

Dietary Iron Is Associated with Bone Mineral Density in Healthy Postmenopausal Women¹

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ABSTRACT Healthy nonsmoking postmenopausal women ($n = 242$; ages 40–66 y) were included in the Bone, Estrogen, and Strength Training (BEST) Study. Bone mineral density (BMD) was measured at five sites (lumbar spine L₂–L₄, trochanter, femur neck, Ward's triangle and total body) using dual energy X-ray absorptiometry (DXA). Mean nutrient intakes were assessed using a 3-d diet record. Regression models were calculated using each BMD site as the dependent variable and iron as the independent variable. Covariates included in the models were years past menopause, fat-free mass, fat mass, use of hormone replacement therapy, total energy intake and dietary intake of protein and calcium. Using linear models, iron was associated with greater BMD at all sites ($P \leq 0.01$), even after adjusting for protein and/or calcium. Increasing levels of iron intake (>20 mg) were associated with greater BMD at several bone sites among women with a mean calcium intake of 800–1200 mg/d. Elevated iron intake was not associated with greater BMD among women with higher (>1200 mg/d) or lower calcium intakes (<800 mg/d). Dietary iron may be a more important factor in bone mineralization than originally thought and, its combined effect with calcium on BMD warrants exploration in future studies. *J. Nutr.* 133: 3598–3602, 2003.

KEY WORDS: • bone mineral density • dietary calcium • dietary iron • postmenopausal women

The association of dietary iron with bone mineral density (BMD)⁴ has not been widely studied and its role in bone mineralization remains largely unknown. It has been established that iron is an important mineral for all cells, including osteoblasts, which are involved in bone formation. Several studies reported that iron overload and iron deficiency are both associated with low bone mass (1–7). However, these reports are based largely on animal models and unique populations of people. To our knowledge, only two large population studies have shown that iron intake is associated with BMD (8,9).

We conducted a preliminary cross-sectional analysis examining the association of many nutrients with BMD individually and in concert with one another in a sample of healthy postmenopausal women. As expected, nutrients such as calcium, magnesium, phosphorus, zinc and vitamin D had posi-

tive significant associations with BMD. Iron was also consistently associated significantly ($P < 0.01$) with BMD across all bone sites studied. Thus, in the analyses reported herein we sought to determine whether this association was clinically significant for bone in a cross-sectional baseline sample of postmenopausal women enrolled in an exercise intervention trial to perform our analysis.

SUBJECTS AND METHODS

Design. The Bone, Estrogen, and Strength Training (BEST) Study was a partially randomized clinical trial of the effects of exercise on BMD in early postmenopausal women. In the main trial, women who were undergoing hormone replacement therapy (HRT, $n = 159$) for at least 1 y and not >5.9 y, and women who had not used HRT (NHRT, $n = 161$) for at least 1 y were randomized to exercise or no exercise conditions. Recruitment is described elsewhere (10). We examined the baseline sample of healthy nonsmoking women for this report. The University Human Subjects Committee approved the study and written informed consent was obtained from all participants before entering the study.

Diet assessment at baseline. A total of 321 women were enrolled in the study. Women who did not have 3-d of diet records ($n = 24$) or a DXA bone scan at baseline ($n = 1$) were excluded from the analyses. Another 52 women whom we determined to have implausible energy intake were also excluded, on the basis of a comparison of estimated energy requirements with self-reported energy intakes taken from diet records. Estimated energy requirements

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⁴ Abbreviations used: BEST Study, Bone, Estrogen, and Strength Training Study; BMC, bone mineral content; BMD, bone mineral density; DXA, dual energy X-ray absorptiometry; HRT, hormone replacement therapy; RDA, recommended dietary allowance.

were determined using the recommended dietary allowances (RDA) (12) at a light-to-moderate activity level for age and gender. The approximate energy allowance for females 25–50 y old is 36 kcal (151 kJ)/kg. The approximate energy allowance for females \geq 51 y old is 30 kcal (126 kJ)/kg (11). The following conditions were then applied: 1) self-reported mean energy intake was within \pm 40% of the RDA for energy requirements for women who perform at a light-to-moderate activity level, or they were excluded; 2) if the self-reported mean energy intake was $<$ 1200 kcal (5021 kJ) the subject was excluded from the data set; 3) any self-reported mean energy intake $>$ 40% of the RDA was accepted. Finally, two women were excluded who had extremely high intakes of iron ($>$ 40 mg/d). After the exclusion criteria were applied, data for 242 women were included in the analysis.

Baseline dietary intake was assessed from three randomly assigned days of diet records, including one weekend day and two weekdays. For the sample size of subjects in our study, 3 d of diet records provided a sufficient data with which to measure major nutrients (12). Subjects completed 1.5 h of diet record training before the recording period. Training consisted of participatory portion size estimation and measurement training, directions on recording food descriptions and evaluation of portion size estimation accuracy. Participants did not receive dietary advice and were instructed not to change their diets. Diet records were reviewed for completeness and accuracy with the participants by trained technicians. The diet records were analyzed for nutrient intake by trained technicians using the Minnesota Nutrient Data System versions 2.8–2.92.

Body composition. Total body and regional BMD (g/cm^2) were measured by dual energy X-ray absorptiometry (DXA). Standing height and weight were measured with subjects wearing lightweight clothing without shoes. BMI in kg/m^2 was calculated from measured weight and height. The percentages of body fat and lean soft tissue mass were obtained from DXA whole-body scans as described above. The percentage of fat was derived as the ratio of fat mass to whole-body mass estimated by DXA. Lean soft tissue measured by DXA is the equivalent of whole-body mass minus the fat and bone masses. Fat-free mass was calculated as the sum of lean soft tissue and total body bone mass. For details of body composition measurements, see Going et al. (10).

Statistical analysis. Statistical analyses were completed using the Statistical Package for the Social Sciences (SPSS, version 10.1, Chicago, IL). Distributions of all variables were examined and nutrient variables were log-transformed to meet the assumptions of statistical tests. Baseline characteristics of BMD and mean nutrient intakes were computed for the entire sample of women. Because HRT was not a randomized component of the study, we also examined the mean differences between women using HRT and not using HRT with the independent samples *t* test.

Several factors have been reported to affect BMD and were considered as confounders in our analyses including age, years past menopause, fat mass, fat-free mass, BMI, HRT use, physical activity, smoking, total energy intake, calcium intake and vitamin D intake. We tested several multivariate models using a variety of these confounders to determine the best, yet most parsimonious prediction model for the overall analysis. The following covariates, which were the most significant predictors of BMD, contributed greatly to the variance of the model and were included in the final model: years past menopause, fat mass, fat-free mass, HRT use (0 = no, 1 = yes) and total energy intake. The inclusion of age and/or years past menopause made no difference in our preliminary analyses as a significant covariate, and we chose to include years past menopause as the proxy covariate for age. Fat mass and fat-free mass were used instead of BMI or % fat because they were significant predictors of BMD, whereas BMI and % fat did not add to the variance of BMD in the model. Because many foods with high iron content also tend to be high in protein (i.e., meat), the association of iron with bone was further examined by another regression using the same covariates mentioned above with the addition of protein.

Pearson correlation coefficients were calculated among bone, iron, calcium and protein intakes. Multiple linear regression was used to test for significance of iron on the BMD of five different bone sites (total body, lumbar spine L₂–L₄, femur neck, femur trochanter and

Ward's triangle) separately, adjusting for the confounding effects of the covariates listed above. We analyzed iron and its association with bone in a series of four regression models. The first model examined the association between iron and BMD, accounting for years post menopause, fat mass, fat-free mass, HRT, and total energy intake. Model 2 used the Model 1 base with the addition of protein, Model 3 used Model 1 with the addition of calcium, and Model 4 examined iron and bone associations as in Model 1 with the addition of both protein and calcium.

To graphically depict the association of iron with bone, quartiles of iron intake were calculated. The adjusted mean BMD at each quartile of the iron was then calculated (adjusted for the variables mentioned above) and BMD differences across the quartile groups were tested for significance using the general linear model procedure, adjusted for the set of confounders described above, with pairwise comparisons of least-squares means.

Finally, because the interactive effect of iron on calcium absorption is well known, we further examined iron and calcium together for their relationship with BMD. We categorized iron into four groups with clinical meaning relative to recommended dietary intake guidelines ($<$ 10 mg, 10–14 mg, 14–20 mg, and 20–40 mg) and calcium into three groups ($<$ 800 mg, 800–1200 mg, $>$ 1200 mg). Two women had iron intakes $>$ 40 mg. Because this is considered an excessively high intake of iron, we deleted these subjects to preserve integrity of the data, thereby eliminating the chance for these two outliers to bias results. The adjusted mean BMD at each iron and calcium category were calculated (adjusted for all covariates) using the general linear model procedure.

Because HRT was a very strong predictor of BMD ($P < 0.001$) and a nonrandom component in the study design, the effects of interactions between iron, protein, calcium and HRT use on BMD were also examined.

RESULTS

Descriptive statistics. The mean age of the women was 54.8 ± 4.6 y; they were 5.8 ± 3.5 y past menopause and were borderline overweight with a mean BMI of 25.4 ± 3.9 . Women who were taking HRT were users for 2.8 ± 1.1 y. These women were slightly younger (54.3 ± 4.3 vs. 55.5 ± 4.9 y, $P < 0.05$) and were fewer years postmenopausal (5.1 ± 3.7 vs. 6.5 ± 3.3 y, $P < 0.01$) than women not taking HRT. The two groups were similar in body composition and weight; however, women using HRT had higher BMD at all bone sites ($P < 0.001$ to <0.05 , **Table 1**).

The mean nutrient intakes of all of the women in this study were compared with the national population mean nutrient intakes and Dietary Reference Intakes. Compared with the population mean, the women in the BEST study tended to have higher energy and nutrient intakes. They also met or exceeded the Dietary Reference Intakes for all nutrients except calcium (811 vs. 1200 mg), potassium (2978 vs. 3500 mg), zinc (10 mg vs. 12 mg), vitamin D (5 vs. 10 μg) and fiber (21 vs. 25 kg). A slightly lower Vitamin D intake is not of concern in women living in the desert Southwest where sun exposure is greater than the national average. There were no differences by HRT status with respect to mean nutrient intakes among the women in the study, suggesting that the diets were similar between the HRT and no HRT groups. HRT interactions with nutrients on BMD were also tested and no significant interactions were found. Thus, the remaining analyses were performed with HRT as a covariate in the regression models.

Correlations between covariates, iron, protein and BMD were tested. The covariate, years postmenopause, was not correlated with any skeletal site. Fat-free mass, fat mass, energy, protein, calcium and iron were positively correlated with all bone sites. Calcium and iron were intercorrelated ($r = 0.34$; $P = 0.01$).

TABLE 1

Baseline characteristics of the postmenopausal women in the study^{1,2}

Characteristic	All women	HRT status	
		NHRT	HRT
Age, y	54.8 ± 4.6	55.5 ± 4.9	54.3 ± 4.3*
Time postmenopause, y	5.8 ± 3.5	6.5 ± 3.3	5.1 ± 3.7*
Time on HRT, y	—	—	2.8 ± 1.1
Height, cm	163.1 ± 6.5	162.8 ± 6.2	163.5 ± 6.7
Weight, kg	67.8 ± 11.6	67.7 ± 11.1	67.8 ± 12.1
BMI, kg/m ²	25.4 ± 3.9	25.5 ± 3.7	25.3 ± 4.1
Fat mass, %	38.3 ± 6.8	38.7 ± 6.7	37.9 ± 6.8
Mean nutrient intake from 3-d diet records			
Energy, kJ	7816 ± 1592	7732 ± 1571	7892 ± 1609
Protein, g	73 ± 20	72 ± 20	74 ± 20
Calcium, mg	811 ± 290	787 ± 290	832 ± 290
Iron, mg	16 ± 6	15 ± 7	16 ± 6
Body composition from DXA			
Fat mass, kg	26.1 ± 8.4	26.3 ± 8.1	25.9 ± 8.8
Fat-free mass, kg	40.6 ± 4.6	40.3 ± 4.6	40.9 ± 4.6
Bone mineral content, g/cm ²	2.22 ± 0.31	2.16 ± 0.31	2.28 ± 0.30*
Total body BMD, g/cm ²	1.11 ± 0.08	1.09 ± 0.08	1.13 ± 0.08*
Femur neck BMD, g/cm ²	0.87 ± 0.12	0.85 ± 0.12	0.89 ± 0.12*
Femur trochanter BMD, g/cm ²	0.74 ± 0.11	0.72 ± 0.11	0.76 ± 0.11*
Ward's triangle BMD, g/cm ²	0.76 ± 0.14	0.74 ± 0.14	0.78 ± 0.14*
Lumbar spine L ₂ -L ₄ , g/cm ²	1.12 ± 0.15	1.09 ± 0.16	1.15 ± 0.14*

¹ Values are means ± SD; all women *n* = 242; NHRT *n* = 128; HRT *n* = 114; * Different from NHRT, *P* < 0.05.

² Abbreviations: NHRT, not on hormone replacement therapy; DXA, dual energy X-ray absorptiometry; BMD, bone mineral density.

Multiple regression with individual nutrients regressed on BMD. We tested the relationship of iron individually (and together with protein and calcium) with BMD at various bone sites using linear regression analyses while adjusting for the effects of HRT, years postmenopause, fat-free mass, fat mass and total energy intake (Table 2). Iron was positively (*P* < 0.01) associated with BMD across all bone sites even after adjusting for all covariates. Adding protein and calcium individually into the models slightly attenuated the relationship of iron with BMD, but associations remained significant. Adding protein and calcium together into the model also attenuated the relationship between iron and bone density but associations were still significant except for total body BMD (*P* = 0.06). Protein and calcium intake, individually or together, contributed to explaining up to 1% of the variance in BMD. These results suggest that although protein and calcium are associated with BMD, iron remains a significant correlate of BMD.

Iron associations with BMD using quartiles. To show graphically the associations of iron with BMD, we displayed

quartiles of iron against the adjusted mean BMD of the corresponding bone site (Fig. 1). Linear regression analysis showed that increases in iron intake were significantly associated with increases in BMD at every site. This corresponded to a 4–14% difference between the lowest and highest iron intake. In contrast, calcium intake was associated with BMD corresponding to a 5–9% difference between the lowest and highest quartiles of calcium intake (results not shown).

Iron and calcium associations with BMD using categories with clinical meaning. The final part of our analyses focused on the effect of the interaction of calcium with iron on BMD. We created four groups for iron and three groups for calcium intakes that have clinical meaning with respect to these nutrients. Women who had mean calcium intakes ranging between 800 and 1200 mg/d had higher BMD with increasing iron intakes. In this group, women with intakes of ≥20 mg iron had the highest mean BMD compared with women with the lowest amount of iron (<10 mg). This was true across all bone sites (similar to Fig. 2). The relationship among the

TABLE 2

Regression of iron on bone mineral density of five bone sites in postmenopausal women

	Model 1 Covariates ¹ + iron			Model 2 Covariates + protein + iron			Model 3 Covariates + calcium + iron			Model 4 Covariates + protein + calcium + iron		
	β ± SD	<i>P</i> -value	<i>R</i> ² *	β ± SD	<i>P</i> -value	<i>R</i> ² *	β ± SD	<i>P</i> -value	<i>R</i> ² *	β ± SD	<i>P</i> -value	<i>R</i> ² *
Total body	0.010 ± 0.039	0.013	0.17	0.083 ± 0.040	0.04	0.177	0.085 ± 0.040	0.03	0.173	0.075 ± 0.040	0.064	0.18
Spine	0.185 ± 0.073	0.012	0.11	0.169 ± 0.075	0.03	0.112	0.177 ± 0.075	0.02	0.108	0.165 ± 0.076	0.030	0.11
Femur trochanter	0.153 ± 0.048	0.002	0.28	0.134 ± 0.049	0.01	0.291	0.143 ± 0.049	0.004	0.283	0.129 ± 0.050	0.010	0.29
Femur neck	0.170 ± 0.058	0.004	0.16	0.148 ± 0.059	0.01	0.165	0.155 ± 0.059	0.01	0.159	0.140 ± 0.059	0.019	0.16
Ward's triangle	0.278 ± 0.068	<0.001	0.13	0.254 ± 0.069	<0.001	0.141	0.251 ± 0.069	<0.001	0.143	0.236 ± 0.070	0.001	0.15

¹ List of covariates: years postmenopause, hormone replacement therapy, fat-free mass, fat mass and total energy intake. * Adjusted *R*².

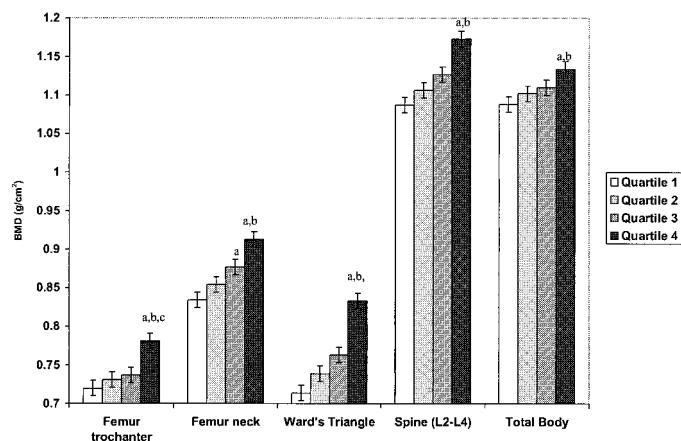


FIGURE 1 Baseline association of iron with five bone sites in postmenopausal women. a: Significantly different from quartile 1; b: significantly different from quartile 2; c: significantly different from quartile 3. Each quartile is one of four equally distributed groups. Differences were considered significant at $P \leq 0.05$. Models were adjusted for years postmenopause, hormone replacement therapy, fat-free mass, fat mass and total energy intake. BMD, bone mineral density

women with lower or higher calcium intakes and BMD was less pronounced. It appeared that iron had a threshold effect at intake ranges of 10–20 mg on BMD among women with lower or higher intakes of calcium. These results show that there is a complex relationship between iron and calcium on BMD.

DISCUSSION

This study examined baseline cross-sectional iron associations with BMD among a healthy, sedentary and nonsmoking population of postmenopausal women. We know of only two other human studies in which iron was examined in relation to bone density (8,9). Angus et al. (8) found a significant relationship between iron and bone in both pre- and postmenopausal women. This relationship with iron was seen in forearm bone mineral content (BMC), a site that we did not include in our analyses. Dual photon absorptiometry, a less accurate method for measuring bone mass, was used to assess bone. Michaelsson et al. (9) examined iron in relation to BMD of the spine, total body and femoral neck in 175 women aged 28–74 y. A food frequency questionnaire and four 7-d records were used to assess diet. In their study, dietary iron, estimated from diet records, was associated ($P < 0.05$) with the BMD at all three bone sites in univariate regression analysis. Multiple regression analyses, adjusted with 1–11 covariates, showed no associations between iron and any bone site. In contrast, we detected significant associations ($P < 0.01$) between dietary iron and BMD at all five bone sites in our study, even after adjusting for confounding variables. When we added other nutrients to the analysis, iron remained significant (Table 2). The results of these regression analyses were evident in the differences in BMD among quartiles of iron intake, with the lowest BMD occurring at the lowest intake of iron. One explanation for this discrepancy in findings may be that we had a larger sample size ($n = 242$ vs. 175), which would allow a better test of the association after adjusting for covariates and a more homogenous population (i.e., narrower age range, nonsmokers, sedentary and postmenopausal women only).

In prior analyses from this study, we found that protein was also positively and significantly associated with BMD. Because protein and iron are found in many of the same food sources,

it is reasonable to assume that iron was significant in associations due to the presence of protein. However, in regression analyses in which iron and protein were entered simultaneously into the model, protein contributed little to the variance of BMD and iron remained highly significant. Thus, iron has a significant association with bone, even after accounting for protein.

Similarly, in preliminary analyses, calcium intake was also significantly associated with BMD. In our current analyses, accounting for calcium when examining iron and bone relationships attenuated the associations slightly, but iron remained significantly associated with bone. Because iron and calcium compete for absorption, we further explored this relationship with bone by creating groups of ranges of calcium and iron intakes that would have clinical meaning related to Recommended Dietary Intake guidelines. We discovered a complex relationship among iron, calcium and bone. Women who consumed 800–1200 mg of calcium had significantly higher BMD with increasing levels of iron intake. However, among women with higher or lower intakes of calcium, the relationship of iron to BMD was not apparent.

Several studies have indicated that there is a possible relationship between iron status and BMD, but the exact mechanisms as well as physiologic and clinical importance of these results have yet to be explored. Iron is essential for the synthesis of collagen structure upon which bone mineralization occurs (13). Iron is also involved in the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, the active form of the vitamin D (14). Vitamin D is required for the proper regulation and absorption of calcium and phosphorus and therefore plays an important role in the mineralization of bone. In one animal study, iron-deficient rats had decreased bone mechanical strength in their femurs compared with normal rats with similar BMD, BMC and dietary levels of calcium (1). The authors suggested that decreased collagen crosslinking (due to decreased iron intake) may contribute to the decreased bone strength rather than calcium. Kipp et al. (15,16) found that iron deficiency resulted in low bone mass and bone volume and more recently found that that long-term iron deficiency altered bone mass and bone structure in growing female rats. In another study, low transferrin levels had deleterious effects on bone biomechanical properties and upset

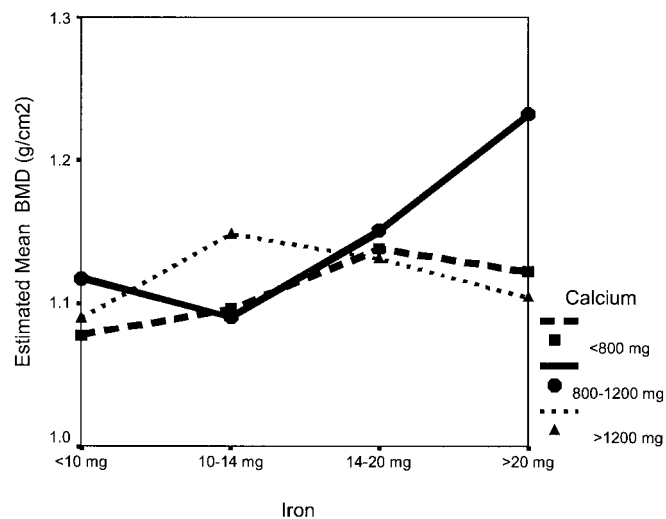


FIGURE 2 Estimated marginal means of spine (L_2 – L_4) bone mineral density (BMD) across categories of calcium and iron intakes in postmenopausal women.

calcium homeostasis. However, dietary iron deficiency had no adverse effect on structural or mechanical properties in rat femurs. A discrepancy in these results could be due to a lack of observed iron deficiency as indicated by hematocrit levels or due to the different species of rats used (2).

Iron overload has also been associated with low bone density (3,4). Excessive levels of iron may lead to depressed osteoblast function (5,6), although only very high iron concentrations (400 μmol , >20 times that in an average American diet) may be required for this to occur (7). Whether excessive dietary iron intake, elevated iron body stores due to disease or other contributing factors (i.e., iron-induced vitamin C depletion) adversely affect osteoblast function has yet to be determined.

To our knowledge, our results present novel findings on the associations of dietary iron with BMD and its interaction with calcium in humans. Our findings concerning the effect of the interactions between calcium and iron on BMD are supported by a recent animal study in which Medeiros et al. (17) found that iron-deficient rats had lower BMD than controls, and the negative effect on bone was exacerbated by a deficiency of calcium in the diet. Several limitations of our present study include small sample sizes in some of the cells in the iron/calcium analyses, use of cross-sectional analysis whereby temporal relationships could not be assessed and no biochemical assessment of iron status in addition to iron intake.

If the iron and calcium relationship exists in relation to bone density in the population in general, dietary recommendations for iron may have to be reassessed. These findings indicate that an increase in iron 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. Further studies are warranted to explore the biological mechanisms and determine what role dietary iron intake or other indicators of iron (i.e., serum ferritin, transferrin, hemoglobin) have on bone mineralization.

LITERATURE CITED

1. Medeiros, D. M., Ilich, J., Ireton, J., Matkovic, V., Shiry, L. & Wildman, R. (1997) Femurs from rats fed diets deficient in copper or iron have decreased mechanical strength and altered mineral composition. *J. Trace Elem. Exp. Med.* 10: 197-203.
2. Malecki, E. A., Buhl, K. M., Beard, J. L., Jacobs, C. R., Connor, J. R. & Donahue, H. J. (2000) Bone structural and mechanical properties are affected by hypotransferrinemia but not by iron deficiency in mice. *J. Bone Miner. Res.* 15: 271-277.
3. Schnitzler, C. M., Macphail, A. P., Shires, R., Schnaid, E., Mesquita, J. M. & Robson, H. J. (1994) Osteoporosis in African hemosiderosis: role of alcohol and iron. *J. Bone Miner. Res.* 9: 1865-1873.
4. Diamond, T., Stiel, D. & Posen, S. (1989) Osteoporosis in hemochromatosis: iron excess, gonadal deficiency or other factors? *Ann. Intern. Med.* 110: 430-436.
5. Van de Vyver, F. L., Visser, W. J., D'Haese, P. C. & DeProe, M. E. (1988) Iron overload and bone disease in chronic dialysis patients. *Contrib. Nephrol.* 64: 134-143.
6. Lynch, S. R., Seftel, H. C., Wapnick, A. A., Charlton, R. W. & Bothwell, T. H. (1970) Some aspects of calcium metabolism in normal and osteoporotic Bantu subjects with special reference to the effects of iron overload and ascorbic acid depletion. *S. Afr. J. Med. Sci.* 35: 45-56.
7. Diamond, T., Pojer, R., Stiel, D., Alfrey, A. & Posen, S. (1991) Does iron affect osteoblast function? Studies in vitro and in patients with chronic liver disease. *Calcif. Tissue Int.* 48: 373-379.
8. Angus, R., Sambrook, P., Pocock, N. & Eisman, J. (1988) Dietary intake and bone mineral density. *Bone Miner.* 4: 265-277.
9. Michaelsson, K., Homberg, L., Mallmin, H., Wolk, A., Bergstrom, R. & Ljunghall, S. (1995) Diet, bone mass, and osteocalcin: a cross-sectional study. *Calcif. Tissue Int.* 57: 86-93.
10. Going, S., Lohman, T., Houtkooper, L., Metcalfe, L., Flint-Wagner, H., Blew, R., Stanford, V., Cussler, E., Martin, J., Teixeira, P., Harris, M., Miliken, L., Figueroa-Galvez, A. & Weber, J. (2003) Effects of exercise on bone mineral density in calcium-replete postmenopausal women with and without hormone replacement therapy. *Osteoporos. Int.* 8: 637-644.
11. National Research Council (1989) Recommended Dietary Allowances, 10th ed. National Academy Press, Washington, D.C.
12. Basiotis, S. P., Welch, S., Cronin, F., Kelsay, J. & Mertz, W. (1987) Number of d of food intake records required to estimate individual and group nutrient intakes with defined confidence. *J. Nutr.* 117: 1638-1641.
13. Propckop, D. J. (1971) Role of iron in the synthesis of collagen in connective tissue. *Fed. Proc.* 30: 984-990.
14. DeLuca, H. F. (1976) Metabolism of vitamin D: current status. *Am. J. Clin. Nutr.* 29: 1258-1270.
15. Kipp, D., Pinero, D. & Beard, J. L. (1998) Low bone mass and volume in iron-deficient rats. *FASEB. J.* 12: A508 (abs.).
16. Kipp, D. E., Beard, J. L. & Lees, C. J. (2002) Mild iron deficiency results in altered bone mass and histomorphometry in growing female rats. *FASEB. J.* 16: A273 (abs.).
17. Medeiros, D. M., Plattner, A., Jennings, D. & Stoecker, B. (2002) The morphology, strength and density are compromised in iron-deficient rats and exacerbated by calcium restriction. *J. Nutr.* 132: 3135-3141.