                               2 times per week
       1-6 times per year
                                                                    NEVER (GO TO QUE STION 93)
        7–11 times per year
                               O 3–4 times per week
        O 1 time per month
                                  5-6 times per week
                                                                                            2 times per week
        O 2-3 times per month
                               O 1 time per day
                                                                    1-6 times per year
                                                                                            O 3–4 times per week
        1 time per week
                                2 or more times per day
                                                                    7–11 times per year
                                                                    1 time per month
                                                                                            ○ 5–6 times per week
                                                                                           O 1 time per day
                                                                    2-3 times per month
                                                                    1 time per week
                                                                                            O 2 or more times per day
                                                             Question 93 appears on the next page.
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Over	the past 12 months		97.	Over	the past 12 months, did	you eat soups?
	92a. How often did the fish or seafood (i	sushi you ate contain raw ncluding shellfish)?	Г	-0 N	O (GO TO QUESTION 98)	
	O Almost never or	never		J Y	ES	=
	O About 1/4 of the t	ime		Y		
	O About 1/2 of the t	ime		97a.	How often did you eat a WINTED?	SOUD DURING THE
	About <sup>2</sup> /4 of the t	ime ralwavs			WINTER:	
	0				O NEVER	-
93.	How often did you eat r	aw ovsters, raw clams, or			○ 1–6 times per winter	O 2 times per week
	other raw fish (not incl	uding raw fish in sushi)?			0 7-11 times per winter	0 3-4 times per week
					1 time per month	5–6 times per week
	NEVER				2–3 times per month	1 time per day
	○ 1_8 times per year	2 times per week			1 time per week	2 or more times
	7–11 times per year	3-4 times per week				per day
	0 1 time per month	0 5-6 times per week		97b.	How often did you eat a	OUD DURING THE
	2–3 times per month	O 1 time per day			REST OF THE YEAR?	. –
	1 time per week	2 or more times per day				-
					O NEVER	
94.	How often did you eat f	ish sticks or fried fish			🔵 1–6 times per year	2 times per week
	(including fried seafood	or shellfish)?			7–11 times per year	3–4 times per week
	C 1151 (5.5				1 time per month	5–6 times per week
	O NEVER				2-3 times per month	0 1 time per day
	○ 1–6 times per year	2 times per week			1 time per week	2 or more times
	O 7–11 times per year	O 3-4 times per week				per day
	0 1 time per month	5-6 times per week		97c.	How often were the sou	ips you ate bean soups? -
	2–3 times per month	<ul> <li>1 time per day</li> </ul>				-
	1 time per week	2 or more times per day			Almost never or never	-
					O About 1/4 of the time	
95.	How often did vou eat a	all other fish or seafood			About 1/2 of the time	
	(including shellfish) that	was NOT FRIED, SMOKED,			<ul> <li>Almost always or always</li> </ul>	ys –
	or RAW ?					-
				97d.	How often were the sou	ips you ate cream
	ONEVER				soups (including chowe	aers)?
	🔾 1–6 times per year	2 times per week			O Almost never or never	-
	O 7–11 times per year	3-4 times per week			O About 1/4 of the time	-
	0 1 time per month	0 5-6 times per week			About 1/2 of the time	-
	1 time per week	2 or more times per day			About %4 of the time	
	- I time per trees	C a of more times per day			U ranos anays or ana	-
				97e.	How often were the sou	ips you ate tomato or
96.	How often did you eat t	ofu, soy burgers, or soy			vegetable soups?	-
	meat-substitutes r				Almost never or never	
	O NEVER				O About 1/4 of the time	
	-				O About 1/2 of the time	)
	1–6 times per year	2 times per week			O About 3/4 of the time	-
	O 7–11 times per year	3–4 times per week			Almost always or always	ys 👘
	0 1 time per month	0 5-6 times per week				
	1 time per week	2 or more times per day				- and the second
	<ul> <li>I mus bei week</li> </ul>	<ul> <li>z or more unles per day</li> </ul>				
			Y			
			Ques	tion 98	appears on the next pag	e.
		■ 1	1			

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102. How often did you eat potato chips (including

    Over the past 12 months...

                                                                          low-fat, fat-free, or low-salt)?
97f. How often were the soups you ate broth soups
           (including chicken) with or without noodles or rice?
                                                                          O NEVER (GO TO QUESTION 103)

    Almost never or never

                                                                                                   O 2 times per week
                                                                           1–6 times per vear
                O About <sup>1</sup>/4 of the time
                                                                           🔿 7–11 times per year
                                                                                                   O 3–4 times per week

    1 time per month
    5-8 times per week
    2-3 times per month
    1 time per day

About <sup>1</sup>/2 of the time
-
                 About <sup>3</sup>/4 of the time

    Almost always or always

                                                                           1 time per week
                                                                                                   O 2 or more times per day
_
                                                                          102a. How often were the potato chips you ate
_
    98. How often did you eat pizza?
                                                                                 low-fat or fat-free chips?
NEVER (GO TO QUESTION 99)

    Almost never or never

           1–8 times per year
                                    O 2 times per week
                                                                                O About 1/4 of the time
                                  3–4 times per week
           ○ 7–11 times per year
                                                                                O About <sup>1</sup>/<sub>2</sub> of the time
           🔾 1 time per month
                                     🔵 5–6 times per week
                                                                                 About <sup>3</sup>/4 of the time
O 2–3 times per month O 1 time per day
                                                                                O Almost always or always
                                    O 2 or more times per day
           1 time per week
98a. How often did you eat pizza with pepperoni,
                                                                    103. How often did you eat tortilla chips or corn chips
             sausage, or other meat?
                                                                          (including low-fat, fat-free, or low-salt)?

    Almost never or never

                                                                           O NEVER (GO TO QUESTION 104)
              O About <sup>1</sup>/<sub>4</sub> of the time
              About <sup>1</sup>/2 of the time
                                                                           🔵 1–8 times per year
                                                                                                   O 2 times per week
                                                                                                   0 3-4 times per week
              About <sup>3</sup>/<sub>4</sub> of the time
                                                                           7–11 times per year
                                                                                                   O 5−6 times per week
                                                                           1 time per month

    Almost always or always

                                                                             2-3 times per month
                                                                                                   O 1 time per day
                                                                           1 time per week
                                                                                                   O 2 or more times per day
    99.
          How often did you eat crackers?
                                                                          103a. How often were the tortilla or corn chips you
           O NEVER
                                                                                ate low-fat or fat-free chips?
○ 1–6 times per year
                                    O 2 times per week

    Almost never or never

                                   O 3–4 times per week
                                                                                O About 1/4 of the time
           7–11 times per vear
           O 1 time per month
                                    ○ 5–6 times per week
                                                                                O About <sup>1</sup>/2 of the time
           2–3 times per month
                                    O 1 time per day
                                                                                O About <sup>3</sup>/<sub>4</sub> of the time
                                    O 2 or more times per day

    Almost always or always

           O 1 time per week
100. How often did you eat corn bread or corn muffins?
                                                                   104. How often did you eat popcorn (including low-fat)?
           NEVER
                                                                           NEVER
           1–8 times per year
                                    O 2 times per week
                                                                           1–6 times per year
                                                                                                   O 2 times per week
           O 7–11 times per year
                                    0 3-4 times per week
                                                                           7–11 times per year
                                                                                                   O 3–4 times per week
                                                                           1 time per month
                                                                                                    🔵 5–6 times per week
           1 time per month
                                    5–6 times per week
O 2–3 times per month
                                   1 time per day
                                                                           2–3 times per month
                                                                                                   O 1 time per day
                                    O 2 or more times per day
                                                                                                   O 2 or more times per day
           1 time per week
                                                                           1 time per week
101. How often did you eat biscuits?
                                                                    105. How often did you eat pretzels?
NEVER
                                                                          NEVER
           🔘 1–6 times per year
                                    O 2 times per week
                                                                           1–6 times per year
                                                                                                   O 2 times per week
           0 7-11 times per year
                                   O 3–4 times per week
                                                                           7–11 times per year
                                                                                                   O 3-4 times per week
                                    ○ 5–6 times per week
                                                                                                   ○ 5–6 times per week
           1 time per month
                                                                           1 time per month
           2–3 times per month
                                    O 1 time per day
                                                                           2–3 times per month
                                                                                                   O 1 time per day
                                    O 2 or more times per day
                                                                           1 time per week
                                                                                                   O 2 or more times per day
           1 time per week
                                                                 18
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How often was the cheese you ate light or Over the past 12 months ... 110a. low-fat cheese? \_ 106. How often did you eat peanuts, walnuts, seeds, or other nuts? Almost never or never About <sup>1</sup>/<sub>4</sub> of the time \_ O NEVER About 1/2 of the time About <sup>3</sup>/<sub>4</sub> of the time 1–6 times per year O 2 times per week Almost always or always 7–11 times per year O 3–4 times per week 1 time per month ○ 5–6 times per week 111. How often did you eat frozen yogurt, sorbet, or 2–3 times per month O 1 time per day ices (including low-fat or fat-free)? -O 1 time per week O 2 or more times per day O NEVER -\_ = 107. How often did you eat granola bars? 🔘 1–6 times per year 2 times per week 7–11 times per year 3-4 times per week NEVER 1 time per month ○ 5–6 times per week \_ 2–3 times per month O 1 time per day -2 times per week 1–6 times per year 1 time per week O 2 or more times per day \_ 7–11 times per year O 3–4 times per week \_ 1 time per month ○ 5–6 times per week \_ 2–3 times per month 1 time per day 112. How often did you eat ice cream, ice cream bars, or sherbet (including low-fat or fat-free)? O 2 or more times per day 1 time per week -NEVER (GO TO QUESTION 113) \_ 108. How often did you eat yogurt (NOT including frozen yogurt)? 1–6 times per year 2 times per week \_ 🔘 3-4 times per week 7–11 times per year \_ O NEVER 1 time per month 5-6 times per week \_ 2-3 times per month O 1 time per day \_ \_ 1–6 times per year O 2 times per week O 1 time per week O 2 or more times per day -7–11 times per year O 3–4 times per week 112a. How often was the ice cream you ate light, -1 time per month 🔘 5–6 times per week low-fat, or fat-free ice cream or sherbet? -2–3 times per month 1 time per day -1 time per week O 2 or more times per day Almost never or never About <sup>1</sup>/<sub>4</sub> of the time -109. How often did you eat cottage cheese \_ About <sup>1</sup>/<sub>2</sub> of the time About 3/4 of the time (including low-fat)? \_ -Almost always or always NEVER 113. How often did vou eat pudding or custard? 1–6 times per year O 2 times per week -7–11 times per year 3–4 times per week O NEVER -\_ 1 time per month O 5–6 times per week 2–3 times per month 1 time per day 1–6 times per year O 2 times per week 🔿 7–11 times per year O 3-4 times per week -1 time per week 2 or more times per day 1 time per month ○ 5–6 times per week \_ -O 2–3 times per month O 1 time per day How often did you eat cheese (including low-fat; \_ O 1 time per week O 2 or more times per day including on cheeseburgers or in sandwiches or subs)? --NEVER (GO TO QUESTION 111) 114. How often did you eat cake (including low-fat or fat-free)? -1–6 times per year O 2 times per week \_ 7–11 times per year O 3–4 times per week NEVER 🔵 1 time per month ○ 5–6 times per week -O 2–3 times per month O 1 time per day 1–6 times per year O 2 times per week -O 3-4 times per week O 7–11 times per year \_ 1 time per week O 2 or more times per day -1 time per month ○ 5-6 times per week O 2-3 times per month O 1 time per day -Question 111 appears in the next column. \_ 1 time per week O 2 or more times per day 19 

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119a. How often was the pie you ate fruit pie (such
   Over the past 12 months ...
                                                                              as apple, cherry, peach, blueberry, or others)?
    115.
          How often did you eat cookies or brownies
           (including low-fat or fat-free)?
                                                                             Almost never or never
                                                                             About <sup>1</sup>/4 of the time
           NEVER
                                                                              About <sup>1</sup>/<sub>2</sub> of the time
                                                                             About <sup>3</sup>/<sub>4</sub> of the time
           1–6 times per year
                                   O 2 times per week

    Almost always or always

           7–11 times per year
                                   O 3–4 times per week
                                   5–6 times per week
           1 time per month
                                                                 120. How often did you eat chocolate candy?
           2–3 times per month
                                   O 1 time per day
                                   O 2 or more times per day
           1 time per week
                                                                        NEVER
116. How often did you eat doughnuts, sweet rolls,
                                                                        1–6 times per year
                                                                                                2 times per week
           Danish, or pop-tarts?
                                                                        7–11 times per year
                                                                                                O 3–4 times per week
                                                                        1 time per month
                                                                                                5-6 times per week
           O NEVER
                                                                        2–3 times per month
                                                                                                1 time per day
                                                                        1 time per week
                                                                                                O 2 or more times per day
           ○ 1–6 times per year
                                   2 times per week
           7–11 times per year
                                   O 3–4 times per week
                                                                 121. How often did you eat other candy?
           1 time per month
                                   🔿 5–6 times per week
           2–3 times per month
                                   1 time per day
                                                                        NEVER
           1 time per week
                                   O 2 or more times per day
                                                                        1-6 times per year
                                                                                                O 2 times per week
                                                                                                O 3–4 times per week
    117. How often did you eat sweet muffins or
                                                                        7–11 times per year
                                                                        1 time per month
           dessert breads (including low-fat or fat-free)?
                                                                                                5–6 times per week
                                                                        2–3 times per month
                                                                                                1 time per day
           O NEVER
                                                                        1 time per week
                                                                                                O 2 or more times per day
           ○ 1-6 times per year
                                   2 times per week
                                  O 3–4 times per week
           7–11 times per year
                                                                 122. How often did you eat eggs, egg whites, or egg
                                                                        substitutes (NOT counting eggs in baked goods and
                                   ○ 5–6 times per week
           1 time per month
                                                                        desserts)? (Please include eggs in salads, quiche,
           2–3 times per month
                                   1 time per day
                                                                        and souffles.)
           1 time per week
                                   2 or more times per day
                                                                        O NEVER (NO TO QUESTION 123)
    118. How often did you eat fruit crisp, cobbler, or
           strudel?
                                                                        1–6 times per year
                                                                                                O 2 times per week
                                                                                                O 3–4 times per week
                                                                        7–11 times per year
           NEVER
                                                                        1 time per month
                                                                                                5-6 times per week
                                                                        2–3 times per month
                                                                                                O 1 time per day
           ○ 1–6 times per year
                                   2 times per week
                                                                        1 time per week
                                                                                                O 2 or more times per day
           7–11 times per year
                                  O 3–4 times per week
           1 time per month
                                   5–6 times per week
                                                                        122a. How often were the eggs you ate egg
                                                                              substitutes?
           2–3 times per month
                                  O 1 time per day
           1 time per week
                                   O 2 or more times per day
                                                                             Almost never or never
                                                                              About <sup>1</sup>/4 of the time
   119. How often did you eat pie?
                                                                             O About <sup>1</sup>/<sub>2</sub> of the time
                                                                             O About <sup>3</sup>/<sub>4</sub> of the time
           O NEVER (GO TO QUESTION 120)

    Almost always or always

                                   2 times per week
           1-8 times per year
                                   O 3–4 times per week
           🔿 7–11 times per year
           1 time per month
                                   5–6 times per week
           2–3 times per month
                                   1 time per day
                                   O 2 or more times per day
           1 time per week
    Question 120 appears in the next column.
                                                                 Question 123 appears on the next page.
```

124a. How often was the iced tea you drank Over the past 12 months ... decaffeinated or herbal tea? \_ 122b. How often were the eggs you ate egg whites only? Almost never or never About <sup>1</sup>/<sub>4</sub> of the time O About 1/2 of the time Almost never or never About <sup>1</sup>/<sub>4</sub> of the time O About <sup>3</sup>/<sub>4</sub> of the time About <sup>1</sup>/<sub>2</sub> of the time Almost always or always About <sup>3</sup>/<sub>4</sub> of the time Almost always or always 125. How many cups of HOT tea, caffeinated or 122c. How often were the eggs you ate regular decaffeinated, did you drink? whole eggs? O NONE (GO TO QUESTION 126) Almost never or never ○ 5–6 cups per week About <sup>1</sup>/<sub>4</sub> of the time Less than 1 cup per month About <sup>1</sup>/<sub>2</sub> of the time. 1 cup per day 1–3 cups per month About <sup>3</sup>/<sub>4</sub> of the time O 2–3 cups per day Almost always or always 1 cup per week 4-5 cups per day O 2–4 cups per week O 6 or more cups per day 122d. How often were the eggs you ate part of egg salad? 125a. How often was the hot tea you drank decaffeinated or herbal tea? Almost never or never Almost never or never About <sup>1</sup>/<sub>4</sub> of the time About <sup>1</sup>/<sub>2</sub> of the time About <sup>1</sup>/4 of the time About <sup>3</sup>/<sub>4</sub> of the time About 1/2 of the time Almost always or always About 3/4 of the time Almost always or always 123. How many cups of coffee, caffeinated or decaffeinated, did you drink? 126. How often did you add sugar or honey to your O NONE (GO TO QUESTION 124) coffee or tea? Less than 1 cup per ○ 5–6 cups per week NEVER 1 cup per day month 1–3 cups per month O 2–3 cups per day C Less than 1 time per 5-6 times per week \_ 1 time per day month 1 cup per week 4–5 cups per day \_ 2–4 cups per week 6 or more cups per day 1–3 times per month 2-3 times per day 1 time per week 4-5 times per day \_ 123a. How often was the coffee you drank d√s−4 times per week O 6 or more times per day decaffeinated? \_ -Almost never or never 127. How often did you add artificial sweetener to \_ your coffee or tea? About <sup>1</sup>/<sub>4</sub> of the time \_ About <sup>1</sup>/<sub>2</sub> of the time \_ -O NEVER About <sup>3</sup>/<sub>4</sub> of the time \_ Almost always or always Less than 1 time per \_ 5–6 times per week month 1 time per day How many glasses of ICED tea, caffeinated or 124. 1–3 times per month -2-3 times per day decaffeinated, did you drink? 1 time per week 4–5 times per day \_ O NONE (GO TO QUESTION 125) 2–4 times per week O 6 or more times per day = Less than 1 cup per ○ 5–6 cups per week 🔵 1 cup per day month O 1–3 cups per month O 2–3 cups per day 1 cup per week 4–5 cups per day O 2–4 cups per week O 6 or more cups per day Question 125 appears in the next column. 21

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131. How often was sugar or honev added to foods you
   Over the past 12 months ...
                                                                      ate? (Please do not include sugar in coffee, tea,
                                                                      other beverages, or baked goods.)
    How often was non-dairy creamer added to your
coffee or tea?
                                                                      O NEVER
_
          NEVER (GO TO QUESTION 129)
                                                                      🔿 1–6 times per year
                                                                                             2 times per week
_
_
          C Less than 1 time per
                                    5-6 times per week
                                                                      🔿 7–11 times per year
                                                                                             O 3–4 times per week
                                     🔿 1 time per day
                                                                      1 time per month
                                                                                              🔿 5–6 times per week
_
             month
          1–3 times per month
                                    O 2–3 times per day
                                                                      2–3 times per month
                                                                                             1 time per day
🔿 1 time per week
_
                                    O 4-5 times per day
                                                                      O 1 time per week
                                                                                             O 2 or more times per day
          2–4 times per week
                                    O 6 or more times per day
_
          128a. What kind of non-dairy creamer did you
                                                               132. How often did you eat margarine on breads, bagels,
                usually use?
                                                                      English muffins, other muffins, pancakes, or waffles?
_
_
                Regular powdered
                                                                      O NEVER (GO TO QUESTION 133)
_

    Low-fat or fat-free powdered

🔘 Regular liquid
_
                                                                      🔵 1–8 times per year
                                                                                             2 times per week
                                                                                             O 3–4 times per week
_
                C Low-fat or fat-free liquid
                                                                      0 7–11 times per year
                                                                                             ○ 5–6 times per week
1 time per month
2–3 times per month
                                                                                             O 1 time per day
   129.
          How often was cream or half and half added to
1 time per week
                                                                                             O 2 or more times per day
          vour coffee or tea?
132a. How often was the margarine you ate on
_
          NEVER
                                                                            these breads low-fat or fat-free?
_
          C Less than 1 time per
                                    ○ 5–6 times per week
                                                                           Almost never or never
_
             month
                                     🔿 1 time per day
                                                                           About <sup>1</sup>/4 of the time
O 1–3 times per month
                                    O 2-3 times per day
                                                                           About <sup>1</sup>/2 of the time
_
          1 time per week
                                    O 4–5 times per day
                                                                           About <sup>3</sup>/4 of the time
_
          O 2–4 times per week
                                    O 6 or more times per day
                                                                           Almost always or always
_
_
   130. How often was milk added to your coffee or tea?
                                                               133.
                                                                      How often did you eat butter on breads, bagels,
                                                                      English muffins, other muffins, pancakes, or waffles?
_
          O NEVER (GO TO QUESTION 131)
_
                                                                      O NEVER (GO TO QUESTION 134)
C Less than 1 time per
                                    5-6 times per week
month
                                                                      1–6 times per year
                                                                                             2 times per week
                                     1 time per day
1–3 times per month
                                    2–3 times per day
                                                                      7–11 times per year
                                                                                             O 3–4 times per week
           1 time per week
                                    4-5 times per day
                                                                      1 time per month
                                                                                             5–6 times per week
_
          O 2–4 times per week
                                    O 6 or more times per day
                                                                      2–3 times per month
                                                                                             O 1 time per day
_
                                                                      1 time per week
                                                                                             O 2 or more times per day
_
          130a. What kind of milk was usually added to
                                                                      133a. How often was the butter you ate on these
your coffee or tea?
breads low-fat or fat-free?
_
                Whole milk
                🔾 2% milk
Almost never or never
1% milk
                                                                           About <sup>1</sup>/4 of the time
Skim, nonfat, or 1/2% milk
                                                                           About 1/2 of the time
                                                                           About <sup>3</sup>/4 of the time
                  Evaporated or condensed (canned) milk
0
                  Soy milk
                                                                           Almost always or always
Rice milk
                O Raw, unpasteurized milk
_
                O Other
_
_
_
_
   Question 131 appears in the next column.
                                                               Question 134 appears on the next page.
_
```

Over the past 12 months ... 136. How often did you eat mayonnaise as a spread or as part of food mixtures? How often did you eat margarine on potatoes. O NEVER (GO TO QUESTION 137) cooked vegetables, rice, grains, or beans? -O NEVER (GO TO QUESTION 135) 2 times per week 1–6 times per year -🔿 7–11 times per year O 3–4 times per week 1–6 times per year 2 times per week 1 time per month 5-6 times per week -O 3–4 times per week 7–11 times per year 2–3 times per month 1 time per day -1 time per month 5-6 times per week O 2 or more times per day 1 time per week -O 2–3 times per month O 1 time per day \_ 136a. How often was the mayonnaise you ate low-fat = 1 time per week O 2 or more times per day or fat-free? -134a. How often was the margarine you ate on these cooked potatoes, cooked vegetables, rice, Almost never or never grains, or beans low-fat or fat-free? O About <sup>1</sup>/<sub>4</sub> of the time -About <sup>1</sup>/<sub>2</sub> of the time -Almost never or never About <sup>3</sup>/<sub>4</sub> of the time About <sup>1</sup>/<sub>4</sub> of the time -Almost always or always About <sup>1</sup>/<sub>2</sub> of the time -About <sup>3</sup>/4 of the time -137. How often did you eat cream cheese? Almost always or always -NEVER (GO TO QUESTION 138) 135. How often did you eat butter on potatoes, cooked vegetables, rice, grains, or beans? 1–6 times per year 2 times per week -O 7-11 times per year O 3–4 times per week -O NEVER (GO TO QUESTION 136) ○ 5–6 times per week 1 time per month -O 2–3 times per month O 1 time per day -2 times per week 🔿 1–6 times per year O 1 time per week O 2 or more times per day 0 3-4 times per week 7–11 times per year -1 time per month 5–6 times per week 137a. How often was the cream cheese you ate -O 2–3 times per month O 1 time per day low-fat or fat-free? 1 time per week O 2 or more times per day Almost never or never -135a. How often was the butter you ate on these About <sup>1</sup>/<sub>4</sub> of the time cooked potatoes, cooked vegetables, rice, O About 1/2 of the time grains, or beans low-fat or fat-free? O About <sup>3</sup>/<sub>4</sub> of the time -Almost always or always -Almost never or never O About 1/4 of the time -O About <sup>1</sup>/2 of the time O About <sup>3</sup>/<sub>4</sub> of the time -Almost always or always -1 ---------Question 136 appears in the next column. Question 138 appears on the next page. -

Over the past 12 months	139. How often did you eat foods with oils added or with oils used in cooking (do not include baked goods or
138. How often did you eat sour cream?	salads)?
O NEVER (GO TO QUESTION 139)	O NEVER
<ul> <li>1-8 times per year</li> <li>7-11 times per year</li> <li>3-4 times per week</li> <li>3-4 times per week</li> <li>5-6 times per week</li> <li>1 time per day</li> <li>2 or more times per day</li> </ul> 138a. How often was the sour cream you ate low-fat or fat-free? <ul> <li>Almost never or never</li> <li>About <sup>1</sup>/4 of the time</li> <li>About <sup>1</sup>/4 of the time</li> <li>About <sup>1</sup>/4 of the time</li> <li>About <sup>3</sup>/4 of the time</li> <li>Almost always or always</li> </ul>	<ul> <li>1-8 times per year</li> <li>7-11 times per year</li> <li>3-4 times per week</li> <li>3-4 times per week</li> <li>2-3 times per month</li> <li>1 time per day</li> <li>1 time per week</li> <li>2 or more times per day</li> </ul> 139a. What kind of oils do you usually eat? (Mark all that apply.) <ul> <li>Olive</li> <li>Corn</li> <li>Canola/rapeseed</li> <li>Other</li> </ul>
	1

Thank you <u>very much</u> for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- Did not skip any pages,
- · Completely blackened-in each answer, and
- · Completely erased any changes you may have made.

Appendix D
## 24-Hour Dietary Recall

DAY: 1 2 3 4 DATE:/	<u>/ DA</u>	Y OF WEEK:	TIME AT START OF	RECALL:	hrs	(2 <u>4 hour c</u>	lock	Universi	ity of Landa
3 Quick list	Time Place	Description of food or drin	k Brand	Amount (P/H/W)	Leftovers (P/H/W)	Food code	P/ H/ W	Portion code	Ν
								L	
								<b> </b>	
							$\vdash$		<u> </u>
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#### **Nutrition Education Specifically for Intervention Group**

In this study, a plant-based diet will include a minimum of 4 cups of vegetables, 3 cups of fruit, 6 ounces of whole grains, 3 cups of dairy including soy products, 1 serving of iron-fortified foods, and 5.5 ounces of protein with more than half of the protein coming from plant-based food sources, focusing on pairing non-heme iron with enhancers.

According to Johnson-Wimbley and Graham (2011), ascorbic acid, citrate, and amino acids from meat and fish enhance the absorption of non-heme iron. Plant phytates found in legumes, rice and grains, tannins found in berries, tea and wine, calcium found in dairy products, tofu and leafy greens, polyphenols found in fruits, vegetables, dry legumes and chocolate, iron overload, and antacids (which decrease stomach acidity) inhibit the absorption of non-heme iron. These inhibitors of non-heme iron will be educated by the research dietitian to be avoided when consuming food sources high in non-heme iron.

#### **Good Sources of Plant-Based Iron:**

• Lentils, chickpeas, beans, tofu, cashew nuts, chia seeds, pumpkin seeds, hemp seeds, leafy greens, tomato paste, potatoes, mushrooms, prune juice, mulberries, amaranth, spelt, oats, quinoa, dark chocolate

#### How to Increase Iron Absorption:

- Eat vitamin C rich foods with high iron foods
- Avoid coffee and tea with iron rich foods
- Soak, sprout and ferment grains and legumes

#### **Meal Ideas:**

- Kiwi with lentil curry
- Broccoli with a tofu stir-fry

• Pepper to a bean chili or green salad

### **Recipe Ideas:**

https://www.onegreenplanet.org/vegan-food/plant-based-iron-recipes/

https://www.verywellhealth.com/iron-rich-vegetarian-meals-4143255

https://www.foodnetwork.com/healthyeats/healthy-tips/2013/06/plant-based-sources-of-iron

#### Nutrition Education Handouts for Intervention and Control Group



# Nutrition for

Follow these winning strategies to help you eat and drink your way to a peak performance

unning requires a mindful approach to food, since everything we drink and eat has a direct impact on our performance and even our enjoy-ment of the sport. It's hard to love being a runner when you're bonking. So we compiled the best advice on how to fuel your runs—including the finest energy sources, smartest hydration strategies, ideal timing techniques, and weight loss tips. Whether you're looking to uncork your perfor-



## **Fuel Rules**

Running keeps you fit. But to lose weight and run your best, focus on what you eat

EAT REAL FOOD Convenience foods have their place: Energy chews during a run or a bottled smoothle afterward provide fast, nutritious fuel. But the bulk of a runner's diet should consist of whole foods. Fish, chicken, vegetables, whole grains, nuts, low-fat dairy, fruit-these healthy staples provide more nutritional value than highly processed options. Plus, preparing meals from real-food sources gives you more control over your sodium, fat, and calorie intake.

#### CHOOSE QUALITY CARBS

Because they fuel workouts and nourish apent muscles, carbs should be the backhone of a runner's diet. But some carbs deliver greater value than others. Make most of your carbs whole rains, fruits, and vegetables. And remember: The less processing a plant receives, the more nutritious it is (think potatoes, not potato chips);

3 WRITE IT DOWN Wite down everything you sat and drink for several days to evaluate your eating habits. Are you snacking more than you realize? Reaching for sweets too often? Keep a ledger to identify areas. where there's room for improvement.

#### INDULGE ON OCCASION

Allow yourself the occasional dessert or cocktail to satisfy cravings and keep those urges from becoming binges. Just keep an eye on portions and frequency.

#### DRINK DELIBERATELY

Fluids are an essential part of any runner's fueling plan: By staying hydrated, you'll boost performance and minimize nuisances like GI distress. But watch the calories: Drinks that are high in sugar can contribute to weight gain. Limit fruit juice, pass on soft drinks, and switch your morning mocha to a cup of tea or coffee.



 $\rightarrow$ 

## TRAINING GUIDE

#### RUNNER'S PANTRY

Stock up on these essentials to ensure an at-the-ready supply of run-fueling foods

#### GRAINS

Sure, pasta's a grain—but it's highly processed, so round it out with unrefined grains such as bulgur, which cooks fast and makes great salads and breakfast cereal. Brown and wild rice provide variety and fiber. Try quinoa, one of the fee sources of complete protein. And don't overlook oats.

#### OLIVE OIL

Choose extra-virgin, which is less procased than other types. Its monoursaturated fat has been shown to lower "bad" cholesterol and improve heart health. Drizzle it over salads, potatoes, pasta.

#### BEANS

Beans are cheap, low in fat, and high in protein, iron, and fiber. Eat beans as side dishes, add them to saladis, and stir them into pasta sauces (where they make a healthy alternative to meat).

#### FRESH HERBS

They elevate other healthy foods from so-so to sensational. Mint freshens up salada, potatoes, even herwages. Basil enhances beans and tomatoes. Rub rosemary into chicken or samon.

#### EGGS

Packed with protein, eggs are inexpensive-and stay fresh for weeks.

LONG-KEEPING VEGETABLES AND FRUITS

Carrots, kale, zucchini, and lemons keep for a week or more; potatoes, onions, and garlic last even longer. Buy frozen spinach and corn to enjoy these fast-fading veggies anytime.

#### CANNED TOMATOES

Indispensable for making superfast sauces for pasta or chicken.

#### ORIED FRUIT AND NUTS

Having these healthy snacks on hand keeps you from oversating at meals. They also make tasty add-ins for salads and grain-based side dishes.



## **Eat This Now**

Eat seasonally for maximum flavor and nutritional value

F000	SEASON	WHAT YOU GET
Rhubarb	March- June	Vitamins C and K. A 26-calorie serving (one cup) also delivers minerals such as calcium, potassium, and manganese, which helps turn protein and carbohydrates into energy.
Pineapple	March- June	Invmunity-hoosting antioxidants. Get your Daily Value of vita- min C in just one cup, along with bromelain—an enzyme that fights inflammation, aids digestion, and reduces swelling.
Arugula	April- October	Bone-strengthening nutrients. One cup contains 2B percent of your Daily Value for vitamin K, plus calcium and folate.
Asparagus	April- early June	B vitamins. One cup provides 65 percent of your Baily Value for folate, a B vitamin that promotes healthy blood celts.
Wild Salmon	May- September	Inflammation-quashing protein. Catch wild coho, sockeye, and salmon for environmentally friendly fish rich in omega-3s.
Tart Cherries	Late June- August	Natural painkillers. Runners who drank tart cherry juice twice daily for a week before and during the 197-mile Hood to Coast Relay reported feeling less pain than placebo drinkers.
Beets	June- September	More stamina. A 2009 study found that cyclists who drank 500 millitters of best juice exercised 16 percent longer than those who drank a placebo.
Apples	September- October	Improved endurance. Apples are among the best food sources of quercetin, an antioxidant that can boost endurance.
Pumpkin Seeds	September- November	Muscle-fueling minerals. Rich in magnesium and iron, protein, vitamin K, and heart-healthy mono- and polyunsaturated fats- all for less than 200 calories per half-cup.
Spaghetti Squash	September- November	A nutritious noodle. One cup contains 42 calories and two grams of fiber; it's also a good source of vitamin $B_{\rm gr}$ vitamin C, manganese, potassium, and iron.

## TRAINING GUIDE



# **How Much Do I Need?**

Your calorie needs vary by body weight and activity level. Here's how to estimate what's right for you

Multiply your goal weight by ID.
2 → Add to that: 20 percent of that number if you're a desk jodiey; 50 percent if you're moderately active; 70 percent if you're moving all day.
3 → Add the calories burned during your workcots.

4 → Reduce the total by 15 percent. S → The final figure = the number of calories you should consume daily to achieve or maintain your goal weight while maintaining enough energy for exercise and your daily activities.

CALORIES BURNED	130 POUNDS	160 POUNDS	190 POUNDS	
12 min/mile	472 cal/hour	582 cal/hour	691 cal/hour	
11 min/mile	532 cal/hour	655 cal/hour	734 cal/hour	
10 min/mile	591 cal/hour	727 cal/hour	864 cal/hour	
9 min/mile	650 cal/hour	800 cal/hour	950 cal/hour	
8 min/mile	709 cal/hour	873 cal/hour	1036 cal/hour	
7 mio/mile	827 cal/hour	1018 cal/hour	1209 cal/hour	
6 min/mile	945 cal/hour	1163 cal/hour 1382 ca		

MEASURE UP Estimate portion size without using a scale—by using your own yardsticks



ONE CLOSED FIST -> One cup of beverage



ONE CUPPED HAND -> A

half-cup of pasta, rice, cut huit, borries, or beans; one ounce of nuts



TWO CUPPED HANDS -> One cup of flaky breakfast cereal, soup, chil, curry; one cunce of chips or pretzels



PALM OF THE HAND I Three ounces of cooked meat, fish, or canned tune



ONE THUMB One ounce of cheese



TWO THUMBS TOGETHER -> One tablespoon of condiments such as pearurt butter, salad dressing, guacamole, or mayonnaise

# RUNNER'S WORLD

## TRAINING GUIDE

#### UNSATURATED FATS (20-35%) CARBOHYDRATES (35-65%) LEAN PROTEN (10-35%) DO-35%)

PLATE To make sure each meal delivers the nutrients you need, give key food groups their place on your plate

CARBOHYDRATES (55-65%) -> Such as hvits, whole-grain breads, pasta, and vegetables UNSATURATED FATS (20-35%) -> Such as

olive oil, walnuts, and avocados LEAN PROTEIN (10-35%) -> Such as chicken,

sirlain, tofu, nuts, and seeds



#### RUNNER'S WORLD