

Spring 2015

A Comparison of Markers of Iron Status between Vegetarian and Non-Vegetarian Female High School Cross Country Runners. Is Routine, Pre-Season Screening Warranted?

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A COMPARISON OF MARKERS OF IRON STATUS BETWEEN VEGETARIAN
AND NON-VEGETARIAN FEMALE HIGH SCHOOL CROSS COUNTRY
RUNNERS. IS ROUTINE PRE-SEASON SCREENING WARRANTED?

A Thesis

Presented to

The Graduate Faculty

Central Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

Nutrition

by

Edward J. Callahan Jr.

May 2015

ABSTRACT

A COMPARISON OF MARKERS OF IRON STATUS BETWEEN VEGETARIAN AND NON-VEGETARIAN FEMALE HIGH SCHOOL CROSS COUNTRY RUNNERS. IS ROUTINE, PRE-SEASON SCREENING WARRANTED?

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Edward J. Callahan Jr.

May 2015

The purpose of the present study is to determine whether the circulating markers of iron status differ between vegetarian or non-vegetarian adolescent female high school cross-country runners. Fifteen non-vegetarian and six vegetarian female cross-country runners completed questionnaires used to obtain anthropometric data, health history, menstrual status, diet history and training history. Dietary intake was determined by self-report, using a food frequency questionnaire and a 3-day diet record. Blood samples were analyzed for serum iron, total iron binding capacity, serum ferritin, and transferrin saturation. No significant difference was found between vegetarians and non-vegetarians for serum iron or transferrin saturation. However, TIBC was significantly higher in vegetarians ($p < 0.05$). Ferritin was lower in vegetarians, trending toward significance ($p < 0.10$). Study results suggest the need for routine pre-season iron screenings as well as nutrition education, aimed at improving iron status for vegetarian and non-vegetarian adolescent female endurance athletes.

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CHAPTER I

LITERATURE REVIEW

The prevalence of “all cause” anemia, worldwide (all ages, ethnicities) is near 25%, or approximately 1.6 billion people.¹ However, this percentage varies significantly from one region to another.¹ In the United States, the prevalence of anemia is relatively low, at nearly 5%.² Though there are several causes for anemia, a dietary deficiency of iron is the most common, worldwide.³

What is Iron?

At approximately 35% of the earth’s mass, iron is the most plentiful element on the planet.⁴ Soils that trees and plants grow in, and many rock formations, contain varying amounts of iron. Iron has been mined from the ground and molded into many useful things, such as tools, weapons, and cookware throughout history. Iron also resides in the waters of streams, lakes, ponds, and rivers, as well as the sediment found therein. Iron that is dissolved in water (ferrous) will not be visibly detectable; however, it shows up as a brownish red color when exposed to oxygen.⁵ Plants absorb iron from the soil and water to use in the formation of chlorophyll which is necessary in order for the plant to produce oxygen, and grow properly.⁶ When animals and humans eat the plants, iron is absorbed into their bodies and the most important role of iron to humans begins.

Functions of Iron in the Human Organism

Iron plays a critical role in the existence of most living organisms. Iron is particularly essential for the day-to-day health and functioning of the human body, as it is a fundamental component of many proteins and enzymes throughout the body.⁷⁻¹⁰ Iron is an integral part of myoglobin, which is a heme containing protein, found in muscles.^{9,10} Iron also functions in the formation of cytochromes, necessary for the production of adenosine triphosphate (ATP) in the electron transport chain.^{9,10} Other important functions that involve iron include: DNA synthesis, growth and repair of cells and muscles, immunity, and as an antioxidant.^{9,10} Of particular concern is the oxygen carrying protein, hemoglobin, the part of the red blood cell which houses two thirds of all the iron in the human body.⁷⁻¹⁰ Hemoglobin depends on iron to carry out its function of transporting oxygen to tissues throughout the body.⁷⁻¹⁰ Iron deficiency (ID) can negatively impact oxygen delivery to cells, and working muscles, resulting in fatigue and poor work performance.⁷⁻⁹

Dietary Iron Sources: Heme vs Non-Heme Iron

As previously stated, iron from the soils is taken up by plants, photosynthesis takes place, and plants grow and thrive.⁶ Animals and humans, in turn, eat the plants and use the iron in their bodies, for many complex functions. Since humans eat plants and animals, two sources of dietary iron are available. Iron from the flesh of animals is called “heme” iron, because it associated with hemoglobin, which is in the blood of animals. Iron found in plant foods, dairy products and even the eggs of animals, however, are

referred to as “non-heme,” because it does not contain a heme group which is where iron is located in hemoglobin.^{7-10,16} More than 80% of the iron found in the average mixed diet consists of non-heme iron.¹⁶ Controversy exists as to which source is better for supplying adequate iron to carry out all the functions in the body. Upon comparison, plant (non-heme) sources, on average, are similar or somewhat higher in iron.¹⁶ The amounts of iron vary by the source of the iron. (Table 1).⁷ The significant difference lies in the bioavailability of the iron in the food,¹¹ as well as the iron stores of the individual.^{13,14} Bioavailability refers to the degree which iron, present in a food or fluid, can be absorbed and used by the body.¹²

Table 1. Select heme and non-heme food sources

Animal Source "Heme"			Plant Source "Non-heme"		
Food	Mg/ serv.	% DV	Food	Mg/ serv.	% DV
Chicken liver 3oz	11	61	Instant Oatmeal, 1 pkg	11	61
Oysters	5.7	32	Soybeans, boiled 1 cup	8.8	48
Beef liver 3oz	5.2	29	Lentils, 1 cup	6.6	37
Beef, roast 3 oz	3.1	17	Kidney beans, 1 cup	5.2	29
Turkey, dark 3 oz	2	11	Lima beans, 1 cup	4.5	25
Beef, ground 3 oz	2.2	12	Black beans	3.6	20
Beef, top sirloin 3 oz	1.6	9	Tofu, raw, 1/2 cup	3.4	19
Tuna, in water 3 oz	1.3	7	Spinach, boiled 1/2 cup	3.2	18
chicken, dark 3 oz	1.1	6	Raisins 1/2 cup	1.6	9

Adapted from, Iron: Dietary Supplement Fact Sheet (2007) National Institutes of Health, Office of Dietary Supplements.

%DV= Percent Daily Value

Iron Metabolism, Excretion, Absorption, Storage

On average the human body contains 2.5-5g of iron, depending on gender and body size (~45 mg/kg of body weight for adult females and ~55 mg/kg of body weight for adult males).^{15,18,20} About two-thirds of the body's iron is found in circulating red blood cells, and another portion is stored (25%), in the liver, spleen, and bone marrow (sternum, pelvis, skull, and shoulder girdle, in adults¹⁷).^{8,15} The remainder is used in myoglobin, enzymes and other proteins.⁸ Iron exists, in the body, in a relatively closed state. The majority of iron gets recycled from old red blood cells (~90%). The other 10% must be absorbed from the diet.¹² Because there are few avenues for excretion of excess iron, the body maintains a tight, homeostatic balance between losses, (~1-2mg/day through desquamation of epithelial cells of skin, urinary & GI tracts, and menstruation), and absorption (~1-2mg/day), which occurs in the duodenum and is affected by several factors.^{15,16,18-20}

Bioavailability is one factor that affects the absorption of iron in the body.¹² Heme iron has a greater bioavailability than non-heme. As Table 1 shows, some vegetarian sources, though higher in iron, provide a smaller net iron absorption compared to meat.¹¹ Individuals that eat only a plant based diet can expect to absorb approximately 5-12% of dietary iron, and a mixed diet, including heme iron will absorb ~14-18%.^{7,12} Some studies give an even wider range, from 1-15% for non-heme and 15-45% for heme iron.^{11,14} This variance in absorptive values is representative of differences in iron stores. There is an inverse relationship: as iron stores decrease, intestinal absorption increases.^{13,14} The upper ends of the absorption range point to lower iron stores and the

lower absorption indicates adequate stores.¹⁴ Other factors that affect iron absorption are often termed inhibitors and enhancers. Enhancers, that can boost the absorption of iron four-fold,¹³ include: vitamin C (ascorbic acid), cooking in cast iron or stainless steel,^{28,60,61} and the inclusion of some type of meat, fish or poultry to the non-heme meal.^{9,12,13} Heme sources enhance the absorption of non-heme, however are, themselves, not affected by enhancers because they are already highly bioavailable.⁸ Since vegetarian diets contain only non-heme iron, the vitamin C enhancement, found in fruits and vegetables, is especially important.^{16,61} Another compounding, “double edged sword” effect of a vegetarian diet, is that there is a substantial amount of iron inhibitors present in plant food. Beans, rice, and legumes, for example, contain phytates that can greatly reduce iron absorption to as little as 2%.^{9,12} Coffee, teas, wines, fruits and vegetables have polyphenols in them that also inhibit iron absorption.^{9,12} Calcium is another potential inhibitor of iron absorption, although results are mixed . In a study by Hallberg et al.²¹ subjects ate bread rolls that had been fortified with varied doses of calcium, and found that even the lowest dose of 40mg of calcium reduced iron absorption by 50%. More recent studies by Mølgaard et al.²² and Ríos-Castillo et al.²³ revealed opposite results. In both long term²² (1 year, with adolescent girls) and short term²³ (1 month, with middle-aged women) studies involving calcium supplementation, no significant negative effects were noted, on iron absorption, in any of the subject groups.^{22,23} Enhancing factors as well as initial iron status of subjects may have been confounding factors in the outcome of the studies.²²⁻²³ Hurrell and Egli¹² concluded that the concurrent intake of vitamin C (such as citrus or tropical fruits) with phytates, polyphenols, or calcium rich foods or

supplements, can have a cancelling effect on the inhibitory nature of these substances.

Individuals who are at risk for potentially low iron status need to plan meals carefully, in order to get adequate iron intake.

Recommendations:

Beginning in May of 1941, the Food and Nutrition Board of the Institute of Medicine (IOM), National Academy of Sciences, Washington, DC has been publishing the Recommended Dietary Allowance.^{24,25} To date, the IOM is still the overseeing body that makes nutrition related recommendation. Even if the source is the CDC, USDA, Mayo Clinic, NIH, MedLine, University websites, Wikipedia, etc, they can usually all be traced back to the IOM. In 1941, there were only nine nutrients that were considered for recommendation: protein, thiamin, riboflavin, niacin, ascorbic acid, vitamins A and D, calcium, and iron.²⁴⁻²⁵ Several of these earliest researched nutrients are important to the present study on iron status, in adolescent, female, vegetarian & non-vegetarian runners.

Current recommendations for iron differ by gender and age.²⁶ Younger individuals (male and female) that are still in stages of growth and maturation require greater amounts of iron.²⁷ Female adolescents having regular menstrual cycles are also candidates for increased iron intake, compared to males.^{8,9,27} Iron recommendations for males aged 14-18 and 19-50 are 11mg/day and 8mg/day respectively. For females in the same two age groups, iron recommendations are 15mg/day and 18mg/day, respectively.^{8,9,26}

In a retrospective cross-sectional study, de Almeida Santos et al.²⁷ assessed the iron status of adolescent male and female subjects (aged 10-20), at different stages of pubertal growth. Results showed that the majority of subjects that presented with low iron status were in a pubertal growth spurt and were also male.²⁷ The authors' rationale for this finding is that sexual maturation requires an increased need for iron, and that muscle development in boys is four times faster than in girls.²⁷

There are several other factors that can alter daily iron recommendations; two of which are actual published recommendations by the IOM. Pregnant females 14-50 years old have an increased recommendation to 27mg/day.^{8,9,26} During pregnancy, placental and fetal growth, as well as increased blood volume, creates a need for increased iron intake.^{8,9,26} During lactation the iron demand is less, due to lack of menstrual bleeding.^{8,26} During lactation, the recommendation is reduce to 10mg/day for 14-18 year olds and 9mg/day for 19-50 year olds.⁸⁻²⁶ Other factors that can alter the need for increased iron intake are vegetarian diet, and endurance exercise, which will be discussed in greater detail later in this discussion.

With so many circumstances requiring either less iron or extra iron in the diet, special care needs to be taken to avoid ID or iron overload. Much like the formula of calories in/calories out for bodyweight maintenance, iron homeostasis is dependent on balancing iron input with iron losses.

Iron Overload

The human body is very efficient at regulating iron levels, in the face of deficiency or overload. It relies on three systems or processes to accomplish this regulation. The recycling of old red blood cells is one way the body maintains a large portion of iron.¹⁰ The use of storage proteins, such as ferritin, is another way to conserve iron in the face of deficit. Regulating the absorption of iron at the intestinal level is the final mechanism available to prevent loss or excess.

Despite the presence of regulatory mechanisms, iron overload can still occur as a result of genetic defects or secondary causes. Genetic iron overload is referred to as hereditary hemochromatosis and has a prevalence of approximately one in every 200 persons in the US.²⁸ This condition is more common in men than women. Secondary iron overload is usually the result of frequent blood transfusions, secondary to another disease process, such as β -thalassemia or sideroblastic anemia.²⁹ In healthy adults, with normally functioning intestines and no tendency toward genetic iron disorders, iron overload, from dietary sources or moderate supplementation, is unlikely.^{9,30} However, iron toxicity may occur in situations of excessive supplementation with oral or parenteral iron which may be administered via intravenous infusion or intramuscular injection.²⁹ Gastrointestinal irritation may occur at doses of 20 mg/kg of elemental iron, and more serious conditions involving the heart, brain, kidneys and liver may occur with doses of 60 mg/kg.^{8,9} “Even though iron overload can and does occur, ID is a much more common problem, globally.

Iron Deficiency

ID is one of the most widespread nutrient deficiencies, among men and women, with prevalences as high as 50% worldwide, in developing countries.³¹ It is also the leading cause of anemia in the world.¹ ID is more common among adolescent and adult females of childbearing age, than among males, and there is a higher frequency in certain ethnic subgroups.^{32,33} In the United States, the prevalence of ID in women aged 12-49 ranges from 9-16%. By contrast, the incidence of ID in men aged 12-49 years ranges from 2-5%.³² Mexican American and non-Hispanic black females were twice as likely to be iron deficient (19-22%), compared to non-Hispanic white females (10%).³³ Other groups that encounter high prevalences of ID include, athletes and vegetarians. Each group has a different explanation for their increased rate of deficiency. In studies involving basketball, soccer, rowing, gymnastics, running and other sports, prevalence rates range from 15% to 57% in both genders.³⁴⁻⁴⁰ The incidence of ID is typically two times higher in females.⁴⁰ According to the Academy of Nutrition and Dietetics, a vegetarian diet that is balanced and nutritionally sound, is suitable for all ages and lifestyles, including athletes.⁴¹ However, in studies that look at iron status of vegetarians, the average prevalence of ID was 44%,⁴²⁻⁴⁵ which is at or above the average for non-vegetarian subjects. It appears that many things, such as age, gender, ethnicity, sports and more, can have a negative effect on a person's iron status.

Etiology of Iron Deficiency

The causes or risk factors of ID are many and varied, but can generally be classified into three key categories: dietary intake, absorption, and increased needs which includes those individuals with increased losses.^{46,48} Lack of adequate dietary intake is the most common of these three. Vegetarian, reduced calorie diets that have little fat or animal protein present a potential risk for decreased iron intake.⁴⁷ The Recommended Daily Allowance (RDA), as mentioned above, is 15 mg/day for females aged 14-18 years and 18 mg/day for females aged 19-50 years.²⁶ A person's current iron status and the type of diet they consume (heme or non-heme) are two key factors in determining how much iron is absorbed. Meat sources of iron are more readily absorbed, compared to plant sources.^{7,11-14} Because of the bioavailability of iron, only about 1-2 mg of the recommended amount is actually absorbed. In an apparently healthy individual, this amount is perfect for replacing the average 1-2 mg that is lost every day.^{15,20} ID occurs when these needs and losses are greater than the dietary intake.

Examples of major iron losses that contribute to increased needs are: menstrual bleeding, blood donation or frequent blood draws.⁴⁸ Assessment of these blood losses is critical in determining total iron losses. The average loss during a normal menstrual cycle is approximately 30 ml of blood loss. Blood loss of greater than 80 ml is excessive and referred to as menorrhagia.^{49,50} Average iron loss, through menstruation, can vary from 0.6-0.7 mg/day or approximately 30 ml of blood loss.⁸ The human body contains about 10 pints of blood. During a blood donation, one unit of blood is taken.⁵¹ This is equivalent to 500 ml or close to one pint of blood. There is roughly 220-250 mg of iron in

one unit of donated blood.⁵² A venipuncture (blood draw), for blood sampling, however, requires only about 5-7 ml of blood per tube.⁵³ Tests for iron studies could require one tube, for a simple test, or several tubes for an extensive analysis resulting in up to 28 ml of blood loss.⁵³

Other minor blood losses can occur in the gastrointestinal (GI) system as a result of ulcers, gastritis and other GI disorders, as well as an overuse of non-steroidal inflammatory drugs (NSAID), like ibuprofen.⁵⁴⁻⁵⁵ According to Lanier⁵⁵ the 15th leading cause of death was from GI bleeding, as a consequence of chronic NSAID use. Use of NSAIDs is especially popular in the athletic community, where blocking pain has the potential to enhance performance. Warner et al.⁵⁶ surveyed NSAID use among high school football players. Results showed that in the three-month period, prior to the survey, 75% of the players claimed to use NSAID's. Of that percentage, 15% were daily users.⁵⁶ Additional iron losses, referred to as basal losses, make up approximately 0.9-1.2 mg/day.⁸ These include losses in the feces, urine, sweat and GI tract.^{8,57}

Increased iron needs can also be seen in pregnancy. In the latter weeks of pregnancy, for example, a woman may need to absorb as much as 5 mg of iron per day.⁸ The above conversation regarding ID, related to the three key categories of risk, can apply to all ages, gender, ethnicity, or activity level. However, three particular groups stand out as having added risk: adolescent females, competitive athletes (especially endurance athletes), and vegetarians.⁸⁰

Adolescent Female

Adolescence is a time of great change within the body. As previously mentioned, women of reproductive age (particularly adolescent females aged 14-18) are at increased risk for ID⁸⁰ due to several factors: onset of menstruation, continued growth and development, and inadequate dietary intake of iron.⁵⁸ In a 4 year, longitudinal study of 354 white females (aged 8-13), Ilich-Ernst et al.⁵⁹ examined the effect that calcium supplements, pubertal growth, and onset of menses had on iron status. Yearly blood samples were analyzed and, at the end of four years, results showed that iron status is negatively affected by regular menses and increased growth and maturation, related to expansion of blood volume and increased lean muscle mass.⁵⁹ The mean serum ferritin level was at 18.4 ± 0.59 $\mu\text{g/L}$ (95% CI: 17.8, 19.0),⁵⁹ which would be considered depleted iron stores or iron deficiency without anemia (IDNA), by many researchers.^{38,49,62-64} It is not uncommon for some adolescent females to lose >80 ml of blood during a menstrual cycle. This happens in approximately 10% of American adolescent girls.⁶⁵ Wang et al.⁶⁵ studied 150, 10-17 year old girls to determine the effects of heavy menstrual bleeding (HMB) on signs and symptoms of ID, chiefly fatigue. Of the total subjects, 48 were considered to have HMB. Subjective measuring surveys were used to assess amounts of menstrual blood loss, as well as severity of fatigue. Blood samples were also taken to evaluate serum ferritin. Results showed median ferritin levels of those with HMB to be 16 ng/ml, with 30% of this group <15 ng/ml.⁶⁵ Further results showed significantly higher fatigue severity scale (FSS) scores among the HMB subjects. This study clearly suggests that HMB results in decreased iron stores, and that fatigue is the

most prevalent symptom among adolescents with ID.⁶⁵ General growth and development, alone, places a burden on the homeostatic iron equation, in young females. Intense physical exercise and athletic performance take it to a higher level.

Endurance Athlete

Endurance exercise poses another ID risk for the adolescent female.⁸⁰ Some research estimates that 40-50% of adolescent athletes have varied levels of iron depletion.⁶⁶ With intense exercise, all of the usual avenues for iron loss are potentially accentuated. Endurance athletes may have greater iron losses through several mechanisms such as gastrointestinal bleeding, as well as increased losses in sweat, urine, and feces.^{39,40,54,57,67,68}

Another mechanism for iron loss that is unique to many athletes is red blood cell hemolysis, commonly called foot-strike hemolysis.⁶⁹ Telford et al.⁶⁹ examined the effect of foot-strike hemolysis in ten male triathletes aged 21-34 years. Subjects completed a cycle ergometer and treadmill test at 75% VO_{2peak} . Each exercise was done one week apart. After blood analysis was complete, the results showed that running on the treadmill produced higher levels of free plasma hemoglobin and decreased levels of haptoglobin, indicative of foot-strike hemolysis.⁶⁹ Haptoglobin is an acute-phase protein that binds to hemoglobin that is released from red blood cells that are broken open during foot-strike hemolysis.⁹⁹ Tissue oxidative damage is reduced through the binding of hemoglobin with haptoglobin.⁹⁹ Another study by Janakiraman et al.⁷⁰ examined the effect hardness of sole in running shoes had on hemolysis. Twenty male runners, 19-23 years of age, were

divided into two groups. Each group ran for one hour on a treadmill wearing either a soft or hard soled shoe. Results suggested that soft soled running shoes produce significantly more hemolysis than the hard soled shoe ($P = 0.017$), however, there was no significant difference in indices of iron status.⁷⁰ Janakiraman et al.⁷¹ found similar results when comparing types of running surfaces. They concluded that the surface, whether grass or asphalt, had equal effect on intravascular hemolysis, but no significant effect on serum ferritin were noted, in twenty male runners.⁷¹

Gastrointestinal iron loss was reported by Stewart et al.⁶⁷ in male and female runners competing in various races, from 10k to marathon. Post race stool samples showed increased hemoglobin, and blood samples showed lower serum ferritin, compared to non-runner controls.⁶⁷ Similar results were reported by Robertson et al.⁷² comparing distance walkers to marathoners. Significant increases in fecal hemoglobin were noted in the marathoners, though considered clinically irrelevant.⁷² Walkers had no significant change from pre-walk test.⁷²

Measuring sweat iron losses is very tedious and difficult.^{57,68,73} Researchers DeRuisseau et al.⁶⁸ attached plastic collection bags on the arms of subjects as they rode cycle ergometers, for 4 -30 minute segments. Collections were recorded after each segment.⁶⁸ Significant results from this study include: males sweat more than females, sweat rates were higher from the first exercise segment through each successive segment, and sweat iron concentrations were higher in the first two 30 minute exercise segments, progressively decreasing in the latter two segments.⁶⁸ Indices of iron status were not assessed in this study. Several theories exist to why iron loss was greater in the first

hour.^{57,68,73} One theory is that iron from cellular debris or iron trapped in sweat glands contaminates the earlier samples, but is not present as sweating continues for longer periods.^{57,68,73} Brune et al.⁵⁷ tried to reduce the possibility of skin contamination by cleaning the skin thoroughly prior to sampling the sweat. In each stage, subjects sat in a sauna for 25-30 minutes, after which time, sweat was collected. Only two of the eleven subjects completed all four stages of the sweat collection analysis.⁵⁷ Results were similar to those found in the Deruisseau et al.⁶⁸ study. Iron loss was significantly greater in the first stage, raising questions about the efficacy of the skin cleaning process used.⁵⁷ No correlation to iron status was made in this study. Estimated iron lost in this study was approximately 22 µg/L of sweat.⁵⁷

As many studies, examining sweat loss, GI bleeding, or foot-strike hemolysis show; iron loss due to these mechanisms is relatively small in most cases.^{57,67-73} However, when compounded with other methods for iron loss, such as menstrual blood loss, adolescent growth and development and dietary intake, they may make a difference. Study after study report that competitive athletes typically have decreased or depleted iron stores, during training and competition.^{34-40,74-76} Many of these studies point to inadequate dietary intake of iron as the culprit.^{34-36,75}

Dietary intake probably has the greatest impact on iron status in the adolescent female athlete. As mentioned previously, the RDA for iron for 14-18 year old females is 15mg/day.^{8,9,26} On average, diets in the US contain 5-7 mg of iron per 1000 calories.⁷⁷ The average diet would be adequate if all adolescent female athletes consumed 2200-3000 calories/day. Estimated energy requirements for adolescent girls can range from

1700 in a sedentary individual to almost 2900 in a very active individual.⁷⁸ Unfortunately, society places greater burdens on females to look and act a certain way.⁷⁹ Adolescent female athletes are not immune to this. Often pressure from parents, and coaches as well as the perceived demand of a sport, causes individual athletes to reduce caloric intake in order to drop weight or maintain a particular physical appearance.⁷⁹ Many times this results in disordered eating.⁸⁰ Examples include: extreme calorie restriction as well as avoiding foods that contain fat, such as meat and dairy products.^{79,83} Some of these individuals may be part of the third group of individuals with increased risk of ID: vegetarians.^{80,81}

Vegetarian

Vegetarians are another group of individuals who are at greater risk for ID.⁸¹ There are many variations of vegetarianism, however the two most common are vegans, who consume plant foods, but do not consume any animal products and lacto-ovovegetarians who eat plant based foods, avoid meat of any kind, but may consume dairy products and eggs.^{81,82} Individuals have different reasons for choosing a vegetarian diet, such as animal welfare, protecting the environment, improved health, and religion or cultural influence.⁸³ For others, though, vegetarianism may be a smokescreen for an underlying eating disorder; a way to reduce caloric intake under the guise of healthful eating.⁸³

There is research that suggests that vegetarian diets have many health benefits such as reducing cardiovascular disease, diabetes, obesity colorectal cancers, and

hypertension.^{84,85} These health benefits are related to the superior nutrient density content of a vegetarian/vegan diet. The diet provides large concentrations of most essential vitamins & minerals, as well as phytochemicals, antioxidants, carotenoids, and fiber.^{84,85} According to the Academy of Nutrition and Dietetics, a vegetarian diet that is balanced and nutritionally sound, is suitable for all ages and lifestyles, including athletes.⁴¹ However, there are a few potential pitfalls to a vegetarian/vegan diet, especially for the vegan athlete.⁸³ Because of the low caloric density and the amount of fiber in plant-based foods, obtaining the daily dietary requirement for energy becomes more difficult for vegan athletes.⁸³ Other pitfalls include; inadequate calcium intake for vegans, lack of B₁₂ and vitamin D, and most importantly the problem with ID among vegetarian and vegan athletes.^{82,83}

Deficiencies in iron status, caused by a vegetarian diet, are generally due to decreased bio-availability (rate or amount of absorption) of iron from plant sources and inadequate quantity of iron consumed.^{81,82} To account for the decreased bio-availability of iron, from plant foods (non-heme), the IOM's Recommended Daily Allowance for iron is 1.8 times greater (~27mg/day) for vegetarian females aged 14-18 years, compared to same aged meat eating females (15mg/day).⁸ This increase in iron needs generally results in the need for increased frequency and portion sizes of plant-based, iron rich meals as well.^{81,82} A study by Snyder et al.⁸⁶ demonstrated the importance of increased dietary iron consumption. This observational study consisted of two groups of female runners, aged 36-49 years. One group of nine incorporated red meat (RM) into their diet, while the other, modified vegetarian (MV) group of nine, allowed dairy, eggs, and some meat into

their diets (primarily fish and poultry).⁸⁶ Three day diet records were recorded and analyzed, as well as serum markers of iron status. Results showed that the average caloric intake was less than 1800 cal/day for both groups. Hematological indices revealed that eight subjects from the MV group were iron deficient, whereas only 2 subjects from the RM group were iron deficient, with ferritin levels < 12 ng/ml.⁸⁶

Donovan and Gibson⁸⁷ looked at iron and zinc status in 122 adolescent females, aged 14-19 years, who were divided into groups based on their dietary intake. Two classifications of vegetarian and one omnivorous group were created. Three day diet records were recorded and a standard venipuncture was used to collect a blood sample from each subject. Upon analysis, 36% of vegetarians and 17% of omnivores had iron status that was indicative of ID.⁸⁷ A recent observational study by Hawk et al.⁴⁵ examined the iron status of 19 vegetarian and 20 non-vegetarian female college students. A three day diet recall and a 12-hour fasted blood sample were analyzed, to give similar results as other studies. Almost 90 percent of the vegetarians had some degree of ID and 75% of the non-vegetarian female college students were iron deficient.⁴⁵ Hawk et al⁴⁵ used different ferritin cut off values than the other two studies mentioned above, however the ratio between vegetarian and non-vegetarians was similar at each cut off. For example, 47% of vegetarian subjects had ferritin levels < 20 ng/ml while 40% of non-vegetarian subjects had ferritin levels < 20 ng/ml. ⁴⁵ Studies in each individual risk category discussed above, provide logical support that the risk for ID is multiplied when all of these three risk categories are considered together. The potential for decrements in athletic performance are increased if ID progresses to iron deficiency anemia (IDA).⁸⁸

Diagnosing Iron Deficiency

ID and IDA, though similar, are not synonymous. There is a progression from one to the other, respectively.^{8,9,30,88} In most research, this progression follows three stages, beginning with iron depletion (stage 1), moving through iron deficient erythropoiesis (stage 2), and ending with IDA (stage 3).^{8,9,30,88} Symptoms vary depending on the stage and the severity of iron depletion.^{8,88} Symptoms are generally the first indication that something is wrong.

IDNA however, may go unnoticed in most individuals, because there are few, if any symptoms.^{8,88} As iron stores continue to drop, possible signs or symptoms of latter stage IDNA may be, decreased power and endurance.^{88,97} But as ID progresses to stage 3 IDA, individuals will experience extreme fatigue and will have difficulty breathing during intense exercise.⁸⁸ Other signs that may occur in some people are dizziness, impaired thermoregulation, brittle nails, swollen tongue, and enlarged spleen.^{46,48,49,88,89} Another unexplained symptom that occurs in some anemic subjects is an unusual desire for non-food items, such as dirt, clay, or ice.^{46, 88,89} Though signs and symptoms help in the initial analysis of the stages of ID and IDA, laboratory blood tests are the definitive means for diagnosis.⁸⁸

In order to best understand each of the stages, it is important to be familiar with some of the indices used for the evaluation of ID. Unfortunately, no two studies agree. The indices used in various stages of iron status, and their respective cut-off values, differ proportionate to the number of researchers.^{8,38,45,49,62-64,90-93} The most common controversy is with ferritin cutoffs used to indicate IDNA. Some researchers state that the

cutoff should be < 12 ng/ml while others state the cutoffs as 10 ng/ml, < 16 ng/ml, < 20 ng/ml, or even < 35 ng/ml.^{8,38,45,49,62-64,90-93}

The most commonly used indice for determining **depleted iron stores** (also called Stage 1 or prelatent stage), is serum ferritin.³⁷ Ferritin is an iron-binding storage protein that is a good indicator of total body iron stores.⁵³ The normal range for ferritin is 10-150 ng/ml in females and varies by lab.⁵³ However, the cutoff used by much of the literature, for stage 1 iron depletion, is < 20 ng/ml^{38,62-64,90,91} while other studies use < 12 mg/ml for the cutoff.^{8,92} Ferritin is an acute phase protein, that increases with various infections and inflammatory states, which may mask an iron deficiency.⁵³ Sometimes transferrin saturation (Tsat) is tested along with ferritin, in stage 1.³⁸ Transferrin saturation, expressed as a percentage, is the amount of all iron-binding proteins saturated with iron.⁵³ Normal values for Tsat are 15-50% in females.⁵³ The Tsat cutoff value for stage 1 is < 16%.^{8,38}

In stage 2, **iron deficient erythropoiesis** (or latent stage), researchers may look at cutoffs for serum iron, ferritin, transferrin saturation, total iron binding capacity (TIBC), and sometimes hemoglobin to rule out anemia.^{8,38,45,62-64, 90-91} Serum iron is an indicator of the quantity of iron bound to transferrin (the most abundant iron-binding protein in the blood).⁵³ Serum iron, located in the hemoglobin molecule, makes up 70% of all the iron found in the body, with normal values between 60-160 µg/dl, in females.⁵³ TIBC measures the quantity of all serum proteins that can bind mobile iron. Normal ranges are from 250-460 µg/dl, in females.⁵³ TIBC levels are increased in the face of iron depletion.⁵³

In the final stage, **iron deficiency anemia** (or manifest deficiency), the primary indicator is decreased hemoglobin.^{8,38,45,62-64, 90-91} Hemoglobin is a late stage ID indicator, and more specifically an indicator of anemia, which may or may not be caused by ID.⁹⁶ Ferritin levels will typically be less than 10-12 ng/ml at this stage; coupled with a hemoglobin of < 12 g/dl it is indicative of IDA.^{53,92} Some authors say that decrements in health and athletic performance can occur with ferritin levels between 12-35 ng/ml (varies by source), which is considered IDNA.^{37,94,95}

Conclusion

The individual link between ID and each of the following: adolescent females, endurance running, and vegetarianism, has been studied in previous studies cited in the literature.^{34-40,42-45,58,75,76} However, the combination of adolescent females, endurance running, vegetarianism, and ID has not been specifically studied. This subset of female athletes has an increased vulnerability toward ID, and IDA which can have detrimental effects on overall health, as well as on the work capacity of these competitive athletes.^{37,88,94,95,97,98}

The purpose of this study is to determine whether the circulating markers of iron status, including serum iron, ferritin, TIBC, and transferrin saturation, differ between vegetarian or non-vegetarian adolescent female high school cross-country runners. The hypothesis for this study is that a vegetarian diet may not provide adequate dietary iron for adolescent female cross country runners, resulting in ID and potentially progressing to IDA and decreased athletic performance. Furthermore, study results may help to target

nutrition education efforts as well as the need for routine pre-season iron screenings, aimed at improving iron status for adolescent female endurance athletes.

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JOURNAL ARTICLE

A Comparison of Markers of Iron Status between Vegetarian and Non-Vegetarian
Female High School Cross Country Runners.

Is Routine, Pre-Season Screening Warranted?

BACKGROUND & INTRODUCTION

As one of the most plentiful elements on the planet, iron plays a critical role in the existence of most living organisms. Iron is particularly essential to the day to day health and functioning of the human body, as it is a fundamental component of many proteins and enzymes throughout the body.¹⁻⁴ Iron is an integral part of myoglobin, which is a heme containing protein, found in muscles.^{3,4} Of particular concern is the oxygen carrying protein, hemoglobin, the part of the red blood cell which houses two thirds of all iron.¹⁻⁴ Hemoglobin depends on iron, to carry out its function of transporting oxygen to tissues throughout the body.¹⁻⁴ Iron deficiency (ID) can negatively impact oxygen delivery to cells, resulting in fatigue and poor work performance.¹⁻³

ID is one of the most widespread nutrient deficiencies, among both men and women with prevalences as high as 50% worldwide. ID is more common among adolescent and adult females than males, and there is a higher prevalence in certain ethnic subgroups.^{5,6} In the United States, the prevalence of ID in women aged 12-49 ranges from 9-16%.

The causes or risk factors of ID are many and varied but can generally be classified into three key categories: dietary intake, absorption, and increased needs.^{7,8} Adolescence is a time of great change within the female body. Ilich-Ernst et al.⁹ reported

that iron status is negatively affected by inadequate dietary iron intake, regular menses and increased growth and maturation, related to expansion of blood volume and increased lean muscle mass. According to the Institute of Medicine, the dietary reference intake (DRI) for non-vegetarian females, 14-18 years of age is 15 mg/day.¹⁰ Endurance exercise poses a further ID risk for the adolescent female.¹¹⁻¹⁶ Some research estimates that 40-50% of adolescent athletes have varied levels of iron depletion.¹⁷ Many studies that examine sweat loss, GI bleeding, or foot-strike hemolysis in athletes show iron loss due to these mechanisms is relatively small in most cases.^{14-16,18-22} However, Woolf et al.²³ suggest that the sum of these losses coupled with menstrual blood loss, may produce a significant iron loss in adolescent female athletes.²³ Vegetarians are also at risk for ID for several reasons. The presence of inhibitors such as phytates and polyphenols inherent in plant foods decreases iron absorption resulting in decreased dietary iron intake.¹⁰

Decreased bio-availability (degree which iron can be absorbed and used by the body) of iron from plant sources also increases the risk for ID.^{24,25} Meat “heme” sources are more readily absorbed.²⁴ Conversely, non-heme ferric iron (Fe^{3+}) that comes from plant, dairy and egg sources^{24,26} must be reduced to heme ferrous iron (Fe^{2+}) in order to be absorbed in the duodenum.²⁷ Because of this decreased bio-availability, the DRI recommendation for iron is 1.8 times greater for vegetarians, at 27 mg/day for females aged 14-18 years.^{1,2}

ID can occur with or without anemia,^{2,3,28,29} and is classified into three stages, as described by Chatard et al.,³⁰ based on signs, symptoms and a variety of hematologic parameters.^{2,29-39} Stages 1 and 2 are considered in most literature as iron deficiency

without anemia (IDNA). Individuals at this stage may show no outward signs and symptoms.^{2,29} However, some studies have reported decrements in health and athletic performance, power and endurance with stages 1 and 2.^{29,40-44} Serum ferritin between 12-20 ng/ml and decreased T_{sat} are indicators of iron depletion in stages 1 and 2.^{31,40,45} Stage 3 is called iron deficiency anemia (IDA). In this stage, ferritin values drop below 12 ng/ml and hemoglobin falls below 12g/dl.^{34,38,46} Because the oxygen carrying protein hemoglobin is negatively affected, symptoms in stage 3 include extreme fatigue due to tissue anoxia. Additionally, a stage 3 individual may experience difficulty breathing during intense exercise, dizziness, and impaired thermoregulation.^{7,8,29,30,47}

ID in populations including: adolescent females, endurance athlete, or vegetarians, has been examined in previous studies.^{11,12,31,32,40,45,48-55} However, the combination of adolescent female vegetarian endurance athlete, has not been specifically studied. This subset of female athletes has an increased vulnerability toward ID, and IDA which can have detrimental effects on overall health, as well as on the work capacity of these competitive athletes.^{29,40-44}

The purpose of the present study is to determine whether the circulating markers of iron status differ between vegetarian or non-vegetarian adolescent female high school cross-country runners. The hypothesis for this study is that a vegetarian diet may not provide adequate dietary iron for adolescent female cross country runners, resulting in ID and potentially progressing to IDA and decreased athletic performance. Furthermore, study results may help to target nutrition education efforts as well as the need for routine pre-season iron screenings, aimed at improving iron status for vegetarian and non-

vegetarian adolescent female endurance athletes.

METHODS:

Participant Selection Criteria

Six vegetarian and fifteen non-vegetarian female high school cross-country runners, aged 14-18 years, were recruited from the rosters of three separate, summer cross country camps, as well as from a local high school cross country team. Over 200 female high school cross-country runners from Washington, Oregon, and Alaska were invited to participate in the study. Each participant was informed of the requirements, benefits, and potential risks of the study, and gave voluntary written assent to participate. Parents of participants, younger than 18 years of age, signed informed consent forms. Approval for this study was granted by the Human Subjects Review Committee at Central Washington University (CWU) prior to commencement of any study procedures.

Participants were classified as vegetarian if their dietary intake did not include meat, fish, and poultry. Non-vegetarians, by contrast, were defined as individuals whose dietary intake included meat, fish, and poultry. Of the six vegetarian participants, three self reported as vegan, two as lacto-ovo vegetarian (allows eggs and dairy), and one reported as a lacto-ovo/pescatarian (allows eggs, dairy, and fish). Participants were ineligible for inclusion in the study for any of the following circumstances (see Table 1).

TABLE 1. Exclusion criteria for vegetarian and non-vegetarian adolescent female cross country runners.

-
- Currently diagnosed with anemia (hemoglobin <12 g/dl)
 - Serum ferritin of <12ng/ml)
 - Currently on prescribed iron supplement therapy*
 - Blood donation, blood transfusion, or receipt of blood products within last three months
 - Active menstrual cycle at time of blood collection
 - Regular heavy menstrual bleeding, (lasting 8-10 days with > 80ml blood loss)
 - Chronic use of non-steroidal anti-inflammatory drugs (NSAIDS) (ie ibuprofen or aspirin)
 - Inflammatory gastrointestinal diseases (such as inflammatory bowel disease, diverticulitis, or ulcerative colitis)
 - Pregnant or lactating
-

*A multivitamin/mineral that included iron, but iron did not exceed 18-27mg/day, was acceptable for this study

Questionnaires

Prior to the cross country running camps, participants completed a questionnaire used to obtain anthropometric data, health history, menstrual status, diet history and training history. A 3DR consisting of two weekdays and one weekend day, as well as a FFQ were completed, before the running camps started, to assess dietary intake. Participants were given instruction on how to accurately complete the 3DR and FFQ.

Study Procedures

After fasting for 12 hours, blood draw procedures were reviewed with participants. Questions about the study were answered, and the 3DR and other questionnaires were reviewed for accuracy. Blood draws began at 7:30am. Approximately 7-10 ml of blood was drawn into a single vacuum tube, from an antecubital vein. Blood samples were centrifuged (Fischer Healthcare, Phillipsburg, PA.)

within one hour of collection, and then stored in an ice chest for transportation back to a local clinic lab for analysis (Interpath Laboratory, Pendleton, OR.). Each sample was analyzed for serum iron, serum ferritin, TIBC, and TSat. TSat was calculated as a ratio of serum iron and TIBC, multiplied by 100.

Dietary Intake Analysis

A FFQ was used to assess daily, weekly, and/or monthly consumption of 25 portion specific food groups that have significance to iron status. Results of the FFQ were converted to estimated daily averages. The 3DR's were analyzed using ESHA Research Solutions Food Processor Diet Analysis software (Salem, OR, 2014). A complete nutrient assessment was performed, to include: caloric intake, macronutrients, micronutrients, fiber, caffeine, and water. Average daily intakes of iron, calcium, vitamin C, and total calories were particularly noted.

Statistical Analysis

Statistical analysis was performed using R.3.13. (R Foundation for Statistical Computing, Vienna, Austria, 2015). Permutation tests were used to assess the mean differences in iron status between vegetarian and non-vegetarian female cross country runners. Results were expressed as means \pm standard deviation (SD) and significance set at $\alpha = 0.05$.

RESULTS

Physical Characteristics

No significant differences were noted in the physical characteristic of non-vegetarian and vegetarian participants for age, height or weight (Table 2).

TABLE 2. Participant characteristics for non-vegetarian and vegetarian adolescent female cross country runners.

	Non-vegetarian (n = 15)	Vegetarian (n = 6)
	Mean \pm SD	Mean \pm SD
Age (years)	15.8 \pm 1.2	14.8 \pm 1.2
Height (inches)	65.1 \pm 2.3	63.5 \pm 2.3
Weight (pounds)	121.5 \pm 19.4	113.7 \pm 28.6

Dietary intake

Daily averages for total caloric intake and select nutrients for vegetarian and non-vegetarian participants are shown in Table 3. Based on statistical analysis of data collected from 3DR's, there was no significant difference in select nutrients related to iron status, or daily caloric intake between vegetarian or non-vegetarian adolescent female cross country runners.

TABLE 3. 3DR results showing average daily intakes of selected nutrients for non-vegetarian and vegetarian adolescent female cross country runners.

Variable*	Non-vegetarian (n = 15)	Vegetarians (n = 6)
	Mean \pm SD	Mean \pm SD
Calories (kcal)	2,437 \pm 673	2,686 \pm 1297
Iron (mg)	17.6 \pm 7.8	24.6 \pm 11.7
Calcium (mg)	941 \pm 386.2	890.7 \pm 354
Vitamin C (mg)	89.7 \pm 71.5	160.4 \pm 102.4

*DRI reference values: Iron (15 mg/day, 27 mg/day), Calcium (1,300 mg/day), Vitamin C (65 mg/day)

Laboratory Assessment

The present study analyzed four markers of early, non-anemic, ID. Participant data for these four markers: serum iron, T_{sat}, TIBC, and serum ferritin are presented in Table 4. Although mean serum iron and transferrin saturation were lower among vegetarians, no significant differences were noted. TIBC levels were significantly higher in vegetarians, compared to non-vegetarians ($p < 0.05$). On average, ferritin, the primary marker for iron storage,⁴⁶ was lower in vegetarians, compared to non-vegetarians, showing a trend toward significance, ($p < 0.10$). (Table 4). Four of the six vegetarians had serum ferritin ≤ 15 ng/ml. Only one vegetarian participant had a normal ferritin level at 51.6 ng/ml. One of the iron deficient vegetarians had a serum ferritin < 12 ng/ml which signifies IDA. Seven non-vegetarians reported ferritin levels below 30 ng/ml. Three of those seven non-vegetarian participants had ferritin levels below 20 ng/ml and two non-vegetarians had ferritin levels < 12 ng/ml which is considered stage 3 IDA.

TABLE 4. Markers of iron status for non-vegetarian and vegetarian adolescent female cross country runners.

	Non-vegetarians (n = 15)	Vegetarians (n = 6)
	Mean \pm SD	Mean \pm SD
Ferritin (ng/ml)	39.3 \pm 19.4	20.2 \pm 16.5*
Serum Iron (μ g/dl)	95.1 \pm 33.8	73.8 \pm 62.6
TIBC (μ g/dl)	363.5 \pm 31.1	410.5 \pm 46.7**
Transferrin Saturation (%)	26.9 \pm 9.0	25.2 \pm 16.9

* $p < 0.10$, ** $p < 0.05$

DISCUSSION

Results of the present study were comparable to research that links ID with etiologies associated with adolescent females, endurance athletes, or vegetarians.^{9,11,12,25,31,32,40,45,49-55} Snyder et al.²⁵ examined the iron status of women runners aged 36 to 49 years, who were either modified vegetarians or who regularly consumed red meat. Iron status was significantly lower in vegetarian subjects in this study.²⁵ In another study, Donovan and Gibson⁵² compared the iron and zinc status of adolescent vegetarians and omnivores aged 14-19 years, who were not athletes. They reported a higher percentage of iron depletion in non-athletic adolescent vegetarians compared to non-athletic omnivorous adolescents.⁵² However, research has yet to examine the exacerbating effect of a vegetarian diet on iron status in adolescent female endurance athletes.

The results of the present study suggest that inadequate intake of iron is directly correlated with total caloric intake. As caloric intake increases, iron intake increases. This is consistent with Snyder et al.²⁵ who reported decreased energy intake resulting in decreased dietary iron intake among female runners. The average American diet contains approximately 6 mg of iron per 1000 calories consumed.²⁵ To meet the DRI iron recommendation of 15 mg/day, non-vegetarian adolescent females, would need to consume about 2500 calories/day. Following the average American diet, vegetarian adolescent females would need to consume 4500 calories/day to meet the DRI iron recommendation of 27 mg/day. In the present study, 6 non-vegetarians and 2 vegetarians participants had an average caloric intake > 2500 kcals/day which is within the DRI recommendation for active individuals (Table 4). Each of these individuals who

consumed > 2500 kcals/day exceeded the DRI recommendation for dietary iron intake. Of the remaining 13 study participants who consumed < 2500 kcals/day, only 3 participants met the DRI for dietary iron intake.

Because of their added energy expenditure, due to physical activity, athletes need to consume even more calories than non-athletes. Energy expenditure is especially very high for competitive adolescent female runners compared to their non-athlete peer counterparts. Yet, on average, adolescent female distance runners do not meet the recommended energy intake proportionate to their total energy expenditure^{25,56,57} which according to Eisenmann and Wickel⁵⁷ averages 2467 ± 426 kcals/day. Only 40% of the non-vegetarians and 33% of the vegetarians exceeded 2500kcals/day.⁵⁷ To keep up with energy expenditures, some adolescent female athletes will need to increase consumption of food to reach the required calorie needs. Unfortunately, many female athletes at this age may be more concerned about cutting calories rather than increasing calories.⁵⁸ Caloric restriction can lead to many nutritional deficiencies, including iron deficiency that may negatively affect an individual's health and athletic performance.⁵⁸

Many variables affect iron status in individuals. Data in Table 5 demonstrates that it is possible to have above average caloric intake and dietary iron intake yet still have poor iron status, as evidenced by decreased ferritin levels. M11 and M12 from the non-vegetarian group and V4 from the vegetarian group each exceeded the DRIs in their

TABLE 5. Energy, iron, vitamin C, and calcium intakes along with select markers of iron status for non-vegetarian and vegetarian adolescent female cross country runners.

	Energy ^a 2300-2900 kcal/d	Iron ^b M15 mg/d V27 mg/d	Vita C ^b 65 mg/d	Calcium ^b 1300 mg/d	Serum Iron ^c 60-160 µg/dl	Serum Ferritin ^d 20-140 ng/ml
M1	1917	19.1	65.6	1110.9	154	89.0
M2	2010	15.6	88.6	500.9	136	41.4
M3	3284	22.7	11.5	861.1	101	32.5
M4	2406	10.6	56.6	446.0	150	33.8
M5	1997	15.0	47.1	613.2	65	14.9
M6	1645	9.1	19.1	479.3	82	28.3
M7	3856	23.0	64.7	1322.3	97	28.3
M8	3179	27.8	49.3	1690.0	78	24.8
M9	2334	7.3	32.9	875.3	108	56.1
M10	2241	12.5	102.3	917.9	45	36.7
M11	2896	23.5	12.7	970.1	66	11.3
M12	2836	26.2	96.0	1101.8	48	11.7
M13	1419	10.3	79.5	524.9	117	28.0
M14	2642	32.1	90.0	1503.1	80	61.6
M15	1897	9.7	50.8	1197.8	100	35.9
V1	959	6.5	60.5	388.2	90	14.9
V2	4902	43.4	56.3	1057.3	162	51.6
V3	2259	18.0	65.5	922.3	38	17.0
V4	2677	28.3	142.3	1377.2	53	13.3
V5	1629	14.6	121.9	525.1	24	3.9
V6	1965	18.8	106.8	571.4	180	15.4

^a Based on DRI for active to very active females aged 14-18 years

^b Based on DRI recommendations

^c Based on Mosby's Diagnostic and Laboratory Test Reference 2014

^d Malczewska J et al.⁵⁹

respective diet group for caloric and iron intake, however, M11 and M12 had serum ferritin levels < 12 ng/ml, indicative of IDA. V4 had a serum ferritin level < 15 ng/ml, representing partial iron storage depletion. In contrast, V2 demonstrated that it is possible to maintain exceptional iron status with a very high caloric intake. The variability seen in

the data in Table 5 may be related to enhancers and inhibitors. Inhibitors such as phytates and polyphenols decrease iron bioavailability. Beans, rice, and legumes, for example, contain phytates that can greatly reduce iron absorption to as little as 2%.^{3,24} Coffee, teas, wines, fruits and vegetables contain polyphenols that also inhibit iron absorption.^{3,24} Calcium is another potential inhibitor of iron absorption, although results are mixed. Hallberg et al.⁶⁰ found that 40 mg of calcium in bread rolls reduced iron absorption by 50%. Conversely, Mølgaard et al.⁶¹ and Ríos-Castillo et al.⁶² found that one year or one month of calcium supplementation respectively, resulted in no significant negative effects on iron status in any of the subject groups.^{61,62}

Iron enhancers, such as vitamin C (ascorbic acid), can boost the absorption of iron four-fold.^{63,64} Hurrell and Egli²⁴ concluded that the concurrent intake of vitamin C (such as citrus or tropical fruits) with phytates, polyphenols, or calcium rich foods or supplements, can have a cancelling effect on the inhibitory nature of these substances.

No direct correlation can be made between vitamin C and iron status. As Table 5 shows, intakes for vitamin C can be well above the DRI in an individual with an adequate caloric and iron intake, yet that individual can still have low serum iron and ferritin, as in M12. Also, an individual can have inadequate caloric intake, poor iron and vitamin C intake, and still present with normal serum iron and serum ferritin. Timing of enhancers and inhibitors plays a critical role in the absorption of dietary iron and the prevention of ID.³

When screening for IDNA, serum ferritin is one of the most commonly used markers for apparently healthy individuals.⁵⁹ However, there is no definite consensus on

which ferritin cutoff best indicates IDNA. Some researchers use 12 ng/ml while others use cutoffs ranging from 10- 40 ng/ml to indicate stage 1 and 2 iron depletion.^{2,30-39} Confounding matters, ferritin is an acute phase reactant protein that may be elevated in times of inflammation.⁴⁶ Due to strenuous training, many athletes have varied levels of inflammation, resulting in elevated ferritin levels. An elevated ferritin in an athletic population may hide an underlying deficiency. For this reason the ferritin cutoff for stage 1 and 2 iron depletion for the present study was set at < 20 ng/ml. This agrees with much of the literature, including protocols for several NCAA Division I Institutions.^{31,34,35-37,65}

In the present study, 83% of the vegetarians had a serum ferritin level < 20 ng/ml (Table 6), representative of Stage 1 and 2 IDNA.^{31,40,45} Only 20% of the non-vegetarian had a serum ferritin < 20 ng/ml (Table 6). TIBC was significantly higher in vegetarians, compared to non-vegetarians (Table 4). Because TIBC is inversely proportional to ferritin, an increased TIBC in vegetarians is indicative of decreased iron stores.

The primary limitation of this study was the small sample size. Additional investigations, with a larger sample size of adolescent female vegetarians runners is needed to increase the power of the statistical analysis. A larger sample size may add to the present study and show a statistical difference in the iron status between vegetarian and non-vegetarian female adolescent endurance athletes.

In conclusion, results of the present study suggest that vegetarian adolescent female endurance athletes have a greater risk of developing IDNA or IDA than their non-vegetarian peers. Because of the prevalence and risk for ID in vegetarian and non-vegetarian female endurance athletes alike, the three stages of prevention should be

implemented. The primary stage must consist of nutrition education focusing on a healthful dietary prophylaxis against IDNA and IDA. A vegetarian diet should not be discouraged, but coaches should be educated about vegetarian nutrition and encourage vegetarian athletes to consume greater quantities of iron rich foods as well as vitamin C rich foods which act as iron absorption enhancers. Secondly, routine pre-season iron screening is warranted in all middle and high schools across the country. Adolescent female athletes in every sport, particularly endurance sports, should be tested for serum ferritin at least two months prior to the start of the competitive season. If IDA is suspected, based on ferritin < 12 ng/ml then other iron markers can be tested to validate the findings. Repeat mid-season screening may be warranted for individuals who have ferritin levels between 20-30 ng/ml at the pre-season screening as declines in serum ferritin levels will generally be noticed over the course of an intense competitive season.⁵⁰ In the tertiary stage, individuals with ferritin levels between 12-20 ng/ml should seek the advice of a healthcare professional such as a Sports Registered Dietitian Nutritionist. Though dietary means is the preferred treatment, iron supplementation may be necessary. Recovery of iron stores may take up to 60 days or longer, depending on the severity of the ID. The goal of the three pre-season stages of prevention is to avoid IDA and have the energy to train and compete safely and successfully.

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