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Bone remodelling is reduced by recovery from iron-deficiency anaemia in premenopausal women

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Abstract Iron-deficiency anaemia (IDA), one of the most common and widespread health disorders worldwide, affects fundamental metabolic functions and has been associated with deleterious effects on bone. Our aim was to know whether there are differences in bone remodelling between a group of premenopausal IDA women and a healthy group, and whether recovery of iron status has an effect on bone turnover markers. Thirty-five IDA women and 38 healthy women (control group) were recruited throughout the year. IDA women received pharmacological iron treatment. Iron biomarkers, aminoterminal telopeptide of collagen I (NTx), procollagen type 1 N-terminal propeptide (P1NP), 25-hydroxyvitamin D, and parathormone (PTH) were determined at baseline for both groups and after treatment with pharmacological iron for the IDA group. IDA subjects were classified as recovered (R) or non-recovered (nR) from IDA after treatment. NTx levels were significantly higher ($p < 0.001$), and P1NP levels tended to be lower in IDA women than controls after adjusting for age and body mass index (BMI), with no differences in 25-hydroxyvitamin D or PTH. After treatment, the R group had significantly lower NTx and P1NP levels compared to baseline ($p < 0.05$ and $p < 0.001$ respectively), whilst no significant changes were seen in the nR group. No changes were seen in 25-hydroxyvitamin D or PTH for either group. IDA is related to higher bone resorption independent of age and BMI. Recovery from IDA has a concomitant beneficial effect on bone remodelling in premenopausal women, decreasing both bone resorption and formation.

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Introduction

Anaemia is one of the most common and widespread health disorders in the world. It can result from a variety of causes; however, globally, iron deficiency is the most significant contributor [37]. This deficiency primarily affects children and women of childbearing age and

constitutes the most prevalent nutritional deficiency in both developing and developed countries [4].

Anaemia is defined as a reduction in erythrocyte or haemoglobin concentration, accompanied by an impaired ability to transport oxygen, causing hypoxia [23]. Iron-deficiency anaemia can lead to reduced physical capacity and affects intellectual performance, as well as the alteration of fundamental metabolic functions, such as temperature regulation and immune function [16].

Moreover, iron-deficiency anaemia has been associated with deleterious effects on bone health. Several studies in rats found that iron depletion reduced bone strength and microarchitecture due to impaired mineralisation [24–26] and alters bone turnover markers [10, 20]. However, very few studies in humans have found links between iron status and bone health. A study in adolescent girls found a trend for a positive association between serum ferritin and bone-mineral density of the radius [18]. In postmenopausal women, dietary iron has been associated with greater bone-mineral density at all sites measured [14]; transferrin levels were negatively correlated with lumbar and femoral neck mineral density and those women with osteoporotic fractures had higher transferrin levels and lower ferritin levels than controls [7]. In older men and women, bone-mineral density was found to be positively associated with haemoglobin levels [6].
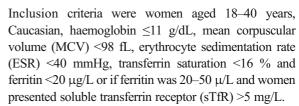
Our research group has evaluated the link between bone health and iron status in premenopausal non-anaemic iron-deficient women and found that consumption of iron-fortified food, which induced a marked increase in iron status, had no effect on bone remodelling parameters. Although bone health was not affected, we also found a high prevalence of vitamin D insufficiency or deficiency in this group of iron-deficient women [5].

Given that premenopausal women are one of the largest groups affected by iron-deficiency anaemia, the present study was carried out in order to know if there are differences in bone turnover markers between a group of iron-deficient anaemic women and a healthy group, and whether recovery of iron status in the anaemic women has an effect on bone turnover markers.

Material and methods

Subjects

Iron-deficient anaemic women were recruited at Complejo Hospitalario de Toledo over a period of 18 months.

Inclusion criteria were women aged 18–40 years, Caucasian, haemoglobin ≤11 g/dL, mean corpuscular volume (MCV) <98 fL, erythrocyte sedimentation rate (ESR) <40 mmHg, transferrin saturation <16 % and ferritin <20 µg/L or if ferritin was 20–50 µ/L and women presented soluble transferrin receptor (sTfR) >5 mg/L.

Exclusion criteria were amenorrhoea, menopause, smoker, pregnancy, breast feeding, non-Caucasian, iron metabolism-related diseases (thalassemia, haemochromatosis), chronic gastric diseases (inflammatory bowel disease, gastric ulcers, coeliac disease, Crohn's disease, haemorrhagic diseases), neoplastic diseases, renal disease or hormone-related diseases independent of iron-deficiency anaemia, C-reactive protein >5 mg/L, creatinine levels >1.0 mg/L (120 µmol/L), abnormal liver tests (>2 times normal), blood donation in the past 3 months or currently taking pharmacological iron supplements.

A group of 84 women were contacted by telephone and asked to participate, of which 36 women were selected according to inclusion and exclusion criteria and agreed to participate, and only 1 did not complete the study.

A control group (n=38) of healthy women was also recruited during the same period. Inclusion criteria were healthy Caucasian adult menstruating women, aged 18–40 years old. Exclusion criteria were ferritin <40 µg/L, haemoglobin <12 g/dL, or any iron metabolism-related diseases (iron-deficiency anaemia, thalassemia, haemochromatosis), amenorrhoea, menopause, smoker, pregnancy, breastfeeding, non-Caucasian, chronic gastric diseases (inflammatory bowel disease, gastric ulcers, coeliac disease, Crohn's disease, haemorrhagic diseases), renal diseases or hormone-related diseases, blood donation in the past 3 months or currently taking pharmacological iron supplements.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. Participants gave signed written consent to participate in the study that was approved by the Clinical Research Ethics Committees of Complejo Hospitalario de Toledo and Hospital Puerta de Hierro, Majadahonda, and the Spanish National Research Council Committee, Spain.

Study protocol

The anaemic women were prescribed pharmacological iron in the form of ferrous sulphate tablets, one tablet (80 mg Fe) daily if haemoglobin >10 g/dL or two tablets per day (160 mg Fe) if haemoglobin ≤10 g/dL. Patients

were instructed to take the tablets in fasting conditions, alone or with orange juice. Treatment duration depended on recovery from anaemia. Patients were considered to have recovered from anaemia when they fulfilled the following criteria: haemoglobin >12 g/dL and ferritin >15 μg/L.

Blood and urine samples for the anaemic and control groups were taken at baseline. In the anaemic subjects, the same samples were taken 1 week after finishing the 8 weeks of treatment, to allow iron levels to stabilise. If patients had not recovered after the first cycle of treatment, a further 8 weeks of treatment was prescribed, and samples were taken 1 week after.

Body weight was measured at baseline using a Seca scale (to a precision of 100 g) and height was measured with a stadiometer incorporated into the scale. Body mass index (BMI) was calculated as weight/height squared (kg/m^2). Baseline dietary intake was assessed with a 72-h detailed dietary report, and monitored throughout the study using a food frequency questionnaire.

Blood and urine sampling and biochemical assays

Blood samples were collected between 8:00 and 10:00 by venepuncture after a 12-h fasting period. Serum was obtained after centrifugation (for 5 min at 1,000 g). Subjects were instructed to provide the second void morning urine for analysis of the bone resorption marker (aminoterminal telopeptide of collagen 1 (NTx)). This urine sample was chosen instead of 24-h urine, both of which are equivalent for NTx determination, as it is easier for the volunteers and lab handling. Serum and urine samples were stored at −80 °C.

Total erythrocytes, haemoglobin, haematocrit, MCV, red cell distribution width (RDW), and ESR were determined in whole blood following standard laboratory techniques using the Beckman Coulter LH780 Analyzer (Beckman Coulter, Brea, California). Serum iron, serum ferritin, serum transferrin, and sTfR, were determined by the Modular Analytics Serum Work Area analyser (Roche, Basel, Switzerland).

Transferrin saturation (in percent) was calculated as: serum iron (in micromoles per litre)/total iron binding capacity (TIBC) (in micromoles per litre) × 100, where TIBC is calculated as 25.1 × transferrin (in grams per litre). Mean corpuscular haemoglobin concentration (MCHC) (in grams per decilitre) was calculated using the formula: haemoglobin (in grams per decilitre)/haematocrit (in percent) × 100.

Serum 25-hydroxyvitamin D (25-hyroxyvitamin D_2 and 25-hydroxyvitamin D_3) was determined with an ELISA technique (25-hydroxyvitamin D EIA, IDS, UK). Sensitivity of this method is 5 nmol/L (2 ng/mL). Intra- and inter- assay coefficients of variation were 5.6 and 6.4 %, respectively. Vitamin D status was defined as deficient for circulating 25-hydroxyvitamin D concentration <50 nmol/L (<20 ng/mL), as insufficient for 51–74 nmol/L (21–29 ng/mL) and sufficient for >75 nmol/L (>30 ng/mL) [17].

NTx, the bone resorption marker, was determined in second void urine by an ELISA test (Osteomark® NTx Urine, Wampole Laboratories, USA). Sensitivity of this method is 1 nM of bone collagen equivalents (BCE). Intra- and inter-assay coefficients of variation were 5.0 and 5.5 %, respectively. The reference range for premenopausal women is 5–65 nmol BCE/millimoles creatinine. Urinary creatinine levels were measured using an autoanalyzer method (modular Roche DDPP). Sensitivity of this method was 0.1 mg/dL. Intra- and inter-assay coefficients of variation of the method were <0.7 and <2.3 %, respectively.

Procollagen type 1 N-terminal propeptide (P1NP), the bone formation marker, and parathyroid hormone (PTH) were determined in the same serum sample by electrochemiluminescence (Elecsys, Roche Diagnostics, USA). Intra- and inter- assay coefficients for P1NP are 2.6 and 4.1 %, respectively, and normal range is 10.4–62 ng/mL. Intra- and inter- assay coefficients for PTH are 2.7 and 6.5 %, respectively, and normal range is 15–65 pg/mL.

Statistical analysis

Results are presented as means with their standard deviations; values greater than the mean plus three times the standard deviation were removed as outliers. Serum ferritin and urinary NTx were log transformed prior to statistical analysis to normalise the data.

To evaluate the effect of recovery from anaemia on bone metabolism, the anaemic group was divided into two subgroups: patients who recovered from anaemia during the study (R) and patients who did not recover (nR).

Data from the control and anaemic groups were compared by analysis of covariance (ANCOVA) using age and BMI as covariates. Spearman's linear correlation tests were performed between baseline vitamin D status (deficient, insufficient and sufficient) [17] and iron biomarkers. Repeated measures analysis of variance

(ANOVA) was used to study time effects. One-way ANOVA was used to compare differences between R and nR anaemic subgroups at baseline and the end of the study. Values of $p < 0.05$ were considered significant. The SPSS statistical package for Mac (version 19.0) was used to analyse the data.

Results

A total of 35 anaemic women and 38 controls completed the study. The age of the anaemic group was 35±5 years whilst that of the control group was 28±3 years. BMI was 25.7±4.1 kg/m^2 for the anaemic group and 22.9 ±3.4 kg/m^2 for the control group. Differences between the two groups in age and BMI were significant ($p < 0.001$ and $p < 0.005$ respectively); therefore comparisons between these groups were adjusted for age and BMI (Table 1).

Table 1 shows iron and bone parameters for anaemic women and controls at baseline. Regarding biochemical bone markers, NTx was significantly higher in the group of anaemic women ($p < 0.001$), however, no significant differences were found between levels of P1NP, serum 25-hydroxyvitamin D or PTH. There were significant positive correlations between baseline vitamin D status and serum iron [Spearman's rho (ρ)=0.498, p=0.002] and between baseline vitamin D status and haematocrit (ρ=0.368, p=0.027) in the anaemic group, but not in the control group.

Food intake, assessed by the food frequency questionnaire, did not change during the assay. Tables 2 and 3 show iron and bone biomarkers of recovered R (n=22) and non-recovered nR (n=13) anaemic women at baseline and end of the treatment. Twelve patients were recovered after 8 weeks of treatment, and 10 patients recovered after two 8-week treatment cycles. Age of the R and nR groups were 35±6 and 36 ±5 years, respectively; BMI was 25.1±2.8 kg/m^2 for R group and 27.7±6.4 kg/m^2 for nR group, without significant differences for either parameter. No differences were found at baseline for iron or bone biomarkers between the nR and R anaemic groups (Tables 2 and 3). Significant changes were seen during treatment in all parameters except for total erythrocytes, which increased significantly only in the recovered subgroup, and RDW, which showed no changes in either subgroup (Table 2).

At the end of the study, R group had significantly higher levels of erythrocytes, haemoglobin, haematocrit and ferritin, and had significantly lower levels of transferrin, TIBC, transferrin saturation, and sTfR compared to nR group. No differences were found between MCV, MCHC, RDW or serum iron (Table 2).

Regarding bone metabolism, P1NP significantly decreased after treatment in the R group ($p < 0.001$) whilst differences in nR were not significant (Table 3). NTx levels were also significantly decreased in the R ($p < 0.05$) but not in the nR group. Differences between the groups after treatment were not significant for P1NP or NTx. No significant changes were observed in 25-hydroxyvitamin D or PTH in either group, nor were there differences in these parameters between the two groups.

Table 1 Iron and bone biomarkers of anaemic women and controls at baseline

	Anaemic	Controls	ANCOVA
Haemoglobin (g/dL)	10.0±1.0	13.5±0.8	$p < 0.001$
Haematocrit (%)	31.1±2.5	39.8±2.4	$p < 0.001$
MCV (fL)	73.1±6.7	92.6±3.3	$p < 0.001$
MCHC (g/dL)	32.0±1.0	33.9±0.7	$p < 0.001$
RDW (%)	17.5±1.8	13.5±0.5	$p < 0.001$
Serum ferritin (ng/mL)	4.2±2.0	72.8±29.6	$p < 0.001$
Transferrin (mg/dL)	381.1±46.9	266.9±42.6	$p < 0.001$
Serum iron (mcg/dL)	24.8±7.9	99.0±34.3	$p < 0.001$
P1NP (ng/mL)	40.4±15.9	47.3±20.0	NS
NTx (nmol BCE/mmol creatinine)	37.8±16.5	21.9±8.4	$p < 0.001$
25-hydroxyvitamin D (nmol/L)	58.0±21.5	54.9±17.6	NS
PTH (pg/mL)	39.3±14.1	38.7±15.2	NS

Values are presented as means with their standard deviations. Comparisons between the anaemic group and the control group were measured using ANCOVA adjusted for age and BMI.

Table 2 Iron biomarkers of recovered (R) and non-recovered (nR) anaemic women at baseline and end of the treatment

	Subgroup	Baseline	End of treatment	ANOVA
Erythrocytes (× 10E12/L)	nR	4.1±0.4	4.3±0.5	NS
	R	4.4±0.3	4.7±0.4*	$p < 0.001$
Haemoglobin (g/dL)	nR	9.7±1.2	12.3±0.9	$p < 0.001$
	R	10.1±0.7	13.8±0.7**	$p < 0.001$
Haematocrit (%)	nR	30.3±3.1	36.7±2.6	$p < 0.001$
	R	31.6±2.0	40.7±2.2**	$p < 0.001$
MCV (fL.)	nR	73.6±8.6	85.4±8.0	$p < 0.005$
	R	72.7±5.7	87.3±3.8	$p < 0.001$
MCHC (g/dL)	nR	31.8±1.2	33.5±1.0	$p < 0.001$
	R	32.1±0.8	33.8±0.5	$p < 0.001$
RDW (%)	nR	17.7±2.3	16.6±3.3	NS
	R	17.5±1.5	18.7±4.4	NS
Serum ferritin (ng/mL)	nR	4.3±2.9	14.3±12.6	$p < 0.001$
	R	4.2±1.3	29.4±19.5**	$p < 0.001$
Transferrin (mg/dL)	nR	380.8±36.0	342.5±34.3	$p < 0.005$
	R	374.7±44.7	285.9±33.3**	$p < 0.001$
Serum iron (mcg/dL)	nR	25.6±10.1	63.9±40.6	$p < 0.01$
	R	24.2±6.6	73.8±23.9	$p < 0.001$
TIBC (mcg/dL)	nR	483.7±45.7	435.0±43.5	$p < 0.005$
	R	475.9±56.8	363.1±42.2**	$p < 0.001$
Transferrin saturation (%)	nR	5.3±2.2	14.7±9.1	$p < 0.005$
	R	5.1±1.4	20.3±6.2*	$p < 0.001$
sTfR (mg/dL)	nR	9.3±4.4	4.5±1.8	$p < 0.005$
	R	7.9±2.8	3.1±0.6***	$p < 0.001$

Values are presented as means with their standard deviations. Time-point differences were analysed by repeated measures ANOVA. Comparisons between nR and R groups at each point were measured using two-sided tests

*$p < 0.05$, **$p < 0.001$; ***$p < 0.01$

Discussion

The present study shows that iron-deficient anaemic women present higher bone resorption compared to controls and describes for the first time some of the changes occurring in bone during recovery from iron-deficiency anaemia in humans. It was found that recovery from anaemia was accompanied by decreases in both formation and resorption biomarkers without changes in PTH.

In this study, volunteers maintained food habits throughout, and were recruited throughout the year, thereby avoiding possible confounding seasonal influences on 25-hydroxyvitamin D levels. It is important to note that average 25-hydroxyvitamin D levels were

Table 3 Bone biomarkers of recovered (R) and non-recovered (nR) anaemic women at baseline and end of the treatment

	Group	Baseline	End of treatment	ANOVA
P1NP (ng/mL)	nR	39.9±13.6	35.3±10.6	NS
	R	41.2±17.5	32.6±14.5	$p < 0.001$
NTx (nmol BCE/mmol creatinine)	nR	34.7±15.7	34.3±15.7	NS
	R	40.0±17.2	31.0±9.9	$p < 0.05$
25-hydroxyvitamin D (nmol/L)	nR	55.3±18.0	50.2±17.5	NS
	R	59.9±23.9	62.0±20.6	NS
PTH (pg/mL)	nR	41.2±16.6	45.9±18.4	NS
	R	37.8±12.9	36.8±12.0	NS

Values are presented as means with their standard deviations. Time-point differences were analysed by repeated measures ANOVA. Comparisons between nR and R groups at each point were not significant.
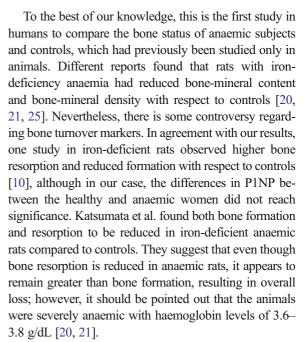
below the 75 nmol/L cut-off value for optimal vitamin D status [17]. Furthermore, 40 % of women in the present study were vitamin D deficient (25-hydroxyvitamin D <50 nmol/L). This result is in agreement with data from Spanish women of different ages [5, 13, 27] and highlights the relevance of research into vitamin D deficiency in Mediterranean countries where sun exposure is presumably high.

Results concerning the association between vitamin D status and iron biomarkers obtained in this study support the relationship between iron and vitamin D deficiencies that has previously been observed by our research group [5]. Studies have also found a relationship between lower vitamin D levels and an increased risk of iron-deficiency anaemia in different population groups [31, 33]. Although the mechanisms are not clear, it is known that iron participates in the activation of 1,25-hydroxyvitamin D and it is suggested that this plays a role in erythropoiesis by stimulating erythroid precursors [33]. In this regard, preliminary results of our group show that consumption of a vitamin D-fortified skimmed milk produces a slight improvement in several haematological parameters in a group of iron-deficient women [34]. Despite the low vitamin D status of the subjects in the present study, which remains unchanged throughout the assay, PTH values for both groups are within the normal range, which can be partially explained by the adequate calcium intake (913.21 ±313.11 mg/day for controls, 930.03±268.38 mg/day for anaemic women).

The values of bone remodelling markers found in the anaemic and control subjects are comparable to those found in other assays of healthy premenopausal women; for similar age groups, P1NP levels found in other studies range from 31.9 to 77.1 µg/L [1, 2, 8, 12] whilst urinary NTx ranges from 24.4 to 40.9 nmol BCE/millimoles creatinine [2, 8, 9, 12]. While the values found in our subjects can be considered normal, the higher levels of NTx seen in anaemic women compared to controls indicate that there is a higher rate of bone resorption. Elevated resorption is considered to adversely affect bone-mineral density and is a source of bone fragility [15]. Therefore, a prolonged situation of iron-deficiency anaemia could result in bone loss and a higher risk of osteoporosis. In agreement with this, there is one assay that studies the iron status of osteoporotic postmenopausal women, which found that patients with osteoporosis had higher transferrin levels and lower, but not significant, ferritin levels compared with controls [7].

To the best of our knowledge, this is the first study in humans to compare the bone status of anaemic subjects and controls, which had previously been studied only in animals. Different reports found that rats with iron-deficiency anaemia had reduced bone-mineral content and bone-mineral density with respect to controls [20, 21, 25]. Nevertheless, there is some controversy regarding bone turnover markers. In agreement with our results, one study in iron-deficient rats observed higher bone resorption and reduced formation with respect to controls [10], although in our case, the differences in P1NP between the healthy and anaemic women did not reach significance. Katsumata et al. found both bone formation and resorption to be reduced in iron-deficient anaemic rats compared to controls. They suggest that even though bone resorption is reduced in anaemic rats, it appears to remain greater than bone formation, resulting in overall loss; however, it should be pointed out that the animals were severely anaemic with haemoglobin levels of 3.6– 3.8 g/dL [20, 21].

In the present study, we also found that when the anaemic women recovered, both P1NP and NTx levels were reduced. It is known that bone formation is coupled with bone resorption, and that elevated bone remodelling in adults after the third decade of life is detrimental to bone health [15, 30, 36], therefore, improvement in iron status leads to an improvement in bone remodelling.

Different mechanisms are suggested to explain the link between iron status and bone health. In iron-deficiency anaemia, this relationship may be due to the unavailability of iron for physiological processes (iron deficiency per se), or due to the situation of anaemia. It is known that iron is an essential cofactor for hydroxylation of prolyl and lysyl residues of pro-collagen. Hydroxylated prolyl residues are essential for the formation of the collagen triple helix, whilst hydroxylated lysyl residues form part of the pyridinoline-type cross-links that stabilise collagen fibres [28, 35]. Disruption of cross-linking can lead to severe dysfunction of bone tissue [22]. Therefore, and given that PTH and 25-hydroxyvitamin D did not change throughout treatment, our results indicate that iron may act directly on bone turnover.

A further possible link between iron-deficiency anaemia and bone health is hypoxia. A state of hypoxia is reached when oxygen supply to tissues is reduced, as occurs in anaemia [29]. Arnett et al. found hypoxia to be a major stimulator of bone resorption by accelerating the formation of large osteoclasts [3]. Moreover, elderly

patients with chronic respiratory failure who did not receive oxygen therapy had reduced bone mass density compared to those who did, and rats that spent 4 weeks in hypoxic air had reduced bone mass density [11]. This mechanism would explain the increased bone resorption in the anaemic women compared to controls, and also, the reduction in resorption after treatment for anaemia.

Furthermore, hypoxia is the main stimulus for erythropoietin synthesis [19], which has been found to induce osteoclast formation followed by osteoblast formation [32]. Therefore, the reduction of erythropoietin levels during recovery from iron-deficiency anaemia [19] may partly explain the reduction in bone resorption observed in the present study. This is supported by the lack of effect of iron recovery on bone remodelling in iron-deficient women who did not present anaemia and therefore should not be affected by hypoxia [5]. Nevertheless, we cannot rule out that the effects observed on bone may also be due to changes in other mediating factors during recovery from anaemia.

In conclusion, this study provides new data that support the link between iron-deficiency anaemia and bone loss and highlights that prevention of anaemia and complete recovery should be promoted in order to improve quality of life including bone health. Further studies on the possible impact of chronic iron-deficiency anaemia on the development of osteoporosis are needed.

Conflict of interest The authors have declared no conflict of interest.

References

1. Adami S, Bianchi G, Brandi ML, Giannini S, Ortolani S, DiMunno O, Frediani B, Rossini M (2008) Determinants of bone turnover markers in healthy premenopausal women. Calcified Tissue Int 82:341–7

2. Ardawi M-SM, Maimani AA, Bahksh TA, Rouzi AA, Qari MH, Raddadi RM (2010) Reference intervals of biochemical bone turnover markers for Saudi Arabian women: a cross-sectional study. Bone 47:804–14

3. Arnett TR, Gibbons DC, Utting JC, Orriss IR, Hoebertz A, Rosendaal M, Meghji S (2003) Hypoxia is a major stimulator of osteoclast formation and bone resorption. J Cell Physiol 196:2–8

4. Bendich A (2010) Iron deficiency and overload. From basic biology to clinical medicine. Humana, NY. ISBN 978-1-934115-22-0

5. Blanco-Rojo R, Pérez-Granados AM, Toxqui L, Zazo P, De la Piedra C, Vaquero MP (2013) Relationship between vitamin D deficiency, bone remodelling and iron status in iron-deficient young women consuming an iron-fortified food. Eur J Nutr 52:695–703

6. Cesari M, Pahor M, Lauretani F et al (2005) Bone density and hemoglobin levels in older persons: results from the InCHIANTI study. Osteoporosis Int 16:691–9

7. D'Amelio P, Cristofaro MA, Tamone C et al (2008) Role of iron metabolism and oxidative damage in postmenopausal bone loss. Bone 43:1010–5

8. De Papp AE, Bone HG, Caulfield MP, Kagan R, Buinewicz A, Chen E, Rosenberg E, Reitz RE (2007) A cross-sectional study of bone turnover markers in healthy premenopausal women. Bone 40:1222–30

9. Del Campo MT, González-Casaus ML, Aguado P, Bernad M, Carrera F, Martínez ME (1999) Effects of age, menopause and osteoporosis on free, peptide-bound and total pyridinium crosslink excretion. Osteoporosis Int 9:449–54

10. Díaz-Castro J, López-Frías MR, Campos MS, López-Frías M, Alférez MJM, Nestares T, Ojeda ML, López-Aliaga I (2012) Severe nutritional iron-deficiency anaemia has a negative effect on some bone turnover biomarkers in rats. Eur J Nutr 51:241–7

11. Fujimoto H, Fujimoto K, Ueda A, Ohata M (1999) Hypoxemia is a risk factor for bone mass loss. J Bone Miner Metab 17:211–6

12. Glover SJ, Gall M, Schoenborn-Kellenberger O, Wagener M, Garnero P, Boonen S, Cauley JA, Black DM, Delmas PD, Eastell R (2009) Establishing a reference interval for bone turnover markers in 637 healthy, young, premenopausal women from the United Kingdom, France, Belgium, and the United States. J Bone Miner Res 24:389–97

13. Groba M, Miravalle A, Gonzalez Rodriguez E, García Santana S, Gonzalez Padilla E, Saavedra P, Soria A, Sosa M (2010) Factors related to vitamin D deficiency in medical students in Gran Canaria. Rev Osteoporos Metab Miner 2:11–18

14. Harris MM, Houtkooper LB, Stanford VA, Parkhill C, Weber JL, Flint-Wagner H, Weiss L, Going SB, Lohman TG (2003) Dietary iron is associated with bone mineral density in healthy postmenopausal women. J Nutr 133:3598–602

15. Heaney RP (2003) Is the paradigm shifting? Bone 33:457–65

16. Hercberg S, Preziosi P, Galan P (2001) Iron deficiency in Europe. Public Health Nutr 4:537–45

17. Holick MF, Chen TC (2008) Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 87:1080S–6S

18. Ilich-Ernst JZ, McKenna AA, Badenhop NE, Clairmont AC, Andon MB, Nahhas RW, Goel P, Matkovic V (1998) Iron status, menarche, and calcium supplementation in adolescent girls. Am J Clin Nutr 68:880–7

19. Jelkmann W (2011) Regulation of erythropoietin production. J Physiol 589:1251–8

20. Katsumata S, Katsumata-Tsuboi R, Uehara M, Suzuki K (2009) Severe iron deficiency decreases both bone formation and bone resorption in rats. J Nutr 139:238–243

21. Katsumata S, Tsuboi R, Uehara M, Suzuki K (2006) Dietary iron deficiency decreases serum osteocalcin concentration and bone mineral density in rats. Biosci Biotec Bioch 70:2547–2550

22. Knott L, Bailey AJ (1998) Collagen cross-links in mineralizing tissues: a review of their chemistry, function, and clinical relevance. Bone 22:181–7

23. McLean E, Cogswell M, Egli I, Wojdyla D, De Benoist B (2009) Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993–2005. Public Health Nutr 12:444–54

24. Medeiros DM, Plattner A, Jennings D, Stoecker B (2002) Bone morphology, strength and density are compromised in iron-deficient rats and exacerbated by calcium restriction. J Nutr 132:3135–41

25. Medeiros DM, Stoecker B, Plattner A, Jennings D, Haub M (2004) Iron deficiency negatively affects vertebrae and femurs of rats independently of energy intake and body weight. J Nutr 134:3061–7

26. Parelman M, Stoecker B, Baker A, Medeiros D (2006) Iron restriction negatively affects bone in female rats and mineralization of hFOB osteoblast cells. Exp Biol Med 231:378–86

27. Rodríguez Sangrador M, Beltrán de Miguel B, Cuadrado Vives C, Moreiras Tuny O (2010) Influence of sun exposure and diet to the nutritional status of vitamin D in adolescent Spanish women: the five countries study (OPTIFORD Project). Nutr Hosp 25:755–62

28. Schulze KJ, Dreyfuss ML (2005) Iron-Deficiency Anemia. In: Caballero B (ed) Encyclopedia of human nutrition, 2nd edn. Elsevier, Oxford, pp 101–109

29. Scientific Advisory Committee on Nutrition (2010) Iron and health. TSO, London

30. Seeman E (2003) Invited review: pathogenesis of osteoporosis. J Appl Physiol 95:2142–51

31. Shin JY, Shim JY (2013) Low vitamin D levels increase anemia risk in Korean women. Clin Chim Acta 421:177–180

32. Shiozawa Y, Jung Y, Ziegler AM et al (2010) Erythropoietin couples hematopoiesis with bone formation. PLoS One 5:e10853

33. Sim JJ, Lac PT, Liu IL, Meguerditchian SO, Kumar VA, Kujubu DA, Rasgon SA (2010) Vitamin D deficiency and anemia: a cross-sectional study. Ann Haematol 89:447–52

34. Toxqui L, Pérez-Granados A, Blanco-Rojo R, Wright I, González-Vizcayno C, Vaquero MP (2013) Intake of an iron or iron and vitamin D-fortified skimmed milk and iron metabolism in women. Ann Nutr Metab (in press)

35. Tuderman L, Myllylä R, Kivirikko KI (1977) Mechanism of the prolyl hydroxylase reaction. 1. Role of co-substrates. Eur J Biochem 80:341–8

36. Watts NB (1999) Clinical utility of biochemical markers of bone remodeling. Clin Chem 45:1359–68

37. World Health Organisation (2004) Assessing the iron status of populations: including literature reviews: report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the assessment of iron status at the population level. WHO, Geneva