Iodine Deficiency Mitigates Growth Retardation and Osteopenia in Selenium-Deficient Rats[1]

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ABSTRACT Selenium deficiency is associated with impaired bone metabolism and osteopenia in rats. However, it is not known how combined selenium and iodine deficiency affects bone metabolism. Therefore, we investigated the effect of selenium and iodine deficiency on bone metabolism in 2nd-generation selenium- and iodine-deficient rats. Selenium-deficient (Se−), iodine-deficient (I−), selenium- and iodine-deficient (Se−/I−), and control rats (Se+/I+), were pair-fed their respective diets until they were 74 d old. Each pair-fed rat was fed a selenium-adequate diet in the same amount as that consumed the day before by its selenium-deficient counterpart, taking food spillage into account. The skeletal phenotype was analyzed by dual energy X-ray absorptiometry, histomorphometry, and bone metabolism markers. Erythrocyte glutathione peroxidase activity (Gpx) and plasma thyroid hormones were measured to assess selenium and iodine status, respectively. In both Se−/I+ and Se−/I− rats, Gpx was reduced by 99% compared with pair-fed Se+/I+ and Se+/I− rats ($P < 0.001$). Iodine deficiency reduced plasma thyroxine by 64% in the 2 iodine-deficient groups ($P < 0.001$). Body weight, tail length, plasma insulin-like growth factor, pituitary growth hormone concentration, and femur and tibia bone mineral density were significantly greater in the Se−/I− rats than in the Se−/I+ rats. This study shows that iodine deficiency mitigated growth retardation and osteopenia in 2nd-generation selenium-deficient rats and suggests that adequate selenium status should be ensured before measures are taken to correct iodine deficiency. J. Nutr. 136: 595–600, 2006.

KEY WORDS: • *selenium deficiency* • *iodine deficiency* • *bone mineral density* • *osteopenia* • *bone metabolism*

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The importance of adequate selenium intake for human health first became apparent when it was shown that Keshan disease, a cardiomyopathy endemic in China, could be prevented by selenium supplementation (1). Several cases of cardiomyopathy attributed to selenium deficiency were also reported in patients administered parenteral nutrition who had low serum selenium concentrations (2). A similar relation between low selenium status and Kashin-Beck disease, an osteoarthropathy endemic in selenium-deficient areas of Tibet, was reported (3). Low selenium status in phenylketonuric children, due to the low selenium content of their diet, has been associated repeatedly with low bone mineral density (BMD)[3] (4,5). In addition, growth retardation in 2nd-generation selenium-deficient rats has been associated with impaired bone metabolism and osteopenia (6). However, the data that support a relation between selenium and bone metabolism are less conclusive than those for Keshan disease. Physiological support for a relation between selenium status and these diverse pathological conditions exists because selenium protects cells against oxidative damage (7–9).

In selenium- and iodine-deficient areas of Tibet, iodine but not selenium status appears to be a risk factor for Kashin-Beck disease (10,11). For Keshan disease, selenium deficiency alone seems insufficient to induce a pathological condition on its own; however, it likely represents a permissive condition that makes bone more sensitive to the action of other factors (12). To elucidate the role of iodine and selenium deficiency in bone physiology, we investigated whether the effect of selenium deficiency on bone metabolism in rats was affected by combined iodine and selenium deficiency as observed in the Kashin-Beck endemic area of Tibet.

MATERIAL AND METHODS

Animals and diets. Female Wistar rats (12 wk old; Iffa Credo) were housed in a light- and temperature-controlled room and fed a selenium-deficient and iodine-adequate diet (Se−/I+), a selenium-adequate

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[3] Abbreviations used: BMC, bone mineral content; BMD, bone mineral density; D2, type 2 iodothyronine deiodinase selenoenzyme; GH, growth hormone; Gpx, glutathione peroxidase activity; IGF-I, insulin-like growth factor; ROS, reactive oxygen species; Se+/+, selenium and iodine adequate; Se+/I−, selenium-adequate/iodine-deficient; Se−/I+, selenium-deficient/iodine-adequate; Se−/I−, selenium and iodine deficient; T3, triiodothyronine; T4, thyroxine; TSH, thyrotropin.

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and iodine-deficient diet (Se+/I−), or a selenium-deficient and iodine-deficient diet (Se−/I−) for a minimum of 42 d before being mated with selenium-adequate male rats. The selenium-deficient diet, the same as that used in previous studies (6,13), contained 0.005 mg selenium/kg and used torula yeast as a protein source. The iodine-deficient diet contained 0.05 mg iodine/kg. The selenium-adequate diet contained 0.19 mg selenium/kg (Hope Farms). The diet composition is presented in **Table 1**. During pregnancy, female rats were housed in individual plastic cages. After delivery, the pups were kept with their dams and fed the same diet. At d 21, male offspring were housed separately and continued to be fed the same diet until the end of the experiment at d 74. A set of control male (Se+/I+) rats was obtained by mating female and male rats fed the same selenium-deficient diet supplemented with 0.19 mg of selenium/kg. From weaning until the end of the experiment, individual pair-feeding was performed every day. A pair-fed control rat was fed a selenium-adequate diet in the same amount as that consumed the day before by its selenium-deficient counterpart, taking food spillage into account.

Starting on d 21, tail length and body weight of 2nd-generation weanling male rats were measured 1 time/wk until the end of the experiment. Rats were placed in metabolic cages on d 73 for a 24-h urine collection to measure deoxypyridinoline, calcium, and phosphate. On d 74, rats were anesthetized with ether; blood was drawn by cardiac puncture using a heparinized syringe, and the rats were then killed humanely by decapitation. Femurs and tibias were removed and cleaned, and pituitaries were dissected and frozen.

The institutional Animal Care Committee of the Free University of Brussels, Faculty of Medicine approved the care and treatment of experimental animals.

Assays. Erythrocyte glutathione peroxidase activity (Gpx) was measured spectrophotometrically at 340 nm, after elimination of leukocytes and platelets, by the decrease in NADPH (0.2 mmol/L) using glutathione (1 mmol/L) and H_2O_2 (0.15 mmol/L) as substrates (15,16). The enzyme unit of Gpx was defined as 1 nmol of reduced NADPH oxidized per minute. Plasma and urinary calcium and phosphate were determined by colorimetry using *o*-cresolphthalein and phosphomolybdate, respectively (Hitachi Modular-P, Roche Diagnostic). Plasma proteins were determined by the Biuret method. Commercial RIA kits were used to measure plasma thyroxine (T4), triiodothyronine (T3) (Amersham), and insulin-like growth factor (IGF-I; Biosource). Osteocalcin was measured using a rat-specific RIA kit (Biomedical Technologies). Urinary deoxypyridinoline was measured by an automated immunoassay with chemiluminescence detection (Immulite, Diagnostic Products) and commercial reagents (Diagnostic Products). The individual pituitaries were homogenized in 150 μL distilled water and frozen. They were later thawed and diluted 20-fold in phosphosaline buffer, pH 7.4, containing 1% bovine serum albumin. For the determination of growth hormone (GH) and thyrotropin (TSH), 20-μL aliquots were used in triplicate. GH and TSH were measured by RIA using rat-specific immunoreactants kindly supplied by the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and by Dr. A. F. Parlow (Harbor-UCLA Medical Center). Concentrations are expressed in weight equivalents of the NIDDK rat GH and TSH RP-2 reference preparation.

Dual energy X-ray absorptiometry. The right femur and tibia were scanned on the Hologic QDR-100 adapted for small animals. The measurement was performed on excised tibias and femurs. The area, length, bone mineral content (BMC), and BMD of the femur and tibia were measured, as well as the BMC and BMD of the distal end (1 cm) of the femur and the proximal end (1 cm) of the tibia.

Histomorphometry. An i.p. injection of calcein (16 mg/kg body weight) was given 11 and 2 d before the rats were killed. The distal end of the left femur was fixed in acetone and embedded, in undecalcified form, in methylmethacrylate/glycolmethacrylate in preparation for morphometric measurements. The distal left femur was sliced longitudinally in the coronal plane and 7-μm thick sections (undecalcified) were stained with von Kossa or left unstained for visualization of calcein labels. The following parameters were measured using a KS300 image analyzer (Zeiss) in a standardized zone of 1.7 mm^2 at a distance of 1 mm from the growth plate: the trabecular bone volume, the trabecular surface (mm/mm^2), the double-labeling surface, and the mineral apposition rate. Sections (5-μm thick) were stained with hematoxylin-eosin for osteoblast counting or for tartrate-resistant acid phosphatase activity for osteoclast. Cells were counted manually under a light microscope, at a magnification of ×250, using an eyepiece equipped with a Zeiss II integration grid.

Statistical analysis. Data are expressed as means ± SEM. Treatment effects were analyzed by 2-way ANOVA (I × Se). When the interaction was significant ($P < 0.05$), the Bonferroni post-hoc test (or Games-Howell test, in the case of heterogeneous variance) was applied to test for differences between groups. A two-factor repeated-measures ANOVA was performed, with time as the within-subjects variable and diet as the between-subjects variable to compare the effect of time and diet and the time × diet interactions for weight and tail length. We considered *P*-values < 0.05 to indicate significant differences. The computer program SPSS 9.0 was used for the analysis.

TABLE 1

Composition of the experimental diets[1]

	Diet			
	Se+/I+	Se+/I−	Se−/I+	Se−/I−
Protein, *g/kg*	190	190	190	190
Fat, *g/kg*	62	62	62	62
Fiber, *g/kg*	67	67	67	67
Sucrose, *g/kg*	50	50	50	50
Cellulose, *g/kg*	36	36	36	36
Mineral mix,[2] *g/kg*	29	29	29	29
Vitamin mix,[2] *g/kg*	10	10	10	10
Selenium, *mg/kg*	0.19	0.19	0.005	0.005
Iodine, *mg/kg*	0.51	0.05	0.51	0.05

1 L-Amino acids (g/kg): 13.40 L-lysine, 4.12 methionine, 1.97 L-cystine, 8.16 L-threonine, 2.45 L-tryptophane, 7.68 L-isoleucine, 9.32 L-arginine, 7.41 L-phenylalanine, 3.47 L- histidine, 12.10 L-leucine, 5.88 L-tyrosine, 8.87 L-valine, 12.65 L-alanine, 17.17 L-aspartic acid, 33.01 L-glutamic acid, 7.72 L-glycine, 6.87 L-proline, 7.72 L-serine.

2 AIN-93M (14).

RESULTS

Selenium status. Erythrocyte Gpx, a marker of selenium nutritional status, was significantly affected by selenium, iodine, and their interaction (**Table 2**). Selenium deficiency decreased Gpx 99% in both the Se−/I+ and Se−/I− rats and iodine deficiency increased Gpx by 13% in the Se+/I− compared with Se+/I+ rats.

Thyroid function. Iodine, but not selenium, affected plasma T4 ($P < 0.001$) (**Table 2**), whereas the interaction between selenium and iodine was not significant ($P = 0.768$). Plasma T4 concentrations were reduced by 64% in the 2 iodine-deficient groups. Plasma T3 concentrations were not affected by either selenium or iodine alone but their interaction was significant, with lower T3 in the Se+/I− compared with Se+/I+ rats and higher T3 in Se−/I− than in the Se−/I+ rats. Both selenium and iodine deficiency significantly increased pituitary TSH concentrations but the interaction was not significant ($P = 0.824$).

Anthropometric data. Despite pair-feeding, both body weight and tail length were lower in the Se−/I+ rats than in the Se+/I+, Se+/I− and Se−/I− rats 1 wk after weaning, and the magnitude of the differences increased with age (**Fig. 1**). In the Se−/I− rats, lower body weight and shorter tail length were observed only 5 wk after weaning. Selenium but not iodine affected body weight and tail length. However, the negative effect of selenium deficiency was different in the

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TABLE 2

Biomarkers of growth and metabolism in 2nd generation selenium- and iodine-deficient rats[1]

	Se+/I+	Se+/I−	Se−/I+	Se−/I−	ANOVA *P*-values[2]		
					Se	I	Se × I
Body weight, *g*	306 ± 3[a]	298 ± 4[a]	255 ± 7[c]	280 ± 3[b]	<0.001	NS[3]	0.001
Tail length, *cm*	18.6 ± 0.1[a]	18.1 ± 0.1[a]	16.7 ± 0.2[c]	17.6 ± 0.1[b]	<0.001	NS	<0.01
Femur length, *mm*	35 ± 0.2	35 ± 0.1	33 ± 0.3	34 ± 0.1	<0.001	0.011	NS
Tibia length, *mm*	39 ± 0.4[a]	39 ± 0.3[a]	38 ± 0.2[b]	39 ± 0.2[a]	0.02	0.05	0.03
Erythrocyte Gpx, *U/mg Hg*	1569 ± 55[a]	1774 ± 73[a]	16 ± 0.5[b]	16 ± 0.6[b]	<0.001	0.031	0.031
Plasma protein, *g/L*	53 ± 0.3	57 ± 0.1	59 ± 0.6	58 ± 0.1	NS	NS	NS
Plasma IGF-I, *nmol/L*	138.5 ± 4.2[b]	107.8 ± 3.3[a]	95.6 ± 2.3[c]	110.9 ± 2.6[a]	<0.001	0.021	<0.001
Pituitary GH, *μg/pituitary*	312 ± 21[a]	278 ± 16[a]	191 ± 15[b]	273 ± 22[a]	0.02	NS	0.04
Plasma T4, *nmol/L*	46.3 ± 1.0	16.7 ± 1.2	46.3 ± 2.3	15.4 ± 0.9	NS	<0.001	NS
Plasma T3, *nmol/L*	0.6 ± 0.05	0.5 ± 0.06	0.4 ± 0.05	0.6 ± 0.03	NS	NS	0.02
Pituitary TSH, *μ*g/pituitary	3.5 ± 0.4	5.1 ± 0.2	5.8 ± 0.7	7.2 ± 0.4	<0.001	0.02	NS

[1] Data are expressed as the means ± SEM, $n = 10$.
[2] Data were analyzed by 2-way ANOVA. Means in a row without a common letter differ, $P < 0.05$.
[3] NS, nonsignificant ($P \geq 0.05$).

iodine-adequate than in the iodine-deficient rats: body weight and tail length were lowest in the Se−/I+ group (Table 2). Selenium deficiency reduced femur length in iodine-adequate and iodine-deficient rats but the interaction was not significant (P = 0.075). Selenium-deficient rats had a shorter tibia length ($P = 0.02$), but there was a significant interaction with iodine. In selenium-deficient rats, tibia length was higher in iodine-deficient compared with iodine-adequate rats.

Dietary selenium ($P < 0.001$) and iodine ($P = 0.021$) affected plasma IGF-I concentrations, and a significant interaction was observed. A selenium-deficient diet significantly lowered plasma IGF-I concentrations, but less so in iodine-deficient than in iodine-adequate rats. Pituitary GH concentration was reduced in selenium-deficient rats ($P = 0.02$) but was not affected by iodine. Again, a significant interaction was observed with a significant reduction in pituitary GH concentration only in the Se−/I+ group, not in Se−/I− rats.

Markers of bone metabolism. Plasma calcium and plasma phosphate concentrations were not affected by dietary selenium or iodine (**Table 3**). Selenium, but not iodine deficiency decreased urinary calcium concentrations, and a significant interaction was observed; the lowest urinary calcium concentrations occurred in the Se−/I+ group. Urinary phosphate concentration was higher in selenium-deficient rats ($P = 0.002$) but was not affected by iodine, and there was no significant interaction. Plasma osteocalcin and urinary deoxypyridoline concentrations were lower in selenium-deficient rats ($P < 0.001$); however, they were not affected by iodine and no interaction was detected.

Bone mineral density. Selenium and iodine significantly affected whole femur and whole tibia BMC and BMD (**Table 4**). Whole femur and whole tibia BMC and BMD were lower in the selenium-deficient groups, but less so in the rats with concomitant iodine deficiency.

Bone histomorphometry. Selenium deficiency ($P = 0.017$) but not iodine affected trabecular bone volume, and a significant interaction was observed (**Table 5**). Trabecular bone volume was decreased less in Se−/I− rats than in Se−/I+ rats. Trabecular bone surface was not affected by selenium or iodine but an interaction was observed ($P = 0.02$): trabecular bone surface was decreased less in Se−/I− than in Se-I+ rats.

Osteoblast and osteoclast numbers were not influenced by selenium or iodine but a significant interaction was present, with higher osteoblast and osteoclast numbers in Se−/I+ rats than in Se−/I− rats. Double labeling of the surface was affected by selenium ($P = 0.031$) but not by iodine with a significant interaction: double labeling of the surface was reduced less in Se−/I− than in Se−/I+ rats. The mineral apposition rate was not affected by selenium or iodine but their interaction was significant, with lower mineral apposition rates in Se−/I+ rats than in Se−/I− rats.

DISCUSSION

In this study, the effect of a diet with combined iodine and selenium deficiency was not to further impair growth and bone mineral density. Despite lower plasma T4 concentrations, iodine deficiency mitigated growth retardation and osteopenia in rats fed a selenium-deficient diet.

The mechanism through which selenium deficiency impairs bone metabolism is not yet fully understood (6). In Se−/I+ rats, osteopenia was associated with increases in osteoblastic and osteoclastic indices, lower plasma osteocalcin concentrations, a marker of osteoblastic function, and lower urinary deoxypyridoline concentrations, a marker of bone resorption. Together, these findings suggest that a defect in osteoblastic and osteoclastic function may contribute to impair bone metabolism in rats fed a selenium-deficient diet.

Because selenium is incorporated into several selenoenzymes, the effect of selenium deficiency on bone cells may be mediated through these enzymes and could affect different metabolic pathways simultaneously. Reactive oxygen species (ROS) are produced during the process of bone remodeling. Selenoproteins, as glutathione peroxidase and thioredoxin reductase, not only protect bone cells against the toxic effect of excessive ROS concentrations but also participate in the control of bone cell function by ensuring redox homeostasis (17,18). Selenium deficiency, by reducing the activity of these selenoenzymes, may modify ROS concentrations and therefore impair bone cell function. The bone loss induced by estrogen deficiency in adult rats was associated recently with a decrease in antioxidant defenses, in particular glutathione and thioredoxin reductase activities (19). This decrease in antioxidant enzymes in estrogen-deficient rats was associated with an increase in osteoclast and osteoblast indices as observed in our group of selenium-deficient rats. Consequently, the diminution

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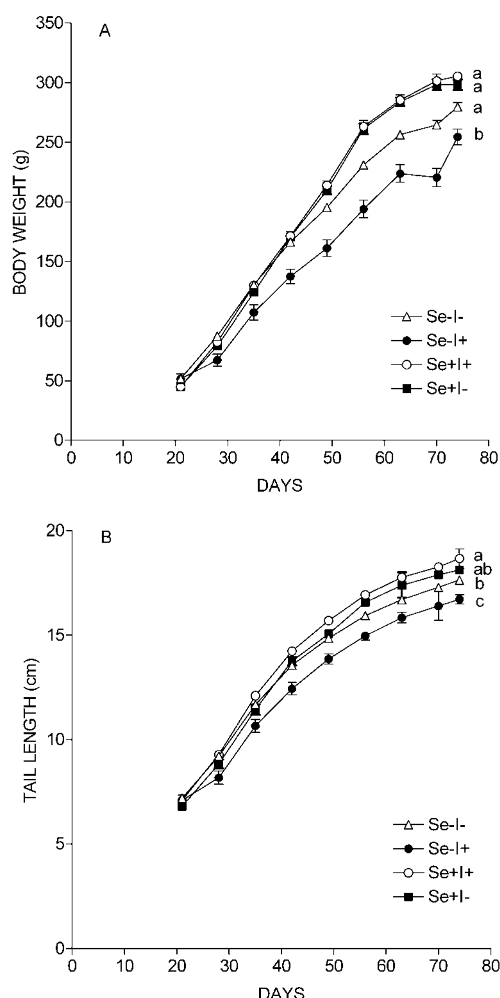

FIGURE 1 Body weights (*panel A*) and tail lengths (*panel B*) in 2nd generation selenium- and iodine-deficient rats. Values are means ± SEM, $n = 10$. The effects of time, time × selenium, time × iodine, and time × selenium × iodine were significant, $P < 0.05$. Means without a common letter differ, $P < 0.05$.

of antioxidant defenses, whether by estrogen deficiency or by selenium deficiency, may modify bone metabolism, underlining the importance that oxidative status could have on bone physiology.

The redox status was reported to be of importance in the intestinal absorption of trace elements and minerals including calcium (20,21). Specifically, glutathione depletion induced by DL-buthionine-*S*,*R*-sulfoximine was reported to decrease the intestinal absorption of calcium (22). A modification of intestinal antioxidant selenoenzymes and ROS concentrations in selenium-deficient rats could also lead to calcium malabsorption and secondary hyperparathyroidism in selenium deficient rats as previously reported (6).

Different groups including our laboratory showed that selenium deficiency is associated with low concentrations of pituitary GH and plasma IGF-I (6,23). GH-IGF-I impairment therefore appears also to be a plausible pathophysiologic mechanism with which to explain the effect of selenium deficiency on growth and bone metabolism.

Osteopenia and growth retardation were more pronounced in selenium-deficient rats than in selenium-and iodine-deficient rats despite hypothyroidism. This is the first study focused specifically on the combined effect of selenium and iodine deficiency on growth and bone metabolism. In previous studies on the effect of selenium and iodine deficiency on thyroid function, lower body weight was reported in rats fed a selenium-deficient diet compared with those fed the combined selenium- and iodine-deficient diet (24–26). Our results suggest that the deleterious effect of selenium deficiency on bone metabolism is mitigated in part by iodine deficiency. Even if experimental selenium deficiency was associated with an impaired bone metabolism, rats fed a selenium-deficient diet or combined selenium- and iodine-deficient diet did not show any sign of arthropathy. These results contrast with the situation in Tibet where, iodine deficiency was a risk factor for Kashin-Beck disease in selenium deficient areas (10). Therefore iodine deficiency in Tibet, even if associated with Kashin-Beck disease, may not be an etiological factor, but rather an indicator of subjects with poor nutrition and precarious living conditions.

What is the underlying mechanism sustaining the protective role of iodine deficiency in selenium deficient rats? The recent detection of type 2 iodothyronine deiodinase (D2) selenoenzyme activity in bone cells may explain the effect of iodine deficiency in selenium-deficient rats (27). D2 is involved in the intracellular conversion of the circulating pro-hormone T4 into T3, in brain, pituitary gland, and brown tissue where a well-regulated local T3 homeostasis was demonstrated extensively. D2 expression is affected by several factors, including selenium and iodine status. Iodine deficiency increases the activity of D2, whereas it is decreased by selenium deficiency (8). The stimulation of D2 activity under iodine-deficient conditions is

TABLE 3

Bone metabolism biomarkers in second generation selenium-and iodine-deficient rats[1]

	Se+/I+	Se+/I−	Se−/I+	Se−/I−	ANOVA *P*-values [2]		
					Se	I	Se × I
Plasma calcium, *mmol/L*	2.5 ± 0.2	2.4 ± 0.05	2.4 ± 0.1	2.6 ± 0.02	NS[3]	NS	NS
Urinary calcium, *mmol/d*	0.07 ± 0.01[a]	0.04 ± 0.004[a]	0.02 ± 0.002[b]	0.03 ± 0.01[ab]	<0.001	NS	0.001
Plasma phosphate, *mmol/L*	2.3 ± 0.2	2.3 ± 0.1	2.2 ± 0.2	2.5 ± 0.1	NS	NS	NS
Urinary phosphate, *mmol/d*	0.34 ± 0.05	0.36 ± 0.01	0.49 ± 0.05	0.51 ± 0.04	0.002	NS	NS
Plasma osteocalcin, *mmol/L*	10.9 ± 0.7	9.1 ± 0.5	7.9 ± 0.5	7.5 ± 0.5	<0.001	NS	NS
Urinary deoxypyridinoline, *nmol/mmol creatinine*	476 ± 27	436 ± 38	288 ± 62	216 ± 31	<0.001	NS	NS

1 Values are expressed as the means ± SEM, $n = 10$.
2 Data were analyzed by 2-way ANOVA. Means in a row without a common letter differ, $P < 0.05$.
3 NS, nonsignificant ($P \geq 0.05$).

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TABLE 4

Bone mineral content and density in 2nd generation selenium- and iodine-deficient rats[1]

	Se+/I+	Se+/I−	Se−/I+	Se−/I−	ANOVA *P*-values[2]		
					Se	I	Se × I
Whole femur							
BMC, *mg*	396 ± 5[a]	385 ± 6[ab]	308 ± 7[c]	367 ± 4[b]	<0.001	<0.001	<0.001
BMD, *mg/cm²*	229 ± 2[a]	228 ± 2[a]	199 ± 2[c]	216 ± 3[b]	<0.001	<0.001	<0.001
Distal femur							
BMC, *mg*	137 ± 1[a]	133 ± 2[ab]	109 ± 7[c]	128 ± 2[b]	<0.001	0.001	<0.001
BMD, *mg/cm²*	244 ± 2[a]	240 ± 3[a]	207 ± 3[c]	229 ± 3[b]	<0.001	0.002	<0.001
Whole tibia							
BMC, *mg*	295 ± 5[a]	292 ± 7[a]	247 ± 6[b]	281 ± 5[a]	<0.001	0.03	0.001
BMD, *mg/cm²*	207 ± 2[a]	206 ± 1[ab]	184 ± 2[c]	197 ± 3[b]	<0.001	0.005	0.003
Proximal tibia							
BMC, *mg*	101 ± 7	102 ± 1	90 ± 4	98 ± 9	0.001	0.021	NS[3]
BMD, *mg/cm²*	206 ± 3	209 ± 2	189 ± 2	201 ± 5	0.001	0.032	NS

1 Values are expressed as the means ± SEM, $n = 10$.
2 Data were analyzed by 2-way ANOVA. Means in a row without a common letter differ, $P < 0.05$.
3 NS, nonsignificant ($P \geq 0.05$).

a compensatory mechanism that maintains local T3 homeostasis to some extent because it was demonstrated in both the brain and the pituitary gland (28–30). Pituitary D2 activity is markedly increased in selenium- and iodine-deficient rats compared with rats deficient in selenium (28), and this may contribute to maintaining pituitary T3 concentrations. The stimulation of GH synthesis by T3 could explain the higher pituitary GH concentration in selenium- and iodine-deficient rats compared with selenium-deficient rats. Because thyroid hormones are an important regulator of bone metabolism, it is tempting to speculate that bone D2 activity, like pituitary D2 activity, is also stimulated by iodine deficiency. Such a compensatory mechanism would maintain bone T3 homeostasis and could also explain why both growth and bone metabolism are less impaired in selenium-deficient rats when iodine deficiency is also present. The potential importance of local bone regulation of T3 homeostasis is underlined by the fact that selenium supplementation improved growth in selenium-deficient rats, whereas T3 infusion did not (31), indicating that restoration of D2 activity may have a a greater effect on bone metabolism than increased circulating T3.

In summary, the effects of selenium deficiency on bone metabolism are likely to be mediated by selenoenzymes implicated in the antioxidant defense system of bone and intestinal cells, and in thyroid hormone metabolism. By decreasing the activity of D2, selenium deficiency may affect both the GH-IGF-I axis and the bone cell function arising from inadequate levels of T3. In combined iodine and selenium deficiency, iodine deficiency is likely to stimulate residual bone D2 activity and thus compensate, to some extent, for D2 dysfunction induced by selenium deficiency. Finally, the decrease in activity of antioxidant selenoenzymes may induce higher bone and intestinal cell ROS concentrations and impair bone metabolism and intestinal absorption of calcium.

In conclusion, iodine deficiency mitigated growth retardation and osteopenia in selenium-deficient growing rats. To avoid aggravating osteopenia, the present study suggests that adequate selenium status should be ensured before measures are taken to correct iodine deficiency.

TABLE 5

Bone histomorphometry in 2nd generation selenium- and iodine-deficient rats[1]

	Se+/I+	Se+/I−	Se−/I+	Se−/I−	ANOVA *P*-values[2]		
					Se	I	Se × I
Trabecular bone volume, *%*	12.7 ± 1.9[a]	6.6 ± 1.1[b]	5.8 ± 1.1[b]	6.9 ± 0.8[b]	0.017	NS[3]	0.01
Trabecular bone surface, *mm/mm²*	6.9 ± 0.8[a]	4.5 ± 0.5[ab]	3.6 ± 0.7[b]	5.5 ± 0.6[ab]	NS	NS	0.002
Trabecular thickness, *μm*	33.5 ± 1.3[a]	28.8 ± 1.2[b]	27.6 ± 0.8[b]	28.3 ± 1.2[b]	0.009	NS	0.025
Osteoblast index, *N Ob/mm trabecular bone length*	10.8 ± 1.6[a]	16.2 ± 4.6[ab]	28.3 ± 5.2[b]	12.8 ± 1.1[a]	NS	NS	0.008
Osteoclast index, *N Oc/mm trabecular bone length*	1.2 ± 0.3[a]	4.6 ± 0.6[b]	4.5 ± 0.7[b]	2.1 ± 0.4[a]	NS	NS	<0.001
Double-labeling surface, *%*	3.6 ± 0.8[a]	1.8 ± 0.7[ab]	0.6 ± 0.4[b]	1.8 ± 0.6[ab]	0.031	NS	0.03
Mineral apposition rate, *μm/d*	1.5 ± 0.3	1.1 ± 0.3	0.4 ± 0.2	1.7 ± 0.5	NS	NS	0.03

1 Values are expressed as the means ± SEM, $n = 10$.
2 Data were analyzed by 2-way ANOVA. Means in a row without a common letter differ, $P < 0.05$.
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