INFLUENCE OF IRON DEFIENCY ON BONE HEALTH

By

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Honors Thesis

Oklahoma State University

Spring 2016

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ABSTRACT

One of today's most prevalent chronic metabolic diseases is osteoporosis, for it is responsible for over 8.9 million fractures annually and approximately one fracture every three seconds. Osteoporosis is characterized by compromised bone strength that substantially increases one's risk of fracture. Along with physical activity, there are several nutrients that play a key role in regulation of bone metabolism and maintaining optimal bone health, including calcium, vitamin D, and iron. In fact, current research has indicated an association between a deficiency in dietary iron status and compromised bone mineral density (BMD), but the details remain unclear. The primary objective of this review was to analyze current knowledge on the impact of iron deficiency on bone health and propose a new hypothesis on the potential mechanisms behind the correlation.

Four in vitro animal studies that assessed the affect of reduced dietary intake on various bone parameters in weanling rats were reviewed. Correlations between results was analyzed and used to determine consistent alterations in bone metabolism due to impaired iron status. The findings indicated a significant association between deficient iron and reduced BMD. Additionally, the decrease in various biomarkers of both collagen synthesis and osteoblast activation implies that these mechanisms may be inhibited when iron is limiting. Therefore, it can be hypothesized that iron deficiency significantly impairs bone metabolism due to an inhibition of osteoblast activation or collagen synthesis that subsequently reduces bone formation and mineralization. However, further research is needed to more specifically examine these associations.

INTRODUCTION

The National Institute of Health defines osteoporosis as a skeletal disorder that is characterized by compromised bone strength, which subsequently increases an individual's risk of fracture [1]. While it is a chronic metabolic disease that is commonly associated with post-menopausal women over the age of 50 due to a decrease in estrogen levels, osteoporotic risk factors begin in adolescence at the mark of peak bone accretion [1, 2]. Overall, osteoporosis causes over 8.9 million fractures annually, equating to approximately one fracture every three seconds [3]. The most common sites of osteoporotic fractures are the hip, vertebrae, and distal forearm [2]. Numerous factors may contribute to an individual's risk of developing osteoporosis, including minimal weight baring exercise, smoking, and various dietary influences. Although calcium and vitamin D are the most widely recognized micronutrients known to be key regulators of bone metabolism, additional nutrients have been shown to participate in the process, such as vitamin K, copper, phosphorus, and iron [2].

Iron is a common cofactor in many of the body's enzymatic processes, with important roles in oxygen transport, vitamin D metabolism, and collagen synthesis. It is a micronutrient of significant importance to proper physiological functioning, so a deficiency can be very detrimental. The most prevalent form of iron deficiency is microcytic anemia, which characterized by a low red blood cell count and low hemoglobin levels. Iron deficiency anemia is considered the most common and widespread nutritional disorder in the world as well as the only nutrient deficiency that is significantly prevalent in industrialized countries [4]. Therefore, it is worthwhile to consider the potential repercussions of iron deficiency on metabolic processes outside of its primary functions. For instance, due to elevated iron needs during puberty, deficiency is particularly common in adolescence, a time period that is also imperative for peak

bone accretion [5]. With the increasing prevalence of osteoporosis, there has been heightened interest as the potential relationship between iron deficiency and bone health.

While several lines of evidence have now revealed that an overload of iron can have damaging effects on bone, particularly through oxidative damage, the interaction between the iron deficiency and bone metabolism remains unclear [5]. Nonetheless, this is an area of vast importance due to the widespread prevalence of both iron deficiency and osteoporosis. Previous studies have indicated an association between deficient dietary iron intake and reduced bone mineral density, but the underlying mechanisms remain unclear. Therefore, the primary objective of this review was to analyze current knowledge on the impact of iron deficiency on bone health and propose a new hypothesis on the potential mechanisms responsible for the association. Specifically, I assessed the findings of four in vitro animal studies on weanling rats, which were fed either an iron-deficient or control diet for a period of time and then anesthetized for assessment of several bone parameters.

METHODOLOGY

Articles were selected for this review that involved an in vitro animal study on weanling rats, which assessed the association between iron deficiency and bone metabolism. Selected were the four studies that most specifically fit these criteria. While the broad organization of each of the studies was the same, they each differed on various details of their methodology.

Iron Deficiency Negatively Affects Vertebrae and Femurs of Rats Independently of Energy Intake and Body Weight

In this study, Medeiros *et al* placed thirty-two female weanling rats into four groups and fed them diets that were 1) control (C), 2) calcium restricted, 3) iron deficient (ID), or 4) pair-fed (PF) to the iron-deficient group. After five weeks, the rats were anesthetized and various bone parameter measures were assessed. Levels of hematocrit were determined as an indicator of iron-deficiency. Bone mineral content (BMC) and bone mineral density (BMD) of excised femur were measured using dual-energy X-ray absorptiometry (DEXA). Micro-CT was used to determine trabecular number, thickness, and separation as well as the structural model index (SMI) of the L-3 vertebra trabecular bone. A Pyrilinks-D kit was used to assess twenty-four hour urine deoxypryidinoline crosslinks (DPD), which is an indicator of bone resorption. An immunoradiometric assay was used to measure serum osteocalcin concentrations, and a radioimmunoassay was used for serum 1,25-dihydroxycholcalciferol levels, which is the bioactive form of vitamin D [6].

Severe Iron Deficiency Decreases Both Bone Formation and Bone Resorption in Rats

In this study conducted by Katsumata *et al*, eighteen three-week-old, male rats were divided into three diet groups, 1) ID, 2) C, or 3) PF to the ID group. After four weeks the rats were anesthetized. Serum samples were used to measure hemoglobin and hematocrit concentrations as indicators of iron deficiency. These samples were also used to assess serum total protein, albumin, and globulin concentrations, as well as serum parathyroid hormone (PTH), insulin-like growth factor-1 (IGF-1), osteocalcin, and 1,25-dihydroxycholcalciferol concentrations. Twenty-four hour urine samples were used to measure urinary C-terminal telopeptide of type 1 collagen (CTx) and DPD excretion. The BMC and BMD of the femur were obtained using DEXA, and the trabecular number, separation, and thickness of the L-2 vertebra were measured along with other bone histomorphometric measures, including the osteoid volume

(OV/TV), mineralizing surface (MS/TS), mineral apposition rate (MAR), and bone formation rate (BAR) [7].

Severe Nutritional Iron-Deficiency Anemia has a Negative Effect on Some Bone Turnover Markers in Rats

This study by Diaz-Castro *et al* divided thirty male weanling rats into either a C group or an ID group. After forty days, the rats were anesthetized, and serum was collected and used to determine hematocrit and hemoglobin concentrations. These samples were also used to assay serum 25-dihydroxycholcalciferol, aminoterminal propeptides of type 1 collagen (PINP), tartrate-resistant acid phosphatase (TRACP 5b), CTx degradation products, and PTH. PINP is an important marker of bone formation, while TRACP 5b indicates bone resorption. The concentration of calcium and phosphorus in the femur was measured with atomic absorption spectrophotometry and used to indicate bone mineralization [8].

Iron Deficiency Negatively Affects Bone Quality And Microarchitecture In Weanling Male Sprague Dawley Rats

In this study by Shawron, thirty weanling rats were divided into one of three groups, 1) ID, 2) C, or 3) PF to the ID diet. After thirty-five days the rats were anesthetized, and serum samples were collected and used for assessment of hemoglobin and hematocrit. BMD and BMC in the tibia were measured with DEXA, and the micro-CT was used to determine trabecular number, separation, and thickness in the L4 vertebrae. The SMI was computed using these measurements [2].

RESULTS

All of the results presented are represented in **Table 1** below.

Indicators of Iron Deficiency

Each of the studies had sufficient biomarkers that revealed the presence of iron deficiency in the ID animals as compared to the C or PF groups. The ID animals in the studies of Katsumata *et al*, Diaz-Castro *et al*, and Shawron each had decreased hemoglobin levels, and the ID animals in all four studies had decreased hematocrit concentrations [6, 7, 8].

Serum Vitamin D, Calcium, and Protein

Both Medeiros *et al* and Katsumata *et al* showed no difference in the concentration of serum 1,25-dihydroxycholcalciferol in the ID compared to C and PF groups [6, 7]. Similarly, 25-hydroxycholcalciferol in the Diaz-Castro *et al* study was not significantly different in the ID group compared to the C group [8]. In the Katsumata *et al* study apparent calcium absorption and rate of absorption were decreased in the in the ID group compared to the PF and C animals. There was also a significant decrease in the concentration of total serum protein, serum albumin, and serum globulin in the ID group in this study [7].

Indicators of Bone Turnover

In the Medeiros *et al* study there was no significant difference in serum osteocalcin concentration or urinary DPD crosslinks in the ID group versus the C or PF groups [6]. In the Katsumata *et al* study levels of serum IGF-1, serum osteocalcin, and urinary DPD excretion significantly decreased in the ID group compared to the PF and C groups, while levels of urinary CT increased. They determined no difference in the levels of PTH between the groups [7]. In the study by Diaz-Castro *et al*, levels of PINP decreased, while levels of CTx, TRACP 5b, and PTH increased [8].

BMC and BMD

Both the study by Medeiros *et al* and the study by Katsumata *et al* revealed a significant decrease BMD and BMC in the ID femur [6, 7]. Similarly, the study by Shawron revealed a significant decrease in the BMD and BMC of both the tibia and lumbar vertebra [2]. Diaz-Castro *et al*'s study revealed significantly less calcium and phosphorus in the femur of the ID rats compared to the control rats [8].

Bone Histomorphometric Parameters of the Lumbar Vertebra

Medeiros *et al* and Shawron both revealed a significant decrease in trabecular number as well as a significant increase in trabecular separation in the lumbar vertebra of the ID animals compared to the PF and C animals [6, 2]. They also revealed a slight decrease in trabecular thickness and an increase in the SMI, indicative of an increased fracture risk [6, 2]. In the Katsumata *et al* study, there was a significant decrease in OV/TV, MS/TS, MAR, and BFR in the ID versus the PF and C group [7].

TABLE 1 Indicators of Iron Deficiency of Bone Metabolism

	Medeiros	Katsumata	Diaz-Castro	Shawron
Iron Deficiency				
Hemoglobin		Decrease	Decrease	Decrease
Hematocrit	Decrease	Decrease	Decrease	Decrease
Serum Concentrations				
1,25-dihydroxycholcalciferol	No Change	No Change		
25-hydroxycholecalciferol			No Change	
Total Protein		Decrease		
Albumin		Decrease		
Globulin		Decrease		
Indicators of Bone Turnover				
Serum Osteocalcin	No Change	Decrease		
uDPD	No Change	Decrease		
uCTx		Increase	Increase	
Serum IGF-1		Decrease		
PINP			Decrease	
TRACP 5b			Increase	
PTH		No Change	Increase	
BMC/BMD				
BMD	Decrease	Decrease		Decrease
BMC	Decrease	Decrease		Decrease
Femur C and P			Decrease	
Bone Histomorphometry				
Trabecular Number	Decrease	No Change		Decrease
Trabecular Separation	Increase	No Change		Increase
Trabecular Thickness	Decrease	No Change		Decrease
SMI	Increase			Increase
OV/TV		Decrease		
MS/TS		Decrease		
MAR		Decrease		
BFR		Decrease		

Table 1 Shows the different bone parameters measured in each study. The primary author of each article is indicated in the columns, and the rows represent each biomarker. Increase means that levels significantly rose with the ID diet compared to the control diet, decrease means they were significantly reduced, and no change indicates that there was no significant difference between the groups.

DISCUSSION

The results from each of these studies reveal a significant decrease in bone mineral density (BMD) and bone mineral content (BMC) in the iron-deficient (ID) animals as compared to the pair fed (PF) or control (C) animals, which is supportive of the proposed idea that iron deficiency may play a dominant cause in bone loss through impaired bone metabolism [6, 7, 8, 2]. However, BMD and BMC were assessed at different anatomical locations in each of the studies, Medeiros *et al* and Katsumata *et al* in the femur and Shawron in the tibia and vertebra, which may have limited the consistency of the results, due to the anatomical differences [6, 7, 2].

Trabecular morphology is another important indicator of bone composition as it provides a more specific look into bone microarchitecture [2]. The articles showed some discrepancy between studies on the influence of iron on the trabecular bone of the lumbar vertebra. Medeiros *et al* and Shawron both indicated a significant increase in the separation and structural model index (SMI), and a reduction in the number and thickness of trabecular lumbar vertebral bone of the iron deficient animals, but Katsumata *et al* found no significant differences in any of these parameters [6, 7, 2]. However, the discrepancy in the data may be due to differences in levels of induced iron deficiency in the studies.

While evidence from the studies of Katsumata *et al* and Diaz-Castro *et al* suggest potential alterations of osteoclast activity iron reduction in animals, there was not consistent evidence of this shown throughout the studies to draw a conclusion [7, 8]. There is, however, much more persistent evidence between all four of the studies that suggests that alterations in osteoblast activity may have caused reduced bone formation in the iron deficient animals. To investigate this association, several important biomarkers of bone formation were measured in

each of the studies including insulin-like growth factor-1 (IGF-1), osteocalcin, and osteoid. IGF-1 is a prominent hormonal regulator of bone formation, which stimulates bone collagen and matrix synthesis as well as the replication of osteoblasts [9]. Osteocalcin is a major noncollagenous protein of the bone matrix that is secreted by osteoblasts and important for maximal bone matrix strength [7]. Osteoid proteins are secreted by osteoblasts and make up the unmineralized, organic portion of the matrix that is composed of both collagen and noncollagenous proteins, including osteocalcin [9].

Katsumata *et al* found a significant decrease in serum IGF-1 and osteocalcin concentrations with iron impairment, as well as a significant reduction in osteoid volume in these animals [7]. The significant decrease in these markers, as well as the negative alterations in mineralizing surface (MS/TS), mineralization apposition rate (MAR), and bone formation rate (BFR) parameters in the ID group, suggests that dietary iron deficiency may have significantly impaired bone formation leading to a decrease in BMD/BMC [7]. Medeiros *et al* did not find a significant decrease in serum osteocalcin concentrations in the ID group versus the PF or C groups. This may have had to do to will the degree of iron deficiency in the animals, for the severity of iron impairment was greater in the study by Katsumata *et al* [6, 7].

Another important biomarker for bone formation assessed by Diaz-Castro *et al* was the level of propeptides of type 1 procollagen (PINP). The decrease in the level of PINP in the anemic group compared to the control group suggests that the amount of bone matrix developed in these animals may have also been limited, which subsequently inhibited their bone mineralization [8]. The increased degradation of C-terminal telopeptides of type 1 collagen (CTx) in these animals also supported this idea as it demonstrated a reduction of cross-linking

the collagen fibers, which may have led to overall weaker collagen fibers, greater bone fragility, and an increased fracture risk [8].

There are a number of molecular mechanisms through which iron deficiency has been suggested to affect bone metabolism. One of the proposed mechanisms considers the role of iron in the second hydroxylation of vitamin D to produce 1,25-dihydroxycholcalciferol, the vitamin's bioactive form that plays an important role in calcium absorption [10]. The enzyme responsible for this hydroxylation, 25-hydroxycholcalciferol 1(alpha)-hydroxylase, is a three-component system composed of a flavoprotein, an iron-sulfur protein, and a cytochrome P-450 [8]. A lack in iron could potentially inhibit this enzyme leading to impairment in calcium absorption [8]. While the study by Katsumata *et al* speculated that a reduction in 1,25-dihydroxycholcalciferol caused by a depletion of iron might have led to the decreased BMD and BMC in the ID rats, the results from both this study as well as the study by Medeiros et al. do not necessarily support this speculation, as neither study showed a significant difference in serum 1,25-dihydroxycholcalciferol concentrations [6, 7]. However, 1,25-dihyroxycholcalciferol has a relatively short half-life, making it a poor indicator of serum vitamin D levels, which could explain the lack of evidence behind this mechanism in the studies.

A second proposed mechanism examines the role of iron as a cofactor in collagen synthesis and maturation. Approximately ninety percent of total bone protein is made of type I collagen, which is the major organic component of bone matrix [8, 10]. Iron serves as a necessary cofactor for prolyl and lysyl hydroxylases that are responsible for catalyzing the crosslinking of type 1 collagen. A situation of limiting iron could inhibit this process, subsequently leading to weaker collagen fibers, greater bone fragility, and an increased fracture risk [10]. Many of the results from the studies in review support this potential mechanism, as

indicated by the decreased levels of PINP and elevated levels of CTx found by Diaz-Castro *et al* [8]. Katsumata *et al* found elevated levels of PINP as well, indicative of a reduction in cross-linking [7]. Weaker collagen fibers would consequently impair bone mineralization leading to the decreased bone mineralization represented in all of the studies [6, 7, 8, 2]. However, other biomarkers, such as decreased levels of osteocalcin and IGF-1 suggest that an iron deficiency may inhibit osteoblast function and bone formation through an alternate mechanism. Therefore, the relationship between iron, osteoblast activity, and bone formation should be examined in future studies.

One mechanism that has yet to be explored is the potential impact of iron on protein metabolism. The results of the study by Katsumata *et al* reveal decreased serum protein, albumin, and globulin concentrations in the iron deficient animals versus the pair fed and control animals [7]. These findings suggest that dietary iron deficiency may impair protein synthesis and/or utilization, which could in turn interfere with proper bone metabolism [7]. However, further research is needed to further examine the relationship between iron and protein metabolism.

CONCLUSION

In conclusion, the evidence presented in each of the studies reviewed is supportive of the proposed association between dietary iron deficiency and altered bone metabolism in mice. While many mechanisms for this correlation have been proposed, the details remain unclear. Several biomarkers of bone formation and resorption were measured in each of the four articles to gain better insight into the impact of iron on bone physiology. While the evidence for bone

resorption seemed somewhat inconsistent, there appeared to be more substantiated evidence in support of a potential association between iron and bone formation, either through collagen synthesis or osteoblast activity. Therefore, it can be hypothesized that iron deficiency significantly impairs bone metabolism due to an inhibition of osteoblast activation or collagen synthesis that subsequently reduces bone formation and mineralization. However, further research will need to be conducted to more specifically assess the effect of iron deficiency on collagen synthesis and osteoblast activation. Future studies are also needed to look into the association between iron and vitamin D and the potential role it could play on bone metabolism, as previous research has been inconclusive. Additionally, further investigations need to be conducted to assess these findings on humans and more specifically look into the role of iron deficiency and risk of osteoporosis.

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