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The relationship between blood iron markers, nutritional intakes and bone mineral density

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The Relationship Between Blood Iron Markers, Nutritional Intakes and Bone Mineral Density

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Abstract

PURPOSE: The purpose of this study was to examine the relationship between serum ferritin (sFer) levels, nutrient intake of iron, calcium and vitamin D and bone mineral density (BMD) in female collegiate athletes. It was hypothesized that if an individual had low sFer (<20) levels, she would also have low BMD. The rationale for this study was that a significant number of female athletes have a predisposition to iron deficiency and low BMD, and although a clear association between iron levels and BMD has not been seen in humans it has been well studied in rats. METHODS: Thirty female freshman athletes (age=18) participating in weight bearing sports (soccer, volleyball, field hockey, lacrosse, softball, basketball, cross country, track and field) completed a blood draw (analyzed for hemoglobin (Hb), Hematocrit, sFer, and high sensitivity C-reactive protein) and dual-energy x-ray absorptiometry (DXA) scans of the total body, left and right hip and lumbar spine (analyzed for BMD). Participants also completed a food frequency questionnaire (analyzed for caloric, iron, calcium and vitamin D intake). RESULTS: Correlation analysis indicated no significant correlation between sFer levels and BMD for total body, lumbar spine, hip or Ward's Triangle, as a whole. In addition, no correlation was found between sFer and dietary intake levels of iron, calcium and vitamin D or between BMD at any site and intake levels of iron, calcium and vitamin D. Also no correlation was observed when the sample was divided into groups having low sFer<20µg/L and high sFer>20µg/L. An independent T-test also showed no mean differences in BMD levels at any site when the group was divided into high and low sFer levels. CONCLUSION: There was no correlation between sFer level and BMD or between BMD and intake levels of iron, calcium or vitamin D, in female collegiate

athletes participating in weight bearing sports. Although a correlation was not found between sFer levels and BMD, this cannot be generalized to other populations, nor can it be assumed from this data that iron deficiency does not influence BMD. In view of the fact that this phenomena has not been studied in a large cross section of athletes or non-athletes, further study is warranted to determine the influence of sFer on BMD.

INTRODUCTION

Two common dietary concerns that may impact performance and injury rate of female athletes are low intake of iron and calcium, resulting in low hemoglobin (ferritin) and low bone mineral density. Inadequate intake of either iron or calcium could pose a problem for female athletes by increasing the chance of iron deficiency and/or anemia. This could also initiate an early onset of osteopenia/osteoporosis, thus increasing the risk for stress fractures.

Iron levels of female athletes, especially cross country runners, has long been a topic of interest (Mercer & Densmore, 2005; Santolo, Stel, Banfi, Gonano, & Cauci, 2008; Suedekum & Dimeff, 2005). Fertile females, particularly athletes, are more prone to developing iron deficiency and/or anemia than males, due to blood loss caused by menstruation and a lower dietary intake of iron. (Dubnov & Constantini, 2004).

An additional topic of interest in female athletes is bone density (Fehling, Alekel, Clasey, Rector, & Stillman, 1995; Meyer et al., 2004; Mudd, Foretti, & Pivarnik, 2007). This often becomes a concern in female athletes due to high amounts of training, poor eating habits and menstrual dysfunctions. Female athletes tend to be more concerned with body image and weight as compared to male athletes. The type of training (weight bearing vs. non-weight bearing) appears to influence the osteogenic response, leading to either higher or lower values of BMD at different sites (Duncan et al., 2002). Higher BMD seems to be associated with strength-based and high-impact sports, whereas non-weight bearing sports had a neutral or negative effect on BMD (Egan, Reilly, Giacomoni, Redmond, & Turner, 2006; Fehling et al., 1995). Zanker et al. have shown that disordered eating and energy deficits are related to low BMD even without menstrual disturbances

(C. L. Zanker & Cooke, 2004). Angues et al. found that iron, zinc and magnesium were positive predictors of BMD at the femoral neck and forearm (Angus, Sambrook, Pocock, & Eisman, 1988). However, they did not find a significant correlation with current calcium intake and BMD at any site, but suggest that higher calcium intake earlier in life correlates with higher BMD later in life (Angus et al., 1988). Mudd and colleagues found no differences in BMD by menstrual status when all sports were considered together. However, it is possible that their study was underpowered to detect significant difference, because strong trends existed for higher total-body, lumbar spine, and pelvis BMD values among the normally menstruating athletes (Mudd et al., 2007). Others (Nattiv, Agostini, Drinkwater, & Yeager, 1994; C. L. Zanker & Cooke, 2004), have reported that menstrual dysfunction is strongly linked to decreased BMD, and that the duration of menstrual dysfunction is related to decreased BMD at the lumbar spine (Cann, Martin, Genant, & Jaffe, 1984; Drinkwater, Nilson, Ott, & Chestnut III, 1986; N. Young, Formica, Szmukler, & Seeman, 1994).

An important area of investigation is the relationship between iron deficiency and bone mineral density, their effect on female athletes, and what factors play into this relationship. This is an important area for investigation because of the known prevalence of iron deficiency and low BMD in female athletes. A known cause of both iron deficiency and low BMD density are inadequate intake levels of nutrients, because this is a known cause, it is important to look at dietary intakes when assessing either of these topics.

Purpose

The purpose of this study was to examine the relationship between serum ferritin levels, nutrient intake of iron, calcium and vitamin D and bone mineral density in female collegiate athletes.

Objectives

Specific objectives of the study were to determine: 1. if there is a relationship between serum ferritin levels and dietary intake of iron, calcium or vitamin D; 2. if there is a relationship between bone mineral density and iron, calcium or vitamin D intake; 3. if there is a relationship between serum ferritin levels and bone mineral density.

Hypothesis

It is hypothesized that individual's with low serum ferritin levels will then to have lower bone mineral density.

Assumptions

Participants will be compliant with time frame of blood draw & DEXA scan.

Delimitations

The research population was restricted to incoming female freshman athletes participating in a weight bearing sport. The rationale for exclusion of female athletes who are not involved in weight bearing sports was to create a sample population that is homogenous with regard to the type and volume of load imposed on the bone. In addition the study participants were limited to incoming freshman thus 17-19 years of age in order to homogenize the level of bone maturation.

Limitations

Limitations of this study included the assumption that participants completed the given surveys correctly; and the blood analysis and DEXA scans were done properly. Other limitations included the low number of participants, and though homogeneity of a sample has its advantages it will also limit the ability to generalize the results of the study beyond similar female athletes.

BACKGROUND

Iron

Iron is a trace mineral and a critical nutrient, important for both athletes and non-athletes, alike (Suedekum & Dimeff, 2005). It plays a key role in energy production as a carrier of oxygen, both in the form of hemoglobin and myoglobin (Akabas & Dolins, 2005). Iron is an important component of hemoglobin, which transports oxygen and carbon dioxide in the blood (Suedekum & Dimeff, 2005). Iron is also present in the muscle as a component of myoglobin, which extracts oxygen from hemoglobin molecules. Iron functions as an antioxidant, and is an important component of the electron transport chain for the production of ATP (Suedekum & Dimeff, 2005). Regular aerobic exercise results in depletion of body iron stores (Blum, Sherman, & Boileau, 1986; Jensen, Weaver, & Sedlock, 1991; Lyle et al., 1992). This is due to a variety of factors, including foot strike hemolysis, gastrointestinal blood loss, and iron loss via sweating. The most bio-available form of iron is found in meat rather than plant-based foods, making vegetarians at an even higher risk of iron deficiency (Suedekum & Dimeff, 2005). Due to these factors, iron deficiency is the most common nutritional deficiency in the United States (Cowell, Rosenbloon, Skinner, & Summers, 2003; Mercer & Densmore, 2005).

Anemia/Iron Deficiency

A low iron level may be caused by bleeding in the gastrointestinal tract or the uterus; vitamin or mineral deficiencies; decreases in red blood cell production; or an increase in red blood cell destruction (Venes, 2001). A lack of iron usually presents as either iron deficiency or anemia. Anemia refers to a reduction in circulating red blood

cells (Venes, 2001) and is commonly assessed measuring the hemoglobin (Hb) levels, however, defining anemia as a specific hemoglobin level is difficult and should be based on an individual's normal level (E. R. Eichner, 2007). A Hb level of <120g/L is often used as a diagnostic cutoff for anemia (Santolo et al., 2008; Sinclair & Hinton, 2005). Dietary iron deficiency causes several health problems such as anemia, hyperlipidemia, lipid perioxidation and changes in vitamin metabolism (Chiba et al., 1998; Ikeda et al., 2002; Uehara, Chiba, Mogi, Suzuki, & Goto, 1997). Dilutional pseudanemia, also known as sports anemia, is a benign temporary condition that develops secondary to training-induced plasma volume expansion and hemodilution (E. R. Eichner, 1992; Mercer & Densmore, 2005; Zoller & Vogel, 2004). Sports anemia is a cardinal feature of aerobic fitness (E. R. Eichner, 1992), and perhaps the most common form of anemia in athletes (Mercer & Densmore, 2005). Dilutional pseudanemia does not require iron supplementation (Suedekum & Dimeff, 2005), and normalization of hemoglobin has been noted within approximately 1 week after cessation of training (Convertino, Brock, & Keil, 1980).

However, true anemia in athletes is frequently due to iron deficiency (Beard & Tobin, 2000). Iron deficiency occurs in 3 stages. Stage I or prelatent anemia is associated with an isolated decrease in serum ferritin (<12μg/L), while hemoglobin levels remain normal, and iron stores become depleted. Stage II or latent anemia, also known as "iron-deficient erythropoiesis," is when iron stores fall further. During State II, transport iron becomes depleted resulting in serum iron and transferrin saturation decreases, and a rise in total iron binding capacity. In Stage III, further depletion of iron stores occurs, and an overt microcytic and hypochromic iron-deficient anemia develops, along with a decrease

in hemoglobin production below 12mg/dL in females (Akabas & Dolins, 2005; Balaban, 1992; Chatard, Mujika, Guy, & Lacour, 1999; Cook & Finch, 1979; Cowell et al., 2003; Herbert, 1992; Mercer & Densmore, 2005).

The main causes of iron deficiency/anemia are nutritional deficiencies due to a restricted diets and/or low intake of iron. This condition is often exacerbated by vegetarian and/or modified vegetarian diets (Mercer & Densmore, 2005). Gastrointestinal bleeding, hematuria, hemolysis leading to hemoglobinuria, thermalhemolysis, and menstrual losses are causes of blood loss and therefore iron loss. (Cowell et al., 2003; Mercer & Densmore, 2005; Suedekum & Dimeff, 2005). Iron loss can also come from sweating and iron malabsorption (Cowell et al., 2003).

Anemia can be particularly detrimental to athletes because the impairment of blood gas transport may decrease physical work capacity and may be a reason for fatigue, weakness, and dizziness (Santolo et al., 2008). A decreased work capacity (i.e. maximal oxygen use, VO_2max) caused by iron deficiency anemia in humans has been well documented (Celsing & Ekblom, 1986; Celsing, Blomstrand, & Werner, 1986; Gardner, Edgerton, & Senewiratne, 1977; Tufts, Haas, Beard, & Spielvogel, 1985). This decreased work capacity is attributed to insufficient oxygen transport by hemoglobin to peripheral tissues. Some contributing factors to anemia include: increased iron demand and loss, intravascular mechanical hemolysis (including foot-strike hemolysis), and dilutional pseudanemia (Beard & Tobin, 2000; Mercer & Densmore, 2005; Shaskey & Green, 2000).

Iron deficiency negatively impacts performance by impairing aerobic exercise, endurance capacity and/or energetic efficiency during submaximal exercise. Performance

is impaired because of reduced concentrations and activities of iron-containing oxidative enzymes. Performance is also impaired by increased muscle fatigability (Beard & Tobin, 2000; Brownlie IV, Utermohlen, Hinton, Giordano, & Haas, 2002; Brownlie IV, Utermohlen, Hinton, & Haas, 2004; Brutsaert et al., 2003; Hinton, Giordano, Brownlie, & Haas, 2000; Zhu & Haas, 1997; Zhu & Haas, 1998). If iron deficiency is undetected or left untreated it can develop into anemia (Santolo et al., 2008).

Screening

Serum ferritin (sFer) is an iron storage protein and it is used to estimate total body iron stores (Vander, Sherman, & Luciano, 2001), as well as an indicator of iron deficiency (Santolo et al., 2008; Sinclair & Hinton, 2005). Ferritin is viewed as the best overall indicator of iron status, since no condition other than iron deficiency has been shown to produce a low serum ferritin, and sFer levels are directly proportional to total iron stores (Cook & Finch, 1979; Herbert, 1992; Newhouse, Clement, Taunton, & McKenzie, 1989). However, being an acute phase protein, ferritin levels increase as a consequence of an infection or inflammation, (Hulthén, Lindstedt, Lundberg, & Hallberg, 1998) disorders of the liver, and malignancies, thus potentially masking actual iron deficiency (Santolo et al., 2008; Suedekum & Dimeff, 2005). Physical activity is accompanied by inflammation-like reactions in the joints and muscles, which may induce a rise in ferritin in plasma persisting for a few days following strenuous exercise (J. Malczewska, Błach, & Stupnicki, 2000; Nikolaidis, Michailidis, & Mougios, 2003; Taylor, Rogers, & Goodman, 1987). Another drawback is that serum ferritin reflects hepatic iron stores and not the functional iron present in myoglobin, iron-dependent

enzymes, or other iron-dependent proteins such as the cytochromes (Sinclair & Hinton, 2005).

While ferritin is a storage protein, transferrin is an iron transport protein, and a transferrin receptor (TfR) is a carrier protein for transferrin. Transferrin is required for importing iron into the cell and is regulated in response to intracellular iron concentration, thus it maintains cellular iron homeostasis (Vander et al., 2001). Soluble transferrin receptors are not an acute phase protein, and have low-biological variability and remain quite stable after exercise or a brief training period. The main limitation of the soluble transferrin receptor concentration is the lack of internationally recognized reference standards and comparable units to make comparisons between different assay kits (Schumacher, Schmid, König, & Berg, 2002).

The clinical presentation of iron deficiency with or without anemia can vary widely (Suedekum & Dimeff, 2005), and can include exertional fatigue, tachycardia, palpitations, headache, nausea and vomiting, diarrhea, and/or poor concentration and sleep (Suedekum & Dimeff, 2005). Also, impaired training and performance, intolerance to exercise, and difficult recovery after exercise may be early symptoms (Suedekum & Dimeff, 2005).

Simply using the cutoff of hemoglobin <12g/dL to define iron deficiency and anemia may not be appropriate, since it ignores the fact that anemia is relative and is best defined as a subnormal hemoglobin level for the individual (R. Eichner, 2001). If hemoglobin was checked without checking serum ferritin, an athlete could be improperly diagnosed with anemia because the low level could be to due to plasma volume expansion from false anemia (Cowell et al., 2003). For these reasons, it is important to

use both hemoglobin and ferritin as part of the screening process. Guyatt et al. found that patients who have ferritin less than 10-15µg/L are nearly always iron deficient (Guyatt et al., 1992). There is, however, no standardized cutoff value. In a survey by Cowell et al. of Division I schools cutoff values for defining iron deficiency included Hgb<12g/dl and serum ferritin<20ng/ml, serum ferritin<20ng/ml with a Hgb>12g/dl and Hgb<12g/dl only (Cowell et al., 2003). What was considered a low serum ferritin varied from <12ng/ml to <50ng/ml (Cowell et al., 2003). The most commonly used serum ferritin cut off value of <20ng/ml may not be stringent enough, since serum ferritin<20ng/ml suggests an absence of iron in the bone marrow (Nuviala, Castillo, Lapieza, & Escanero, 1996). Chatard and Nielsen suggests that serum ferritin levels between 30-35ng/ml need to be supplemented and may be a better cutoff value to use (Chatard et al., 1999; Nielsen & Nachtigall, 1998). Change in lean body mass was a significant predictor of serum ferritin, with a negative influence on iron status, that is, higher gains in lean body mass (LBM) were associated with lower ferritin concentrations (Ilich-Ernst et al., 1998). Other considerations in assessing iron deficiency are an athlete's BMI, sex, and sport, as it has been demonstrated that hematological values vary by these factors (Telford & Cunningham, 1991).(S. J. Young, Ross, & O'Dell, 2000)(S. J. Young, Ross, & O'Dell, 2000)

Prevalence

The prevalence of iron deficiency with and without anemia has been well documented. Iron deficiency without anemia is more common than iron deficiency anemia, affecting between 12-16% of premenopausal adult women and 2% of adult men (A. D. Looker, Cogswell, & Gunter, 2002; A. C. Looker, Dallman, Carroll, Gunter, &

Johnson, 1997). The prevalence of iron deficiency in adolescent and adult females was 25-35% for athletes competing in a variety of sports (Constantini, Eliakim, Zigel, Yaaron, & Falk, 2000; Dubnov & Constantini, 2004; J. Malczewska, Raczynski, & Stupnicki, 2000; J. Malczewska, Szczepańska, Stupnicki, & Sendecki, 2001).

Santolo and colleagues studied the effects of regular physical activity on iron status. The purpose of the study was to compare frequency of anemia and iron deficiency in a sample of women aged 18-35 who were not taking supplements containing iron, had no menstrual dysfunction and participated in 11.1±2.63h/wk of physical activity. They found that athletes had a significant decrease in serum iron and transferrin saturation (TfS), while transferrin and ferritin did not differ between groups. Physical activity was not related to anemia, iron deficiency anemia, low-hematocrit, low-ferritin levels, or elevated transferrin. Conversely, physical activity was associated with a three-fold higher frequency of low-serum iron, and with a two-fold higher frequency of transferrin saturation. From these result, Santolo et al. observed a rate of 15.7% anemia, 27.1% iron deficiency, 8.6% iron deficiency anemia (Santolo et al., 2008). Sinclair et al. evaluated the prevalence of iron deficiency with and without anemia in males and females aged 18-41, who endurance trained for an average of 10.5±6.3hrs/wk. Seven females and one male had iron deficiency with anemia. They found that 29% of females and 4% of males had iron deficiency when determined by a serum ferritin level of ≤16µg/L. When using the transferrin receptor-ferritin index they found 36% of females and 6% of male to be iron deficient without anemia (Sinclair & Hinton, 2005).

In a study of 85 female marathon runners, 16% had low ferritin level of <40ng/mL; only 2% had iron deficiency anemia (Matter, Stittfall, & Graves, 1987). In a

group of trained adult swimmers, 57% of women were found to be iron deficient based on serum ferritin levels lower than 15μg/L, while only 1 out of the 15 women had iron deficiency anemia (Selby & Eichner, 1986). Hallberg et al. determined that the prevalence of iron deficiency in adolescent girls in Sweden, could be up to 40% using the cut off value of 16 μg/L serum ferritin (Hallberg et al., 1993).

It appears that the prevalence of iron deficiency anemia is greater in athletes than the general public and greater in women than men (11-14 vs. 3-5% in women childbearing age and 2-3 vs. <1% in young men, respectively) (Dubnov & Constantini, 2004; A. C. Looker et al., 1997; Sinclair & Hinton, 2005).

Treatment

It is agreed that athletes who have documented iron deficiency anemia should be treated with iron replacement therapy, typically with oral ferrous sulfate (Mercer & Densmore, 2005). Of the institutions surveyed by Cowell and colleagues all reported providing iron supplements to female athletes that were found to be iron deficient (Cowell et al., 2003). Ferrous sulfate is the most often used, followed by a combination of ferrous sulfate and a multivitamin containing iron (Cowell et al., 2003). Thirty-nine milligrams elemental iron per day (125mg ferrous sulfate) is sufficient to maintain serum ferritin (Beard & Tobin, 2000). Thirty-seven percent reported a dose of >300mg/d (>60mg elemental iron as ferrous sulfate), 26% reported providing between 51-100mg/d (Cowell et al., 2003). However, noncompliance with treatment can be a significant issue with doses of >50mg of elemental iron per day, since such doses are associated with increased side effects, such as nausea and constipation. The effect of iron supplementation is variable from one person to another, so the length of treatment is most

often individualized (Newhouse et al., 1989; Nuviala et al., 1996). However, it has been suggested that iron repletion takes at least 3 months, even in those without pathological iron loss (Nielsen & Nachtigall, 1998). Although improvement in hemoglobin and ferritin levels may only take several weeks, a minimum of 6-12 months of therapy is usually required (Mercer & Densmore, 2005).

Dietary Advice

Inadequate dietary iron intake is considered the primary cause for iron deficiency in both female athletes and non-athletes (Nuviala et al., 1996); therefore, adequate iron intake through diet should be the primary tool for preventing iron deficiency (Beard & Tobin, 2000; Centers for Disease Control and Prevention, 1998; Centers for Disease Control and Prevention, 1998; Peterson, 1998). Overall the schools surveyed by Cowell at al. reported providing dietary advice to the athletes (Cowell et al., 2003). Some information commonly provided to athletes includes: literature regarding iron rich foods, individual counseling concerning iron rich foods, guidelines on how to take the supplements, information on foods that inhibit iron absorption, and encouragement to include more meat in the diet (Cowell et al., 2003). Three major nutrients/compounds known to inhibit iron absorption are tannins, (found in tea, wine, and some fruits, berries and nuts); phytates (found in whole grains and also affect the absorption of other minerals including calcium, magnesium, and zinc) and calcium (found dairy products). Hallberg et al. found that the interference that calcium causes in iron absorption is largely dose dependent and that the effect seems to be similar between heme and non-heme iron (Hallberg, Rossander-Hulten, Brune, & Gleerup, 1992). Thus, the composition of a meal and/or a supplement may contribute to mineral deficiencies (Gropper, Blessing, Dunham,

& Barksdale, 2006; Hallberg et al., 1992). Three proposed ways to manage the interaction calcium has on iron absorption are as follows: reducing the calcium content of the diet assuming requirements are still met; increase iron intake and/or increasing the bioavailability of the dietary iron by reducing the intake of foods containing other inhibitors and/or changing meal composition in order to keep calcium intake low in the meal(s) that provide the most dietary iron (Hallberg et al., 1992). Ascorbic acid (found in citrus fruits and juices) and meats enhance iron absorption. However, meat is unique in that it enhance the absorption of both types of iron, while other dietary factors that influence iron absorption only effect non-heme iron and have no effect on heme iron (Gropper et al., 2006).

Iron and Bone Mineral Density

Bone health depends on mechanical, hormonal, nutritional, and genetic factors (Pepper & Saint-Phard, 2007). While, calcium is the nutrient most thought of when talking about bone health, there are several key factors that contribute to bone health. These include vitamin D, vitamin C, copper, iron, protein, estrogen and weight bearing exercise (Heaney, 2000; Hunter et al., 2001; Medeiros, Plattner, Jennings, & Stoecker, 2002; South-Paul, 2001; Swaminathan, 1999). Several studies have been conducted on rats demonstrating an interaction between iron deficiency and bone physiology. However, fewer studies have been done showing the same association between dietary iron intake and bone mineral density (BMD) in human populations and to our knowledge have only been done in healthy postmenopausal women (Harris et al., 2003; J. Malczewska et al., 2000).

There are two hypothesized mechanisms by which iron deficiency could decrease BMD and mechanical bone strength; however there could be more, that are yet to be understood. Iron is a required cofactor for prolyl and lysyl hydroxylase enzymes in collagen synthesis. This step is essential for lysyl oxidase activity, which then catalyzes the cross-linking of adjacent collagen fibers, and is an important part of collagen maturation (Tuderman, Myllyla, & Kivirikko, 1977). Thus, in iron deficiency there may be less iron available to the prolyl and lysyl hydroxylase enzymes, which could result in decreased cross-linking activity and weaker collagen fibers (Tuderman et al., 1977). In addition, renal 25-hydroxyvitamin D hydroxylase, which converts 25- hydroxyvitamin D to the active form of vitamin D, is located in the mitochondria and is a three-component system involving a flavoprotein, an iron-sulfur protein, and a cytochrome P-450 (DeLuca, 1976). Consequently, these iron-dependent enzyme activities might become lower, causing bone loss in iron-deficient anemia (Katsumata, Tsuboi, Uehara, & Suzuki, 2006).

Serum osteocalcin concentrations were measured as a biochemical maker of bone formation (Katsumata et al., 2006; Katsumata, Katsumata-Tsuboi, Uehara, & Suzuki, 2009). Osteocalcin is a major noncollagenous protein of the bone matrix and is synthesized and released from osteoblasts (Brown, Delmas, & Malaval, 1984). Serum osteocalcin concentration in the iron deficient individuals can be reduced to about 56% of normal (Katsumata et al., 2006), suggesting that dietary iron deficiency might decrease bone formation via a decrease in osteoblast function (Katsumata et al., 2006). This finding supports the hypothesis that iron deficiency may decrease bone metabolism through the renal 25-hydroxyvitamin D hydroxylase response (Katsumata et al., 2006).

The assessment of bone resorption was evaluated using urinary C-terminal telopeptide of type I collagen (CTx) and urinary deoxypyridinoline (DPD) markers, which are released into the body fluids during type I collagen degradation. Urinary excretions of CTx and DPD are useful for showing changes in bone resorption (Bonde, Qvist, Fledelius, Riis, & Christiansen, 1994; Calabresi et al., 1994). However, Katsumata et al. had conflicting results when evaluating bone resorption by CTx and DPD. The reason for this discrepancy is not clear; as a result Katsumata et al could not determine bone resorption from these measurements exclusively. Therefore, they needed to measure the number of osteoclast by bone histomorphometry in iron deficiency to evaluate bone resorption (Katsumata et al., 2006).

In order to clarify the manner in which dietary iron deficiency decreases BMD, Katsumata et al. conducted another study investigating the changes in bone formation and bone resorption by measuring the markers of bone turnover and bone histomorphometry in rats fed an iron-deficient diet (Katsumata et al., 2009). Bone histomorphometric parameters, such as mineralizing surface (MS/BS), mineral apposition rate (MAR), bone formation rate (BFR/BS), and adjusted apposition rate (Aj.Ar.) were significantly lower in the iron deficient group than in the control and pair-fed groups (Katsumata et al., 2009). These results suggested that dietary iron deficiency clearly decreases BFR/BS, which causes decreases in BMC and BMD (Katsumata et al., 2009).

Another important factor in bone formation is IGF-I, which is secreted in the bone (Katsumata et al., 2009). Katsumata et al. found that serum IGF-I concentration was significantly lower in the ID group than in the control and pair fed groups (Katsumata et al., 2009). In a previous study, IGF-I deficient mice showed reductions in bone size and

BFR/BS compared with wild-type mice (Bikle et al., 2001). Therefore, it can be assumed that the decreased serum IGF-I concentration might be one of the factors suppressing bone formation in the iron deficient rats (Katsumata et al., 2009).

A decrease in food intake and final body weight in iron-deficient rats was thought to have contributed to the decreases in BMC and BMD, and mechanical strength of the femur (Katsumata et al., 2006). However, the level of iron used in Parelman et al. study did not result in body weight change, eliminating this potentially confounding variable (Parelman, Stoecker, Baker, & Medeiros, 2006). Katsumata et al. completed a follow up study that supported Parelman et al. findings by indicating that an iron-deficient diet could be a dominant cause of bone loss independent of decreases in food intake and body weight, as supported by the lower BMC and BMD values in the iron deficient group compared with the pair-fed group (Katsumata et al., 2009).

Iron's biological importance to bone is related to its role as a cofactor for hydroxylases in collagen synthesis (Prockop, 1971). It has also been postulated that iron deficiency could decrease hydroxylation of 1,25 dihydroxcholecalciferol and lead to a decline in calcium absorption from the gut (Medeiros et al., 2002). 1,25 dihydroxcholecalciferol is the active form of vitamin D found in the body. Vitamin D increases the absorption of calcium and phosphate from the GI tract and kidneys; it also inhibits the release of calcitonin (Venes, 2001).

Iron and Bone Mineral Density in Humans

Multiple animal studies have demonstrated the effect of iron deficiency on bone physiology (Katsumata et al., 2006; Katsumata et al., 2009; Parelman et al., 2006), but fewer studies in the human population have been completed showing the same

relationship between dietary iron intake and bone mineral density (Harris et al., 2003; J. Malczewska et al., 2000). A cross-sectional analysis (a set of measurements of a population at a particular point in time (Thompson & Subar, 2008) indicated that dietary iron intake was positively associated with baseline BMD in healthy nonsmoking postmenopausal women (Harris et al., 2003). In a longitudinal study of women on hormone replacement therapy (HRT) a significantly higher baseline BMD at all 5 bone sites (lumbar spine L2-L4, femur trochanter, femur neck, Ward's triangle, and total body) was noted compared to women who were not on HRT (Maurer et al., 2005). Iron was positively associated with changes in BMD at the greater trochanter and Ward's triangle, but not with the femoral neck. After calcium intake was added to the model iron remained positively associated with change in BMD at the greater trochanter and Ward's triangle, indicating that iron is independently associated with change in BMD at these bone sites (Maurer et al., 2005). Iron and iron plus calcium associations with 1-y BMD change was maintained at the greater trochanter and Ward's triangle in women using HRT. However, BMD was not maintained in women who were not using HRT (Maurer et al., 2005). When looking at the association of calcium on BMD changes in all women, they found that calcium was only associated with femoral neck BMD (Maurer et al., 2005). After adding iron to the model, the associations of calcium and of BMD change, remained significant, indicating that calcium has an independent effect on the femoral neck (Maurer et al., 2005). However, after adding iron, there was a negative effect on the greater trochanter, leading to the conclusion that iron may modify the relationship between calcium and BMD (Maurer et al., 2005).

The results from examining the calcium relations by HRT status support the hypothesis of Maurer et al. that HRT use influences the relationship of nutrients on BMD changes (Maurer et al., 2005). In women not using HRT, calcium was positively associated with change in femoral neck BMD, but negatively associated with change in the greater trochanter BMD. However, there was no significant relationship found between calcium and women using HRT, suggesting perhaps that HRT may override any effect of calcium on BMD (Maurer et al., 2005). Regardless of HRT use, iron alone or in combination with calcium, accounted for between 3 and 9% of variance in BMD change, while calcium alone or in combination with iron, accounted for between 6 and 10% of variance in BMD regardless of HRT status (Maurer et al., 2005).

Harris et al. found that fat-free mass, fat mass, and intake of energy, protein, calcium and iron were positively correlated with all bone sites (Harris et al., 2003), along with calcium and iron being intercorrelated (r=0.34; P-0.01) (Harris et al., 2003). Iron had a threshold effect at an intake range of 10-20mg on BMD among women; these results show that there is a complex relationship between iron and calcium on BMD (Harris et al., 2003). D'Amelio et al. found that iron metabolism correlated with lumbar and femoral neck BMD, and with calcium and phosphorus metabolism as well as transferrin being significantly correlated with lumbar and femoral neck BMD (D'Amelio et al., 2008).

A clear association between bone and iron status has not been demonstrated in humans (D'Amelio et al., 2008). However, a trend between BMD at the radius and serum ferritin has been reported in adolescent females (Ilich-Ernst et al., 1998). Also, two human studies have reported a beneficial effect of iron on adult BMD (Angus et al.,

1988; Michaelsson et al., 1995). Angus et al. found a positive association of iron intake on femoral neck BMD and forearm BMC in premenopausal but not postmenopausal women, however, they used a less precise method of measuring BMD (dual photon absoptiometry) (Angus et al., 1988). Michaelsson et al. also found a positive association between iron intake from weighed dietary records with femoral neck and lumbar and total bone mineral density (Michaelsson et al., 1995). However, neither study discussed the biological significance or the potential mechanism(s) behind these associations of iron with BMD (Maurer et al., 2005).

There is a complex relationship between iron and calcium as they compete for absorption and their roles in BMD (Harris et al., 2003). Women who consumed 800-1200mg of calcium had significantly higher BMD with increasing levels of iron intake, whereas women with higher or lower intakes of calcium, the relationship of iron to BMD was not apparent (Harris et al., 2003). If the iron and calcium relationship exits in relation to bone density in the general population, dietary recommendation for iron may have to be reassessed (Harris et al., 2003). Thus, an increase in iron intake may be necessary to prevent stress fractures especially in special populations such as the elderly, elite female athletes and female military recruits who have compromised iron status (Harris et al., 2003). Iron and calcium might not be the only predictors of BMD levels.

Other major determinants of BMD are age and BMI and possibly genetic constitution (Mundy, 1994). Mudd et al. found that among the possible explanatory variables (age, gynecologic age, height, mass, BMI, sport, and oral contraceptive use) only mass and sport consistently predicted BMD (Mudd et al., 2007). Mass and sport were significant predictors of total-body, pelvic, and average leg BMD, while mass,

sport, and gynecological age predicted lumbar spine BMD in female collegiate athletes (Mudd et al., 2007).

Nutritional Aspects

Even if an athlete is consuming the correct amount of micronutrients (especially if she is supplementing with a vitamin-mineral supplement), if energy requirements are not being met, athletic performance will most likely be suboptimal (Volpe, 2007). Exercise type (ie. weight-bearing vs. non-weight bearing) appears to influence the osteogenic response and can lead to either higher or lower values of BMD at different sites (Duncan et al., 2002). Weight bearing exercise in female athletes usually increases BMD and lean body mass, which may help to prevent stress fractures and osteoporosis later in life (Creighton, Morgan, Boardley, & Gunnar Brolinson, 2001; Fehling et al., 1995; Meyer et al., 2004; Quintas, Ortega, López-Sobaler, Garrido, & Requejo, 2003; Taaffe et al., 1995). Strength based and high impact sports also seem to be associated with higher BMD, where as non-weight bearing sports have a neutral or negative relationship (Egan et al., 2006; Fehling et al., 1995). Decreased BMD increases the risk for stress fractures (Myburgh, Hutchins, Fataar, Hough, & Noakes, 1990) and osteoporosis later in life (Cann et al., 1984; Drinkwater, Bruemner, & Chesnut III, 1990; N. Young et al., 1994). Petrie et al. identified female distance runner as athletes at highest risk for mineral deficiencies (i.e. iron and calcium) (Petrie, Stover, & Horswill, 2004).

Swimmers and divers tend to have lower average leg BMD values than other athletes (Mudd et al., 2007) (Creighton et al., 2001; Fehling et al., 1995). It would be expected that distance runners would have higher BMD because of the weight bearing nature of the sport. However, runners also demonstrated lower BMD at several sites

when compared with athletes in the other sports and had the lowest total-body, lumbar spine and pelvis BMD values (Mudd et al., 2007). Some possible explanations for this discrepancy are despite that fact that running is a high impact weight bearing sport, female runners tend to have a low calcium intake, disordered eating, or insufficient energy intake relative to energy expenditure or a combination of these factors (Mudd et al., 2007).

Acute or chronic energy deficiency elicits metabolic aberrations which can lead to inadequate bone formation or excessive bone resorption (Grinspoon, Baum, Peterson, & Klibanski, 1995; Laughlin & Yen, 1996). Mal-nutrition and its metabolic consequences are responsible for precipitating a bone remodeling imbalance which may lead to bone loss in young women with exercise associated amenorrhea (Zanker, 1999). However, it has been shown that disordered eating and energy deficits are related to low BMD even without menstrual disturbances (Zanker & Cooke, 2004). Disordered eating behavior has been identified as the predominant factor in causing osteopenia (Miller, 2003). Menstrual status determined the bone mineral density at three measurement sites; and therefore the effect of disordered eating to reduce BMD was explained by its relationship to menstrual irregularities (Gibson, Mitchell, Harries, & Reeve, 2004). Active amenorrhoeic women will continue to lose bone while their menstrual disturbance persists, however their BMD will stabilize or increase if their menses resumes (Drinkwater et al., 1986).

No significant correlation was found between bone mass and current calcium intake for either premenopausal or postmenopausal women (Angus et al., 1988). An explanation for this is that peak bone mass is achieved, usually by the fourth decade, and is likely to be an important determinant of risk of fracture in later life (Garn, Rohmann, &

Wagner, 1967; Krolner & Nielsen, 1982). Therefore, the assessment of calcium intake up to early adulthood may be more relevant with regard to effects on bone mass than calcium intake in later life (Angus et al., 1988). Michaelsson et al. found no statistically significant relation between nutrients calculated from the dietary records and total body BMD after multivariate adjustment (Michaelsson et al., 1995). They did find a higher dietary intake of protein and calcium among women with a higher total body BMD (Michaelsson et al., 1995). However, when participants were stratified according to menopausal status, the association with calcium persisted only among the premenopausal women (Michaelsson et al., 1995). Protein was positively related to BMD of the femoral neck, and total body (Michaelsson et al., 1995).

Angus et al. found that iron intake was positively correlated with forearm BMC in premenopausal women (Angus et al., 1988). The recommended daily allowance of iron for premenopausal women is 18mg daily(Akabas & Dolins, 2005), with a tolerable upper limit set at 45mg daily for all adults to avoid the gastrointestinal distress often experienced with doses above this amount (Institute of Medicine, Food and Nutrition Board, 2001). Gropper at al. found that 25% of athletes did not consume two thirds of the recommended daily allowance (RDA) for iron. These athletes displayed suboptimal serum ferritin, iron, or transferrin saturation concentrations (Gropper et al., 2006). Athletes whose serum ferritin concentration were 15μg/dL or less also displayed a serum iron concentration of less than 60μg/dL and transferrin saturation of less than 16%, both below normal (Volpe, 2007). Serum ferritin showed no significant association with BMD or content of the total body at baseline or during the first three years of follow up, however a positive association was seen during the fourth year between ferritin and bone

variables that was nearly significant, but only in the placebo group (Ilich-Ernst et al., 1998). The association between serum ferritin concentration and bone mineral density of the radius was significant only at baseline although no significant associations were found between bone mass and iron stores (Ilich-Ernst et al., 1998).

Calcium in food or as a supplement may decrease iron absorption by as much as 50% (Hallberg et al., 1992; Monsen & Cook, 1976). However, the inhibitory effect of calcium is influenced by the compounds containing calcium and iron, the amount consumed, and whether the minerals are consumed separately or as part of a meal (Ilich-Ernst et al., 1998). Ilich-Ernst et al found no effect of calcium supplementation in the form of calcium citrate malate on the iron status of adolescent girls, as assessed by serum ferritin and red blood cell indexes (Ilich-Ernst et al., 1998).

Vitamin C was positively correlated with serum ferritin in a study of girls who were at the beginning stages of puberty (stage 2). (Ilich-Ernst et al., 1998). Hallberg et al. noted that ascorbic acid significantly increased the absorption of non-heme iron by an average of 20%, but did not affect the absorption of heme iron (Hallberg & Sölvell, 1967). While Hunt et al. found that vitamin C supplementation did not improve iron absorption in women between the ages of 20- 45 years (Hunt, Gallagher, & Johnson, 1994).

Spinal BMDs of vegetarians were similar to those of the non-vegetarians, while femoral neck BMDs of vegetarians was slightly lower than non-vegetarians, although not significantly (Kim, Choi, & Sung, 2007). No significant differences were seen in serum levels of macrominerals such as calcium, phosphorus, magnesium, and iron between vegetarians and non-vegetarians (Kim et al., 2007). Vegetarians' serum levels of zinc,

copper, and ferritin were significantly lower than those of non-vegetarians (Kim et al., 2007). While urinary level of DPD, a biochemical marker of bone resorption, were significantly higher in non-vegetarians than in vegetarians (Kim et al., 2007). In vegetarians iron and copper intakes were positively correlated with BMD of the femoral neck (Kim et al., 2007).

Calcium Assessment

When investigating bone health it is important evaluate dietary calcium intake (Henry & Almstedt, 2009). The main purposes of the rapid assessment method (RAM) are to: be user-friendly for the participants; accurately evaluate daily calcium intake; and to be cost and time effective (Henry & Almstedt, 2009). Vitamin and mineral supplements represent a significant component of dietary intake in some individuals and especially in athletes, making it important to include supplementation in questionnaires and RAMs (Henry & Almstedt, 2009). Roetert found that 89% of 203 collegiate athletes surveyed had taken or were currently taking dietary supplements (Roetert, 2006). Henry and Almstedt developed the modified Loyola Marymount University (LMU) RAM, designed for young, athletic individuals who frequently consume supplement. To validate their RAM they compared it to a three day diet record (3DR) in college athletes and controls. The mean calcium intakes did not differ significantly between the two methods, a strong positive correlation was seen between calcium intake measured with the LMU RAM and 3DRs, and agreement between the two was good (Henry & Almstedt, 2009). From these results is appears that the LMU RAM is a valid tool for use in assessing calcium intake.

Summary

Both iron deficiency/anemia and low bone mineral density in female athletes has been well documented (Constantini et al., 2000; Dubnov & Constantini, 2004; J. Malczewska et al., 2000; J. Malczewska et al., 2001). A relationship between low iron and BMD has been seen in rats and in postmenopausal women (Harris et al., 2003; Katsumata et al., 2009); however the same relationship has not been studied in female athletes (D'Amelio et al., 2008). Since iron deficiency and low BMD are both concerns in the female athlete and a relationship has been seen in other populations it is logical to examine this relationship in female athletes.

METHODOLOGY

Participants

Sixty-six female Division I collegiate athletes participating in weight bearing sports, who entered James Madison University as a freshman were invited to participate in the current study. Thirty (n = 30) of these individuals volunteered to participate in the study. Participants were excluded from the study if they were currently taking an iron supplement, had missed more than 3 consecutive days of training during the weeks prior to the study, were pregnant within the past year or ammenoreic for 3 or more consecutive months and/or had a recent illness in the past month that resulted in a fever that inhibited their current training schedule. All risks and procedures were fully explained, participation in this study was strictly voluntary and a letter of informed consent was obtained from all participants. Approval for the study was received from the Institutional Review Board for the use of Human Subjects in Research at James Madison University.

Research Design

Participants who met the selection criteria during pre-participation physical exams had their blood drawn by a trained health professional. The blood draw and DEXA scan occurred within 7 days following the end of the participant's monthly menses. Menstrual history and osteoporosis questionnaires were completed at the time of recruitment. Participants completed a 7-day physical activity recall and a food frequency questionnaire at the time of the blood draw and the DEXA scans. DEXA scans were performed to determine bone mineral density. Participant's height was measured to the nearest 0.25 inch and weight to the nearest 0.5lb. All measurements were taken using standard techniques.

Blood Analysis

Finger Prick

A finger prick was utilized to collect blood for the measurement of hematocrit (Hct), and hemoglobin (Hb). A 10µl whole blood sample was collected and analyzed for hematocrit and hemoglobin (HemoPoint H2 machine, Stanbio Laboratory).

Venous blood draw

A venous blood draw was done via venapuncture of the antecubital vein while the participants were either seated or lying down. Approximately 20mL of blood was obtained from the blood draw, and was divided into two 10mL serum collection tubes. After sitting for at least 30 minutes, the tubes were centrifuged for 20 minutes at 3000 RPM and 4°C. Serum was extracted from the samples and frozen in serum tubes at -80ºC. Samples were stored and later analyzed for serum ferritin and hsCRP.

Assays

Serum ferritin was analyzed using serum ferritin receptor enzyme immunoassays. Serum ferritin levels were measured through an enzyme immunoassay procedure (Ramco Laboratories; Stafford, TX). Manufacturer guidelines dictated the immunoassay analysis procedure. High sensitivity C reactive protein was be analyzed using an enzyme-linked immunosorbancy assay (ELISA). Manufacturer guidelines were followed for analysis (Oxis International Inc., Foster City, CA).

DEXA Scan

Dual-energy x-ray absorptiometry (DEXA) was used to assess bone mineral density as well as make estimates of bone, fat, and muscle tissue. Four DEXA scans were utilized to

determine bone mineral density. A full body scan was utilized to evaluate total bone mineral density (BMD) and body composition. Scans of the right and left hip were taken as well as of the lumbar spine. Female endurance athletes are at increased risk for low BMD and these scans provided specific information related to the risk for osteoporosis in the right and left hip. The fourth scan was of the lumbar spine, which is often the first site for the development of low BMD (osteopenia-osteoporosis). Participants were asked to wear clothing that did not contain any type of metal. Each participant was additionally asked to remove all metal jewelry, watches, eye glasses etc. before the scans took place.

Forms and questionnaires

Participants completed a food frequency questionnaire that was utilized to determine average dietary iron, calcium, vitamin D and caloric consumption. Additionally, participants were asked to complete a 7-day physical activity recall, a pre-participation form, a menstrual history form and an osteoporosis form to determine study eligibility and menstrual cycle information.

The Food Frequency Questionnaire (Appendix A) was used to estimate total caloric intake, calcium intake, iron intake, and vitamin D intake. The **7**-day physical activity recall (Appendix C) was used to determine physical activity levels. The Menstrual Status Questionnaire (Appendix B) was used to determine if a participant had a menstrual dysfunction. The Osteoporosis Questionnaire (Appendix D) was used to determine family history of osteoporosis.

Statistical Analysis

Statistical analysis was carried out using Statistical Package of Social Sciences (Version, 19). Descriptive statistics on anthropometric characteristics were calculated for the total

group. Pearson correlation coefficients were calculated for sFer, BMD of 4 different bone sites (total body, lumbar, hip and Wards triangle), and nutritional intake of iron, calcium and vitamin D. A correlation was consider significant at an alpha level of $p<0.05$.

RESULTS

Thirty female athletes from 8 teams (soccer [n=4], volleyball [n=2], field hockey [n=1], lacrosse [n=3], softball [n=6], basketball [n=1], cross country [n=8], track and field [n=5]) participated in the study. Eight subjects withdrew due to various reasons including injury, placement on iron supplement, or amenorrhea. Participant characteristics are shown in Table 1. A review of subject's demographics, including BMI reveals that the subjects were of normal height and weight for age and sex (50^{th} percentile for BMI). General health status markers are listed in Table 2 and nutritional intake is summarized in Table 3. Correlation analysis indicated there were no relationships between serum ferritin levels and BMD for total body, lumbar spine, hip or Ward's Triangle. There was also no correlation between serum ferritin and dietary intake levels of iron, calcium and vitamin D. In addition no correlation was found between BMD at any site and intake levels of iron, calcium and vitamin D in the study participants. The correlation values are reported in Table 4. Due do the potential of a non-linear correlation, the sample was divided into 2 groups, those with sFer levels below 20µg/L(Malczewska et al., 2000), and those with sFer levels above 20µg/L. No correlation was seen with the sample divided. An independent T-test was also done to evaluate if a threshold effect was present between the group with sFer below 20µg/L and sFer above 20µg/L. This showed no significant difference in mean BMD at any site, compared to sFer levels.

Table 1 Demographic Characteristics (n=30)

Characteristics	Mean ± SD	Min	Max
Age	18.1±0.3	17	19
Height (in)	65.98±3.00	60.75	74
Weight (lbs)	138.7±23.4	92	199.5
BMI (kg/m^2)	22.3±2.8	17.472	30.62
Body Fat (%)	24.11±4.87	15.4	33.3

Table 2 Health Status

	Mean ± SD	Min	Max
Hemoglobin (g/dL)	12.5±1.2	9.7	14.8
Hematocrit (%)	38.6±2.5	35	44
Ferritin (µg/L)	24.34±9.90	13.69	57.43
Total BMD (g/cm^2)	1.180±0.078	1.012	1.320
BMD Left Hip (g/cm^2)	1.083±.0134	0.9	1.497
BMD Left Ward's Triangle (g/cm^2)	0.947±0.172	0.753	1.402
BMD Right Hip (g/cm^2)	1.081±0.1239	0.886	1.363
BMD Right Ward's Triangle (g/cm^2)	0.933±0.189	0.623	1.432
BMD Lumbar Spine (g/cm^2)	1.069±0.0984	0.8250	1.289

n=16 for hematocrit measurements due to equipment errors

Table 3 Nutritional Intake (n=28)

	Mean ± SD	Min	Max
Total Kcals	2929±966	1245	5644
Protein (g)	123.4±45.9	33.8	239.8
Carbohydrates (g)	361.6±112.4	103.4	648.7
Fat (g)	116.0±49.7	54.5	256.0
Iron (mg)	26.0±8.5	5.3	39.3
Calcium (mg)	1342.4±374.8	539.0	2129.4
Vitamin D (ug)	5.1±2.4	0.05	9.7

Table 4 Correlations Coefficients (R values) Relationship between sFer, nutritional intake and BMD

	Ferritin	Iron Intake	Calcium Intake	Vitamin D Intake
Ferritin	-	-0.027	-0.089	0.199
Total BMD	-0.038	-0.056	-0.050	0.004
BMD Left Hip	0.055	0.174	-0.191	-0.106
BMD Left Ward's Triangle	-0.147	0.137	-0.178	-0.189
BMD Right Hip	0.008	0.214	-0.236	-0.166
BMD Right Ward's Triangle	-0.066	0.064	-0.210	-0.162
BMD Lumbar Spine	0.157	0.054	0.029	-0.018

No significant correlations ($p>0.05$)

DISCUSSION

The purpose of this study was to determine if there was a relationship between serum ferritin levels and bone mineral density (BMD) for total body, lumbar, hip and Ward's triangle in college age female athletes who participated in weight bearing sports. In addition the study was designed to determine if there was a relationship between dietary intake of iron, calcium and vitamin D to BMD at specific anatomical sites that are prone to fractures in women.

No correlation was found between serum ferritin (sFer) levels or iron intake and BMD for total body, lumbar, left or right hip or Ward's triangle. To the author's knowledge this is the first investigation to explore the potential relationship between sFer level and BMD in humans. However, Katsumata et al. have thoroughly evaluated this relationship in rats (Katsumata et al., 2006). They investigated the effects of dietary iron deficiency on bone metabolism by measuring markers of bone turnover. To do this they fed one group of rats a control diet and another group an iron-deficient diet for 4 weeks. They reported that dietary iron deficiency decreased BMD and bone mineral content (BMC) in rats. Serum osteocalcin concentration, (a maker of bone formation) in the iron deficient group was reduced to approximately 56% of the control group (Katsumata et al., 2006; Katsumata et al., 2009). This suggests that iron deficiency might decrease bone formation via a decrease in osteoblast function. The results from the Katsumata et al. studies, led to the development of the hypothesis for the current study.

Results of previous studies indicated that twenty-five to thirty-five percent of adolescent and adult females athletes that competed in a variety of sports were iron deficient (Constantini et al., 2000; Dubnov & Constantini, 2004; Malczewska et al.,

2000; Malczewska et al., 2001). A slightly higher percentage was found in the current participants, with a rate of 37% being ID when the cut-off value of 20µg/L sFer was used (Dubnov & Constantinni, 2004; Malczewska et al., 2000). Participants in the current study had comparable height, weight, age and BMIs of those in other studies referenced above. Although a correlation was not found between sFer levels and BMD, this cannot be generalized to other populations, nor can it be assumed from our data that iron deficiency does not influence BMD. The lack of a correlation between sFer and BMD in the current study may be due to the adequate caloric, iron, and calcium intake of the participants. In view of the fact that this phenomena has not been studied in a large cross section of athletes or non-athletes, further study is warranted to determine if sFer levels are related to BMD.

From the results of the food frequency questionnaires it appears that the participants in the current study are entering the university with eating habits that provide adequate nutrition, as their intake of the assessed micro and macro nutrients either met or exceed recommended values. The results of previous studies of female athletes' dietary behaviors have indicated that they tend to have inadequate caloric intake, which results in intakes of iron and calcium below the recommended daily allowances (Angus et al., 1988; Hawley, Dennis, Lindsay, & Noakes, 1995; Perron & Endres, 1985). Perrson and Endres found that in a group of adolescent female athletes the mean caloric intake was less than recommended and that iron and calcium intakes were less than 67% of the RDA (Perron & Endres, 1985). However, on average, the participants in the current study met all the micro-nutrient requirements that were examined; this could be largely attributed to their adequate intake of calories. That is, if caloric needs are being met it is more likely

that micronutrient needs will also be met. Therefore, this sample was not at an increased risk of having iron deficiency and/or anemia or for low BMD, due to nutritional deficiencies. It is important to note that when self reporting dietary intake, there is a 10-15% potential error for over-reporting intakes. With this taken into consideration, the participants in the current study still met the RDAs for critical nutrients. It is quite common to see nutritional deficiencies and inadequate intakes in female athletes, especially in image focused sports such as gymnastics, and figure skating. This, however, was not the case with the current sample, whose average caloric intake was 2929 Kcals/day.

No correlation was found between iron intake and BMD at any site in the current study. At the present time there is a paucity of studies that have examined the relationship between BMD and iron intake in humans (Angus et al., 1988; Harris et al., 2003; Michaelsson et al., 1995). Angus and colleagues found a significant positive relationship between iron and BMC at the forearm in pre-menopausal women (Angus et al., 1988). However, Angus et al. used a dual photon absorptiometer (DPA) to measure BMD, which is considered a less accurate method of measuring bone mass compared to DEXA utilized in the current study. Wahner et al. compared DEXA and dual photon absorptiometry for bone mineral measurements of the lumbar spine (Wahner, Dunn, Brown, Morin, & Riggs, 1988). They obtained measurements of phantoms and lumbar spines of patients to study accuracy, precision, limitations, and compatibility of results between instruments. For lumbar bone mineral measurements, the DEXA instrument had a shorter scanning time and higher resolution images, superior precision and reduced influence of thickness for patients measurements compared to the DPA system. For measurements of the

regions of interest, accuracy was better with the DEXA instrument, as well. Bone mineral density measurements in patients were consistently lower with the DEXA instrument because of better accuracy in area specific measurements. Michaelsson et al. examined iron in relation to BMD of total body, spine and femoral neck in women ranging in age from 28 to 74 years. Dietary iron was associated with BMD at all three sites; however, after adjusting for covariates, such as age, adiposity, smoking, physical activity, postmenopausal hormonal replacement therapy, menopausal status, and menopausal age, no association was seen (Michaelsson et al., 1995). Whereas, in a study conducted by Harris et al. a significant association was observed between dietary iron intake and BMD at all five sites (total body, spine, Ward's Triangle, femoral neck and femoral trochanter), and this association was still observed after adjusting for confounding variables (Harris et al., 2003). Iron intake levels (Angus: 10.9±0.4mg/day, Harris: 16±6mg/day, Michaelsson: 11.4±3.9mg/day) in these three studies were considerably lower than in the population in the current study (26.0±8.5mg/day). Possible reasons for this variation are differences in how nutritional intake was assessed, whether through a food frequency questionnaire or a dietary log, and that Angus and Michaelsson's studies were not conducted in United States and eating habits and patterns could be different in foreign countries. Participants in the current study were meeting the recommended daily allowance of iron intake of 15-18mg/day. Therefore, in view of the adequate iron intake it is no surprise that 63% of the sample had sFer levels within the normal range and not below the cutoff value of 20µg/ml for iron deficiency and/or anemia. This could mean that a relationship may only exist if there are more significant deficiencies in iron and/or if these deficiencies are prolonged. Also a relationship may only exist if intake is below the suggested levels.

No correlation was observed between BMD at any site and calcium (Ca) intake in the current study. Similar to iron intake, calcium intake (1342±375mg/day) was greater than the recommended daily allowance (RDA 1,000-1,300mg/d) in the current population than in previously cited studies (Angus: 738±320mg/d; Harris 811±290mg/d; Michaelsson 785±285mg/d). Most evidence suggests no correlation between current Ca intake and BMD (Angus et al., 1988; Freudenstein & Johnson, N. E. Smith, E. L., 1986; Sandler, Slemenda, & LaPorte, 1985; Sowers, Wallace, & Lemke, 1985). However, Harris et al. (Harris et al., 2003) found a significant association between Ca intake and BMD and Michaelsson (Michaelsson et al., 1995) saw a weak relationship between Ca intake and total body BMD. This may suggest the current intake of calcium (i.e. as an adult) is not as important as once thought. Angus et al. analyzed the relationship between bone mass and early calcium intake by an analysis of variance. They found that postmenopausal women who consumed more than 600 milliliters (ml) of milk per day prior to age 20 had a significantly higher mean forearm BMC than women who drank less than 300ml of milk per day. In conjunction with the previous findings this suggests that calcium intake earlier in life may be more important to bone health than calcium intake later in life. Peak bone strength does not develop until approximately 25 years of age. Given the age of current population being studied (17-19 years) and their high calcium intakes, it is not surprising there was little evidence of low BMD in our sample.

There was also no correlation found between BMD and vitamin D intake. Vitamin D intake was slightly above the RDA (5μg/d) in the population studied. Because of the known interaction between vitamin D and calcium absorption one would assume that if a correlation was not seen between calcium and BMD that there would also be no

correlation between vitamin D and BMD. However, no other study has evaluated this relationship to our knowledge.

Since, the studied population was homogeneous in age, and gender, this minimized the ability to detect trends because values were so close to one another. Therefore, expanding the studied population to a wider age range within college age female athletes may make trends easier to see. Also, it may be beneficial in future studies to restrict the population to groups of athletes that are known to have a higher frequency of iron deficiency and dietary issues, such as cross country, track, and gymnastics.

In conclusion, this study demonstrated that there was no correlation between sFer and BMD or between dietary intake of iron, calcium or vitamin D and BMD when sFer levels were normal or low and nutrient intake was adequate. Further studies are needed to determine if these relationships exists when nutrient intake is insufficient. Participants of the current study had an adequate nutritional foundation and were not at an increased risk for iron deficiency and/or anemia or low BMD as expected. It would be beneficial to do a follow-up study with the same population following their freshman year, to determine how their eating habits and training load change with time. A follow-up study could possibly provide insight into when iron deficiency develops and when bone mass changes occur, therefore, indicate when screening and intervention would need to be implemented. It may also be beneficial to look at injury rates throughout their career to see if more stress fractures occur later and if and when iron deficiency develops.

Appendix A

FOOD FREQUENCY QUESTIONNAIRE for Nutritionist Pro

FOOD QUESTIONNAIRE:

This questionnaire asks about your eating patterns during the past year. For each food item listed, respond by indicating your usual intake of that food per Day, Week or Month. For example: Eggs. If you eat 2 eggs every day, respond 2 Daily. If you think you average 2 eggs a week over the year, respond 2 Weekly. If you do not eat the food or if you have it once or twice a year, then do not mark a frequency or interval. This questionnaire will take about 20 minutes to complete. The accuracy of your nutrition report depends on the accuracy of your answers.

For example, if you eat 2 eggs per week, you would write 2 under the week column.

Description	Amount	Unit	Daily	Weekly	Monthly
Whole egg	1.00	item	2		

Breads, Cereals and Grain Products

Description	Amount	Unit	Daily	Weekly	Monthly
Whole grain breads (whole wheat, rye, pumpernickel)	1.00	sl.			
White breads (French-1 slice, burger/hot dog bun-1/2 item)	1.00	svg.			
English muffin, bagel, pita bread	0.50	item			
Whole grain crackers: Triscuits, Wheat Thins, etc. (4-6 each)	5.00	item			
Other crackers: Saltines, Ritz, etc. (4-6each)	5.00	item			
Tortilla, corn, 6 inch diameter (medium)	1.00	item			
Muffins	1.00	item			
Pancakes (2), waffles (1 - 7 inch diameter)	1.00	svg.			
Whole grain hot cereal: rolled oats, rolled wheat, Roman Meal	0.50	c.			
Instant or quick hot cereal: cream of wheat, cream of rice	0.50	c.			
Cold cereals: shredded wheat, raisin bran, or bran flakes	0.75	c.			
Cold cereals: Frosted Flakes, Sugar Smacks, etc.	0.75	c.			
Rice, cooked	0.50	c.			
Pasta, cooked	0.50	c.			

Fruits and Juices

Description	Amount	Unit	Daily	Weekly	Monthly
Apple or pear, fresh, medium	1.00	item			
Banana, medium	1.00	item			
Orange (1 item) or grapefruit (1/2 item)	1.00	svg.			
Peach (1), nectarine (1/2), or apricots (2)	1.00	svg.			
Berries (in season)	0.75	c.			
Cantaloupe, medium (in season)	0.25	item			
Other melon (watermelon, honeydew, casaba)	1.00	c.			
Pineapple, fresh	0.50	c.			
Raisins (2 Tbsp), dates (2), prunes (2), dried apricots (4)	0.25	c.			
Canned fruit or frozen fruit	0.50	c.			
Orange or grapefruit juice	0.50	c.			
Tomato juice or vegetable juice	0.50	c.			
Other juices: apple, grape, pineapple, or cranberry	0.50	c.			
Fruit drinks: lemonade, punch, Koolade	0.50	c.			

Fats and Oils

Description	Amount	Unit	Daily	Weekly	Monthly
Vegetable oils: corn, safflower, soy, etc.	1.00	T.			
Olive oil	1.00	T.			
Shortening	1.00	T.			
Lard	1.00	T.			
Margarine	1.00	t.			
Butter	1.00	t.			
Mayonnaise	1.00	T.			
Regular salad dressing	1.00	T.			
Low-calorie dressings	1.00	T.			
Sour cream	1.00	T.			
Cream cheese	1.00	T.			
Half & Half, table cream	1.00	T.			

Milk, Yogurt and Cheeses

Description	Amount	Unit	Daily	Weekly	Monthly
Skim milk or low fat milk	1.00	c.			
Whole milk	1.00	c.			
Chocolate milk	1.00	c.			
Yogurt	1.00	c.			
Cheese: cheddar, Colby, American, Monterey jack, etc.	1.00	oz.			
Other cheeses: Swiss, mozzarella, ricotta, string, etc.	1.00	oz.			
Cottage cheese	0.50	c.			

Vegetables

Description	Amount	Unit	Daily	Weekly	Monthly
Salads: lettuce, celery, green peppers, onions	1.00	c.			
Dark green leafy vegetables, raw or cooked	0.50	c.			
Carrots, raw or cooked	0.50	c.			
Tomatoes, fresh, medium	1.00	item			
Starchy vegetables, cooked: corn, peas, mixed vegetables	0.50	c.			
Other vegetables, cooked: green beans, beets, zucchini	0.50	c.			
Cauliflower, broccoli, brussels sprouts, cabbage	0.50	c.			
Winter squash, cooked: acorn, butternut, hubbard	0.50	c.			
White potato, baked, boiled, or mashed	1.00	item			
Sweet potatoes or yams, cooked	0.50	c.			

Beverages

Description	Amount	Unit	Daily	Weekly	Monthly
Cola drinks (1 can = 12 fl. oz)	12.00	fl.oz.			
Diet cola drinks (1 can = 12 fl. oz)	12.00	fl.oz.			
Non-cola drinks: 7-U , Sprite, Slice, etc. (1 can = 12 fl. oz)	12.00	f1.oz.			
Diet non-cola drinks (1 can = 12 fl. oz)	12.00	fl.oz.			
Coffee or tea (1 cup = 8 fl. oz)	8.00	fl.oz.			
Decaffeinated coffee or teas: Sanka, herbal tea, etc.	8.00	fl. oz.			
Hot chocolate or cocoa	1.00	c.			
Beer 1 can=12fl.oz	12.00	fl.oz.			
Wine, dry or table (red, white or blush)	4.00	fl. oz.			
Liquor: vodka, whiskey, gin, rum, etc.	1.50	fl.oz.			

Protein Foods

Description	Amount	Unit	Daily	Weekly	Monthly
Legumes: lentils, pinto beans, navy beans, cooked	1.00	c.			
Nuts and seeds: peanuts, almonds, sunflower seeds, etc.	0.25	c.			
Peanut butter, nut butters	1.00	T.			
Tofu or other meat substitutes	3.00	oz.			
Beef: rib roast, steak, pot roast, veal, etc.	3.00	oz.			
Beef, ground, cooked	3.00	oz.			
Pork: chops, roast, ham	3.00	oz.			
Lamb: chops, roast	3.00	oz.			
Poultry: chicken, turkey, duck	3.00	oz.			
Fish, canned with oil: tuna, sardines	3.00	oz.			
Tuna, water pack	3.00	oz.			
Fish, fresh or frozen, no breading: trout, halibut, sole, etc.	3.00	oz.			
Shellfish: shrimp, scallops, lobster, clams	3.00	oz.			
Eggs, whole, large	1.00	item			
Egg substitutes or egg whites	0.25	c.			
Lunch meats: bologna, salami, etc.	1.00	oz.			
Frankfurters or sausage link (4 in x 1 1/8 in)	1.00	item			

Desserts and Sweets

Description	Amount	Unit	Daily	Weekly	Monthly
Cookies: chocolate chip, oatmeal, peanut butter. etc.	2.00	item			
Brownies, 2 in.	1.00	item			
Doughnut or sweet roll	1.00	item			
Cake, 1/l2 of 9in.	1.00	sl.			
Granola bars (1 item) or granola (1/2 cup)	1.00	item			
Pie, 1/8 of whole pie	1.00	sl.			
Gelatin, flavored	0.50	c.			
Pudding or custard	0.50	c.			
Ice cream	0.50	c.			
Ice Milk	0.50	c.			
Sherbet	0.50	c.			
Candy bar, chocolate bar (1 bar), M&Ms (1 pkg.)	1.00	item			
Hard candy, gum drops, Lifesavers	1.00	item			

Miscellaneous

Description	Amount	Unit	Daily	Weekly	Monthly
Fast food – pizza	1.00	sl.			
Fast food: hamburger or cheeseburger	1.00	item			
Fast food - burrito or taco	1.00	item			
Bacon	2.00	sl.			
Popcorn, popped	2.00	c.			
Potato chips, corn chips, tortilla chips	1.00	oz.			
Catsup or chili sauce	1.00	T.			
Tomato based sauce (spaghetti sauce)	0.50	c.			
Pickles or pickle relish (1 Tbsp)	1.00	T.			
Olives	5.00	item			
Avocado (1/8 item)	0.13	item			
Sauces: soy sauce, steak sauce, barbecue sauce	1.00	T.			
Brown gravy, giblet gravy, or white sauce	0.25	C.			
Soups, vegetable or noodle type	1.00	c.			
Soups, cream	1.00	c.			
Chewing gum	1.00	item			
Sugar, honey, jam, jelly, syrups	1.00	T.			

Appendix B

Menstrual History Review — Sample Questionnaire Items

Have you ever had a menstrual period? ___Yes___ No
If yes –How old were you when you had your first menstrual period? _____
When was your last period? _____
How many days are there between your periods from the first day of your menstrual cycle to the first day of your next cycle? _____ 3 days _____ more than 3 – 10 days_____ more than 10 days
How many periods have you had in the past 12 months? _____ In the past 6 months?_____
Have you ever missed 3 or more consecutive months of your menstrual periods? ___Yes ___ No
If yes, how many consecutive months have you missed your period? _____
Does your menstrual cycle change with a change in the intensity, frequency or duration of training? ___ Yes ___ No
If yes, does it become (circle below)…
Lighter / Heavier / Shorter / Longer / Disappear
Do you ever have trouble with heavy bleeding? ___ Yes ___ No
Do you ever experience cramps during your period? ___ Yes ___ No
If yes, how do you treat them? _____
Are you on birth control pills or hormones? ___ Yes ___ No
If yes, were they prescribed for (circle below)…
Irregular periods / No periods / Painful periods / Birth control
When was your last pelvic examination? _____
Have you ever had an abnormal Pap smear? ___Yes ___ No
Have you ever been treated for anemia (low hemoglobin or iron)? ___Yes ___ No
Is there any history of osteoporosis (thinning of the bones) in your family? ___Yes ___ No

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Appendix C

Seven-Day Physical Activity Recall

1: On the average, how many hours did you sleep each night during the last five weekday nights, Sunday through Thursday?

Enter a numeric value (0 if not applicable) ___ ___ . ___

2: On the average, how many hours did you sleep each night last Friday and Saturday nights?

Enter a numeric value (0 if not applicable) ___ ___ . ___

3: How many hours did you spend during the last five weekdays doing these moderate activities or others like them?

Enter a numeric value (0 if not applicable) ___ ___ . ___

4: How many hours did you spend last Saturday and Sunday doing these moderate activities?

Enter a numeric value (0 if not applicable) ___ ___ . ___

5: How many hours did you spend during the last five weekdays doing these hard activities or others like them?

Enter a numeric value (0 if not applicable) ___ ___ . ___

6: How many hours did you spend last Saturday and Sunday doing these hard activities?

Enter a numeric value (0 if not applicable) ___ ___ . ___

7: How many hours did you spend the last five weekdays doing these very hard activities, or others like them?

Enter a numeric value (0 if not applicable) ___ ___ . ___

8: How many hours did you spend last Saturday and Sunday doing these very hard activities?

Enter a numeric value (0 if not applicable) ___ ___ . ___

9: Were you employed outside the home during the last seven days? If no, put zeros for questions 9-13. If yes, how many days?

Enter a numeric value (0 if not applicable) ___ ___ . ___

10: How many hours per day?

Enter a numeric value (0 if not applicable) ___ ___ . ___

11: How many of these hours per day were spent doing moderate activities?

Enter a numeric value (0 if not applicable) ___ ___ . ___

12: How many of these hours per day were spent doing hard activities?

Enter a numeric value (0 if not applicable) ___ ___ . ___

13: How many of these hours per day were spent doing very hard activities?

Enter a numeric value (0 if not applicable) ___ ___ . ___

14: Compared to your physical activity over the past three months, was last week's physical activity more, less, or about the same?

1-More 2-Less 3-About the same

Moderate Activities (3-5 METs)

These activities involve modest increases in heart rate & breathing—e.g., many household & home repair tasks.

- Calisthenics without weights
- Carpentry
- Cleaning, heavy (such as vacuuming, sweeping)
- Croquet
- Cycling—leisure, 5.5 mph mild
- Electrical work
- Feeding farm animals, manual milking
- Fencing
- Forestry—slow ax chopping, power sawing, stacking firewood, weeding
- Frisbee playing
- Gardening—hedging, raking, planting, mowing
- Golf—no power cart
- Gymnastics
- Horseback riding
- Locksmith
- Machine tooling—lath, punch press, tapping & drilling, welding
- Mopping floor
- Motor-cross
- Mowing lawn—push & power mower
- Music—playing drums
- Painting—outside
- Planting seedlings
- Plastering
- Sailing & board sailing
- Scraping Paint
- Stock clerking
- Surfing
- Sweeping
- Swimming-mild
- Grocery shopping
- Table tennis
- Laundry-heavy
- Childcare
- Window cleaning
- Walking on firm level surface, 3-4 mph – Average to fairly brisk
- Yoga
- Tai-chi
- Bowling
- Horse shoes
- Grocery shopping
- Heavy cooking

Hard Activities (5.1-6.9 METs)

Most people will have noticeable increases in breathing and will likely perspire—e.g., vigorous household, home repair and gardening tasks, heavy industrial work, and some construction and vigorous sports.

- Aerobic Dance
- Badminton
- Climbing hills with no load
- Coal shoveling
- Cycling—leisure, 9.4 mph (moderate)
- Farming—shoveling grain
- Fast Walking
- Folk Dancing
- Forestry—hoeing, planting by hand
- Karate or Judo
- Roller skating
- Scrubbing floors
- Skiing, water or downhill
- Tennis, doubles
- Walking on level Brisk or striding, firm surface @ 4.5 mph
- Weight lifting or training (count only lifting time)
- Swimming-moderate

Appendix D

Osteoporosis Questionnaire

	Yes/No	**Comment**
Female		
Family History		
Over age 45		
Small frame/Thin body		
Sedentary Lifestyle		
Caucasian or Asian		
Frequently drink alcohol (greater than 2 drinks/day/week)		
Had a fracture as adult		
Currently smoke, or smoked in past		
Eating Disorders		
Drink caffeinated beverages (greater than 2 drinks per day)		
Experienced menopause, amenorrhea or low testosterone levels		
Take steroidal or thyroid medication		
DO NOT take calcium supplements or consume less than 3 servings of dairy per day		

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