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Effects of selenium and iodine deficiency on bone, cartilage growth plate and chondrocyte differentiation in two generations of rats[1]

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Summary

Objective: The purpose of the current study was to investigate the roles of combined selenium and iodine deficiency in bone development as a possible experimental model of Kashin-Beck osteoarthropathy.

Methods: Sprague–Dawley rats ($n=48$) were randomly divided into selenium deficiency (−Se+I), iodine deficiency (+Se−I), combined selenium and iodine deficiency (−Se−I), and selenium and iodine sufficient (+Se+I) groups. Growth of bone and cartilage, and the expression of type X collagen (ColX) and parathyroid hormone-related peptide (PTHrP) were measured in two generations of rats (F_0 and F_1).

Results: The tibial length in −Se−I rats was significantly shorter in F_1 generation. In +Se−I of F_1 rats, the thickness of the growth plate cartilage, and the proliferative zone was smaller, while in −Se−I rats the growth plate, and the proliferative and hypertrophic zones were also thinner in F_1 generation. In articular cartilage, ColX expression was increased in the deep zone in −Se−I rats of F_0 generation, and in −Se+I, +Se−I and −Se−I rats of F_1 generation. PTHrP expression was increased in the middle zone of −Se+I, +Se−I and −Se−I rats of both F_0 and F_1 generations. In the growth plate cartilage, ColX and PTHrP were expressed in the hypertrophic zone. ColX expression was significantly weaker in −Se+I and −Se−I rats in both F_0 and F_1 generations, while PTHrP expression was stronger in −Se+I, +Se−I and −Se−I rats in both F_0 and F_1 animals.

Conclusions: Combined selenium and iodine deficiency impaired the growth of bone and cartilage. The changes in the expression of ColX and PTHrP induced by combined selenium and iodine deficiency were compatible to measurements of ColX and PTHrP in Kashin-Beck osteoarthropathy.

Key words: Selenium deficiency, Iodine deficiency, Bone, Cartilage.

Introduction

Kashin-Beck osteoarthropathy (Kashin-Beck Disease, KBD) affects maybe 2.5 million people of the 30 million people living in endemic selenium-deficient areas of China (PR)[1]. Despite the past 150 years of research, the etiology of KBD is still under debate. Three major environmental hypotheses have been proposed as follows: (1) selenium deficiency, (2) cereal contamination by mycotoxin-producing fungi, and (3) high humic acid levels in the drinking water. Geographically, the disease occurs mainly within a distinct wide belt with a low soil selenium content, running obliquely from the northeast to the southwest of China. The basic pathological feature of KBD is a focal necrosis of chondrocytes in the hypertrophic zone of growth plate cartilage and in the deep zone of articular cartilage, which can result in growth retardation, secondary osteoarthritis, and disability in advanced stages[2,3]. Because the growth plate cartilage is the growth center of bone, the developmental deformities are most likely a result of impaired chondrocyte differentiation and endochondral ossification.

In 1972, hepatocyte necrosis was observed in rats fed with a low selenium diet from KBD areas[4]. Since then, epidemiological investigations have indicated that most of the inhabitants in areas with KBD have a low selenium nutritive status due to low selenium contents in cereals, soil, and drinking water[5]. This results in low selenium contents in hair, blood and urine[5]. In children from 7 to 13 years of age, a significantly negative correlation between the pathological changes in metaphysis of phalanges on hand X-ray films and selenium contents in urine and hair, but not in serum, has been reported[5]. Using Se-supplementation by oral sodium selenized tablets, spraying selenium onto the wheat and selenium-rich salts, low selenium nutrition of children in KBD areas has been improved as in a normal Se nutritive status, the repaired rate of pathological changes in metaphysis of phalanges in KBD children on X-ray films increased,

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and the prevalence also reduced[6]. Meanwhile, some investigations showed that iodine deficiency coexisted with low selenium in KBD areas in Tibet and Gansu province, the western part of China since 1998[7,8]. Iodine deficiency with low selenium was suggested as risk factors in environment associated with KBD. In animal experiments, growth retardation[9,10] was observed in rats fed with low selenium diet, and the impaired bone development with iodine deficiency[11].

The histological and morphological changes seen in articular cartilage of KBD[12,13] include significant alterations in chondrocyte phenotype based on major changes in collagen distribution and growth factors. The collagen types synthesized by chondrocytes can be used as specific markers to define various differentiation states of this cell type[14]. Type X collagen (ColX) is a specific marker for hypertrophic chondrocytes[15] where focal necrosis of chondrocytes appeared mainly in KBD cartilage. Numerous growth factors have been shown to be involved in the control of cartilage growth and chondrocyte differentiation at various stages of chondrocyte development. One of them, parathyroid hormone-related peptide (PTHrP) is mainly expressed in prehypertrophic zone between proliferating and hypertrophic zones[16]. PTHrP stimulates proliferation and hypertrophy of chondrocyte in those zones and delays terminal differentiation of chondrocytes during endochondral bone development[17,18]. However, it is little known whether or not selenium and iodine deficiency had an effect on the expression of ColX and PTHrP in cartilage. Therefore, the effects of selenium and iodine deficiency on the growth of bone and cartilage, and the expression of ColX, PTHrP in both growth plate cartilage and articular cartilage were observed from two generations of rats, as an experimental model of Kashin-Beck osteoarthropathy.

Materials and methods

GROUPS AND DIETS

Weanling Sprague–Dawley (SD) rats ($n = 48$) were randomly divided into four groups and fed with one of the four kinds of diets: (1) selenium deficient diet (−Se+I, selenium: <0.02 μg/g, iodine: 0.4–0.5 μg/g); (2) iodine deficient diet (+Se−I, selenium: 0.1–0.3 μg/g, iodine: 0.04 μg/g); (3) combined selenium and iodine deficient diet (−Se−I, selenium: 0.01 μg/g, iodine: 0.04 μg/g), and (4) selenium and iodine adequate (control) diet (+Se+I, selenium: 0.1–0.3 μg/g, iodine: 0.4–0.5 μg/g)[19]. The parental rats were mated in each group 8 weeks after the beginning of the experiment (Fig. 1). The experiment was approved by Animal Ethics Committee, Xi'an Jiaotong University School of Medicine.

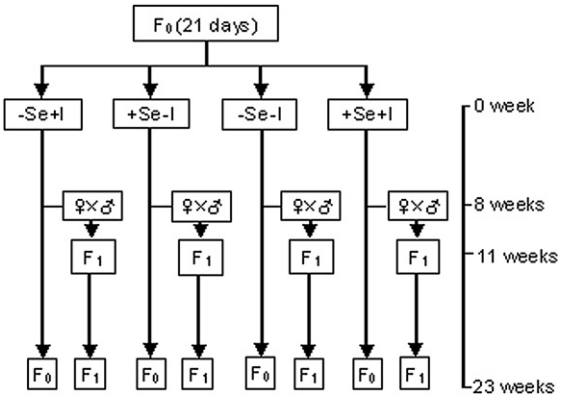

Fig. 1. Planning of the diet and of the experimental processes. Weanling SD rats were randomly divided into four groups: the selenium deficiency group, iodine deficiency group, combined selenium and iodine deficiency group, and selenium and iodine adequate (control) group. The parental rats were mated in each group 8 weeks after the beginning of the experiment. The experiment was ended 23 weeks after the beginning of the experiment when the F_0 animals were about 6 months and the F_1 rats were about 3 months old. Blood samples, right tibias and left knee joints were collected for measurements.

SAMPLE PREPARATION

Blood was collected from the tail vein when the F_0 animals were about 6 months and the F_1 rats were about 3 months old, and right tibias and left knee joints were collected under the general anesthesia. The right tibias were stored at −20°C before measurements. The left knee joints were immediately fixed in 4% (w/v) polyformaldehyde for 2–3 days and decalcified in 10% (w/v) ethylene-diamine tetraacetic acid (EDTA) for 4 weeks. And then the left knee joints were transferred into phosphate buffer (pH 7.4), embedded in paraffin and cut into 6–8 μm thick sections for immunohistochemistry and for hematoxylin and eosin (HE) staining.

SERUM SELENIUM LEVELS AND T_3, T_4, CONCENTRATIONS

Serum selenium level was measured by fluorescent atomic absorption spectrometry[20]. Serum T_3 and T_4 concentrations were determined by radioimmunoassay (FM-2000, China).

GROWTH OF BONE AND CARTILAGE

Right tibial length, mid-shaft tibial diameter, and articular cartilage diameters were measured by vernier caliper. Knee joint sections of rats were deparaffinized, rehydrated, and stained with HE. Thickness of the growth plate cartilage (10×), layers of proliferative and hypertrophic chondrocytes (40×) in growth plate cartilage of tibia were measured under microscope. Ten sites in one section were measured for each sample, and average values were calculated for analysis.

ANTIBODIES AND REAGENTS

Mouse monoclonal antibodies against human recombinant ColX (X53) were kindly donated by professor Klaus von der Mark in the Institute of Experimental Medicine I, University of Erlangen-Nürnburg (Germany), and the specificity of the antibody was tested by enzyme-linked immunosorbent assay (ELISA), Western blotting, and by immunostaining on test tissues[21]. The polyclonal immunohistochemistry kits for PTHrP (1–34) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and their specificity was tested by ELISA, Western blotting, and immunostaining on test tissues[22,23].

IMMUNOHISTOCHEMISTRY

For ColX immunostaining, deparaffinized cartilage sections were incubated with testicular hyaluronidase (2 mg/ml) in phosphate-buffered solution (PBS), pH 5, for 30 min at room temperature, followed by protease type XXIV (Sigma, 0.02 mg/ml) digestion in PBS, pH 7.3, for 30 min at room

temperature[24]. Primary monoclonal antibodies to ColX were incubated overnight at 4°C and visualized using alkaline phosphatase-labeled secondary antibodies. Color development was continued for 30 min at room temperature using 3-hydroxy-2-naphthoic acid 2,4-dimethylanilide (Naphthol AS-MX) as a substrate. Finally, nuclei were counterstained with hematoxylin.

For PTHrP immunostaining, deparaffinized cartilage sections were briefly pretreated in a microwave oven to expose the antigens, and incubated in 3% H_2O_2 for 10 min according to the protocol recommended by the manufacturer of the immunohistochemical staining kits for PTHrP (Santa Cruz Biotechnology). Sections were incubated overnight at 4°C with primary antibodies and visualized using alkaline phosphatase-labeled secondary antibodies. Color development was continued for 30 min at room temperature using Naphthol AS-MX as a substrate. Finally, nuclei were counterstained with hematoxylin.

Cartilage sections were examined under the light microscope, and counted for pericellular and cytoplasmic staining for ColX and PTHrP. Four to six randomly selected fields in each zone were counted at 40× magnification.

CLASSIFICATION OF CARTILAGE ZONES

Similar to the fetal growth plate, chondrocytes of articular cartilage were divided into five zones to define three cell morphologies by light microscopy criteria, namely, (1) the superficial zone, (2) the upper zone (corresponding to the reserve or resting zone of the fetal growth plate), (3) the middle zone (corresponding to the proliferating zone of the fetal growth plate), (4) the deep zone (corresponding to the hypertrophic zone of the fetal growth plate), and (5) the calcified cartilage zone below the tidemark. Chondrocytes in superficial zone are relatively small and flat, and oriented with the long axis parallel to the surface. Chondrocytes of the upper and middle zones are larger and show the typical rounded cellular profile of hyaline cartilage. They are randomly distributed in a matrix with fibers running in oblique directions; cells in the deep zone were of increasing size and arranged in columnar manner perpendicular to the surface, similar to the proliferating zone of the fetal growth plate[25].

STATISTICAL ANALYSIS

Data are expressed as means ± SE. Differences among the group means were examined by Analysis of Variance (ANOVA). Values of $P < 0.05$ were considered to indicate statistically significant differences.

Results

SERUM SELENIUM LEVELS AND T_3 AND T_4 CONCENTRATIONS

Serum selenium analysis was performed to make sure that restricted availability of selenium in the diet was reflected in the body level. It could be confirmed that dietary selenium deficiency effectively limited its intake, since the serum selenium level was significantly lower in the −Se+I and −Se−I groups both in F_0 and F_1 (Table I).

In F_0 rats, T_3 concentrations were significantly lower in −Se+I and −Se−I groups, but there was only a slightly decreased trend in +Se−I group. T_4 concentrations were significantly lower in +Se−I and −Se−I groups (Table I).

In F_1 rats, T_3 concentrations were no different among the four groups, whereas T_4 concentrations were significantly lower in −Se−I groups (Table I).

TIBIAL GROWTH

There were no significant differences between the groups in F_0 rats with respect to tibial length, half frontal plane diameter of tibia, and frontal articular cartilage diameters. However, in F_1 rats the tibial length was significantly decreased in −Se−I group, and coronal articular cartilage diameters were increased in +Se−I rats (Table II).

CARTILAGE GROWTH

In F_0 rats, no significant differences between the groups were found in the thickness of the growth plate cartilage, or in the number of layers of the proliferative and hypertrophic chondrocytes. In F_1 rats, the thickness of the growth plate cartilage and the numbers of the proliferative chondrocytes in +Se−I and −Se−I groups, and layers of hypertrophic chondrocyte in −Se−I group were significantly decreased. But the degree of difference between the reference group +Se+I and the other groups is quite small, except for the number of layers of proliferative chondrocytes in the −Se−I group (Table II).

ColX LOCALIZATION IN THE GROWTH PLATE AND THE ARTICULAR CARTILAGE

In negative control articular cartilage, ColX is not visualized [Fig. 2(I)]. In all four groups of F_0 [Fig. 2(A−D)] rats submitted to diet, pericellular staining for ColX was deposited in middle and deep zones in rat articular cartilage. In F_1 rats, the staining was observed only in the deep zone except for −Se−I group, which had staining both in the middle and the deep zones [Fig. 2(E−H)]. In F_0 rats, the percentage of ColX staining was not different in the middle zone, but was significantly increased in the deep zone of −Se−I group. In F_1 rats, the percentage of ColX staining in deep zone of −Se+I, +Se−I and −Se−I groups was significantly increased (Table III).

In negative control growth plate cartilage, ColX is not visualized [Fig. 2(R)]. In all four groups of both F_0 [Fig. 2(J−M)] and F_1 [Fig. 2(N−Q)] rats submitted to diet, pericellular staining of ColX appeared in the hypertrophic zone, and the percentage of ColX staining in −Se+I and −Se−I groups was significantly lower in both F_0 and F_1 rats (Table III).

Table I
Contents of selenium, T_3 and T_4 in serum in F_0, F_1 rats. Data are expressed as mean ± SE

Groups	*n*	Selenium (μg/l)	T_3 (nmol/l)	T_4 (nmol/l)
F_0				
−Se+I	12	30.28 ± 32.1†	0.55 ± 0.09*	28.12 ± 3.33
+Se−I	12	345.83 ± 29.55	0.62 ± 0.06	24.11 ± 2.29*
−Se−I	12	30.33 ± 41.18†	0.55 ± 0.05*	20.66 ± 1.93†
+Se+I	12	358.64 ± 30.50	0.75 ± 0.08	36.15 ± 2.74
F_1				
−Se+I	12	43.95 ± 9.75†	1.04 ± 0.16	51.26 ± 5.06
+Se−I	12	245.24 ± 9.95	1.01 ± 0.18	41.05 ± 6.38
−Se−I	10	35.40 ± 3.16†	1.05 ± 0.07	27.95 ± 2.82*
+Se+I	12	236.50 ± 9.75	1.05 ± 0.14	48.30 ± 4.49

*$P < 0.05$, †$P < 0.01$ as compared with the control group (+Se+I) by ANOVA.

Table II
Comparisons of bone and cartilage growth in F_0 and F_1 rats among four groups. Data are expressed as mean ± SE

	−Se+I	+Se−I	−Se−I	+Se+I
F_0				
n	12	12	12	12
Tibial length (mm)	41.78 ± 0.73	41.45 ± 0.95	42.22 ± 1.3	40.40 ± 0.83
Tibial frontal diameter (mm)	3.41 ± 0.10	3.25 ± 0.14	3.56 ± 0.09	3.26 ± 0.12
Coronal diameter of articular cartilage (mm)	6.18 ± 0.11	6.07 ± 0.22	6.10 ± 0.17	5.72 ± 0.27
Frontal diameter of articular cartilage (mm)	7.05 ± 0.12	6.99 ± 0.15	7.14 ± 0.15	7.07 ± 0.15
Thickness of the growth plate cartilage (mm)	1.49 ± 0.05	1.70 ± 0.08	1.67 ± 0.06	1.56 ± 0.11
Layers of proliferative chondrocytes	7.83 ± 0.36	7.74 ± 0.38	7.77 ± 0.71	6.85 ± 0.42
Layers of hypertrophic chondrocytes	4.83 ± 0.15	4.81 ± 0.34	5.00 ± 0.29	4.26 ± 0.31
F_1				
n	12	12	10	12
Tibial length (mm)	33.17 ± 0.34	34.02 ± 0.25	32.30 ± 0.87†	34.12 ± 0.32
Tibial frontal diameter (mm)	2.45 ± 0.04	2.48 ± 0.05	2.49 ± 0.13	2.48 ± 0.04
Coronal diameter of articular cartilage (mm)	4.97 ± 0.09	5.45 ± 0.16*	5.36 ± 0.11	5.13 ± 0.07
Frontal diameter of articular cartilage (mm)	6.41 ± 0.09	6.53 ± 0.11	6.60 ± 0.10	6.56 ± 0.09
Thickness of the growth plate cartilage (mm)	3.03 ± 0.10	2.90 ± 0.09*	1.60 ± 0.18†	3.19 ± 0.09
Layers of proliferative chondrocytes	15.59 ± 0.37	13.75 ± 0.33*	8.54 ± 0.81†	14.94 ± 0.36
Layers of hypertrophic chondrocytes	6.60 ± 0.31	6.84 ± 0.20	4.95 ± 0.37†	6.64 ± 0.26

*$P < 0.05$, †$P < 0.01$ as compared with the control group (+Se+I) by ANOVA.

PTHrP LOCALIZATION IN THE GROWTH PLATE AND THE ARTICULAR CARTILAGE

In negative control articular cartilage, PTHrP is not visualized [Fig. 3(I)]. In all four groups of both F_0 [Fig. 3(A−D)] and F_1 [Fig. 3(E−H)] rats submitted to diet, PTHrP staining distributed to all of the zones. The percentage of PTHrP staining in the chondrocytes of the middle zone was increased in all of the experimental groups in both F_0 and F_1 (Table III).

In negative control articular growth plate cartilage, PTHrP is not visualized [Fig. 3(R)]. In all four groups of both F_0 [Fig. 3(J−M)] and F_1 [Fig. 3(N−Q)] rats submitted to diet, PTHrP staining was seen only in the hypertrophic zones, and the percentage of PTHrP staining

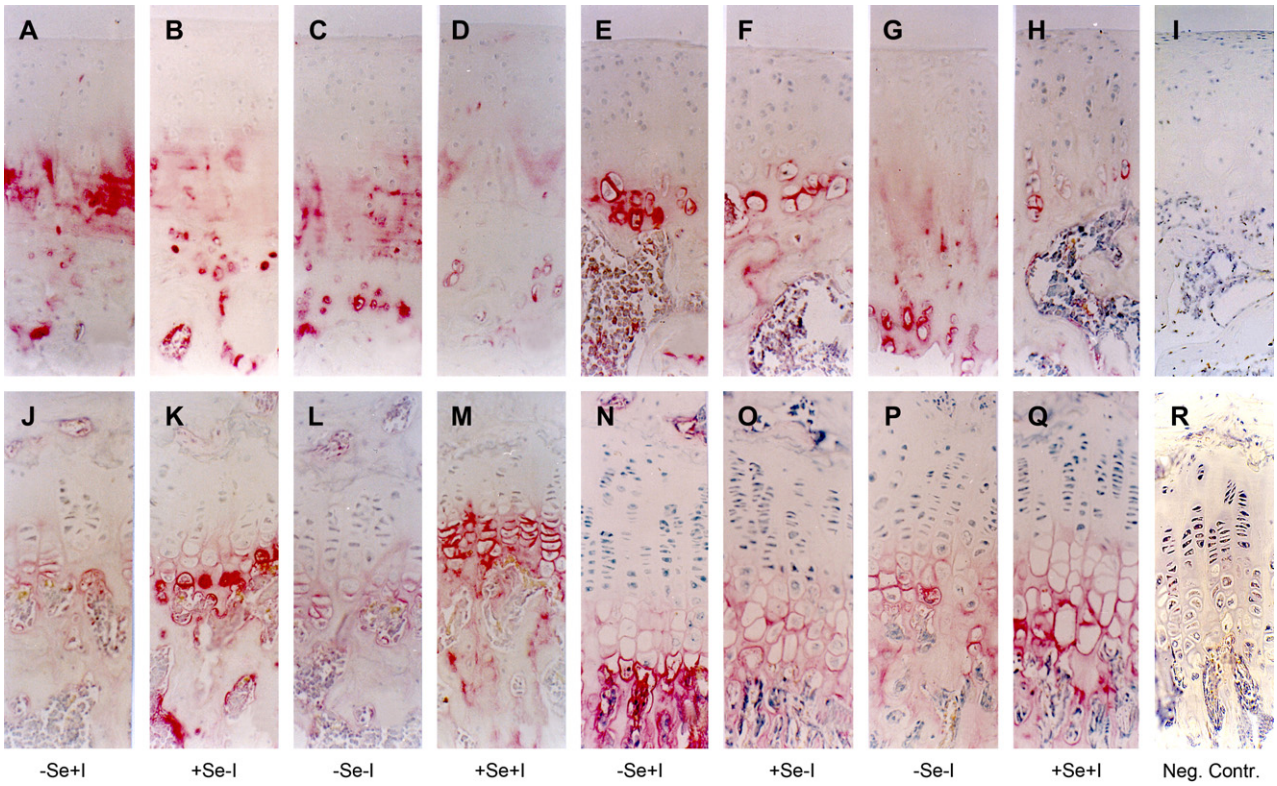

Fig. 2. Immunohistochemical staining of ColX in the articular cartilage and the growth plate cartilage. Pericellular staining of ColX in middle and deep zones of articular cartilage in F_0 rats of −Se+I (2A), +Se−I (2B), −Se−I (2C), +Se+I (2D) and in F_1 rats of −Se−I (2G), in deep zone of articular cartilage in F_1 rats of −Se+I (2E), +Se−I (2F) and +Se+I (2H) groups. Pericellular staining of ColX in the hypertrophic zone of growth plate cartilage in F_0 and F_1 rats of −Se+I (2J,2N), +Se−I (2K,2O), −Se−I (2L,2P) and +Se+I (2M,2Q) groups. 2I and 2R show negative controls (20×).

Table III
The percentage of ColX and PTHrP staining in the chondrocytes of the articular cartilage and the growth plate cartilage in F_0 and F_1 rats (%). Data are expressed as mean ± SE

Groups		Articular cartilage			Growth plate cartilage
	n	Upper zone	Middle zone	Deep zone	Hypertrophic zone
F_0					
Col X					
−Se+I	12	–	51.36 ± 2.66	57.15 ± 2.81	71.36 ± 1.83†
+Se−I	12	–	51.40 ± 1.27	68.38 ± 1.65	76.14 ± 0.63
−Se−I	12	–	57.53 ± 1.62	71.34 ± 2.85*	67.55 ± 1.16†
+Se+I	12	–	49.12 ± 2.56	59.34 ± 2.60	78.63 ± 1.27
PTHrP					
−Se+I	12	82.85 ± 1.93	62.41 ± 1.55*	63.93 ± 1.77	75.95 ± 2.52†
+Se−I	12	82.73 ± 1.45	64.83 ± 2.02†	60.86 ± 0.87	70.57 ± 2.96†
−Se−I	12	84.02 ± 1.90	62.21 ± 1.96*	60.09 ± 2.52	77.79 ± 2.55†
+Se+I	12	79.00 ± 1.20	54.21 ± 1.96	59.87 ± 1.37	55.01 ± 1.61
F_1					
Col X					
−Se+I	12	–	0.00 ± 0.00	47.76 ± 2.64*	76.41 ± 1.16†
+Se−I	12	–	0.00 ± 0.00	47.74 ± 2.23*	80.56 ± 1.24
−Se−I	10	–	26.07 ± 1.47†	52.25 ± 1.71†	64.99 ± 1.37†
+Se+I	12	–	0.00 ± 0.00	39.57 ± 1.63	81.57 ± 1.15
PTHrP					
−Se+I	12	78.05 ± 0.98	78.63 ± 0.86†	80.23 ± 1.04	75.98 ± 1.13*
+Se−I	12	75.91 ± 0.85	78.65 ± 1.08†	78.50 ± 1.24	77.18 ± .87†
−Se−I	10	78.80 ± 2.31	78.93 ± 1.54†	56.95 ± 1.29†	76.52 ± 1.00*
+Se+I	12	77.53 ± 0.86	73.29 ± 0.80	79.19 ± 1.06	69.9 ± 2.73

*$P < 0.05$, †$P < 0.01$ as compared with the control group (+Se+I) by ANOVA.

was increased in all of the experimental groups in both F_0 and F_1 (Table III).

Discussion

EFFECTS OF SELENIUM OR/AND IODINE DEFICIENCY ON THE GROWTH OF BONE AND CARTILAGE

The results showed that combined selenium and iodine deficiency only caused a moderate hypothyroidism in rats. It decreased T_3 and T_4 concentration in F_0 and T_3 concentration in F_1 moderately.

This study shows that selenium deficiency had no obvious adverse effect on bone and cartilage growth in F_0 and F_1 rats. Decreased number of layers containing proliferating chondrocyte and thinner growth plate cartilage was observed in +Se−I group of F_1 rats, but the degree was quite limited. These results indicated that iodine deficiency starting from the embryo period may retard the chondrocyte differentiation in the growth plate cartilage. Iodine is an essential element of T_4 which plays an important role in regulating bone growth and chondrocyte differentiation. Contrary to the bone growth retardation, chondrocyte layer irregularity and thinner proliferation layer of the growth plate cartilage observed in rats treated with low iodine diet obtained from low soil iodine areas of China[26,27], the present experiment showed that retardation of chondrocyte differentiation occurred in the first filial generation rats, but not in the parental one. The explanation for differences in these results may lie in the fact that there was only iodine deficiency in the diets of the present experiment, while the food directly from iodine deficiency areas may contain some other components that are risk factors for cartilage and bone growth in the diseased areas. Therefore, one generation suffering from iodine deficiency from embryonal development period until maturity showed impaired chondrocyte differentiation in the proliferative zone, resulting in the reduced thickness of the growth plate cartilage.

The growth retardation of bone and cartilage in −Se−I rats was remarkably more serious than that in −Se+I and +Se−I groups in F_1 rats. Selenium plays an important role in the biological pathways by forming selenoproteins. Glutathione peroxidase (GSH-Px), one of the best characterized selenoproteins, acts as a catalyst in the breakdown of a wide range of organic and inorganic peroxides and, thus, protects cells against oxidative stress. Iodine is an essential substrate for T_4. On the other hand, selenium is required for the expression of the selenoenzymes type I and type II iodothyronine deiodinase, which are crucial in the generation of the active hormone T_3. Selenium may also play an indirect role in the control of T_4 synthesis because it is required by GSH-Px. In the thyroid, GSH-Px is thought to be the main antioxidant system for neutralizing cytotoxic H_2O_2 and its oxidative by-products[28]. Hydrogen peroxide is produced by thyroid as a co-factor in T_4 synthesis. Although some studies showed long term nutritional selenium deficiency had only marginal effects on the thyroid T_4 and T_3 content and on the activity of the selenoenzyme type I deiodinase (5'D-I) in the thyroid gland[29], combined selenium and iodine deficiency may have an adverse effect on the thyroid gland function. It has been suggested that selenium deficiency is a co-factor to iodine deficiency in the pathogenesis of myxedematous cretinism[30], and can further associate with the adverse effects of iodine deficiency[31].

EFFECTS OF SELENIUM OR/AND IODINE DEFICIENCY ON ColX

Two representative alterations in ColX expression were observed in cartilage. Firstly, an increased ColX expression was present in the articular cartilage in the groups with single selenium or iodine deficiency and combined deficiency

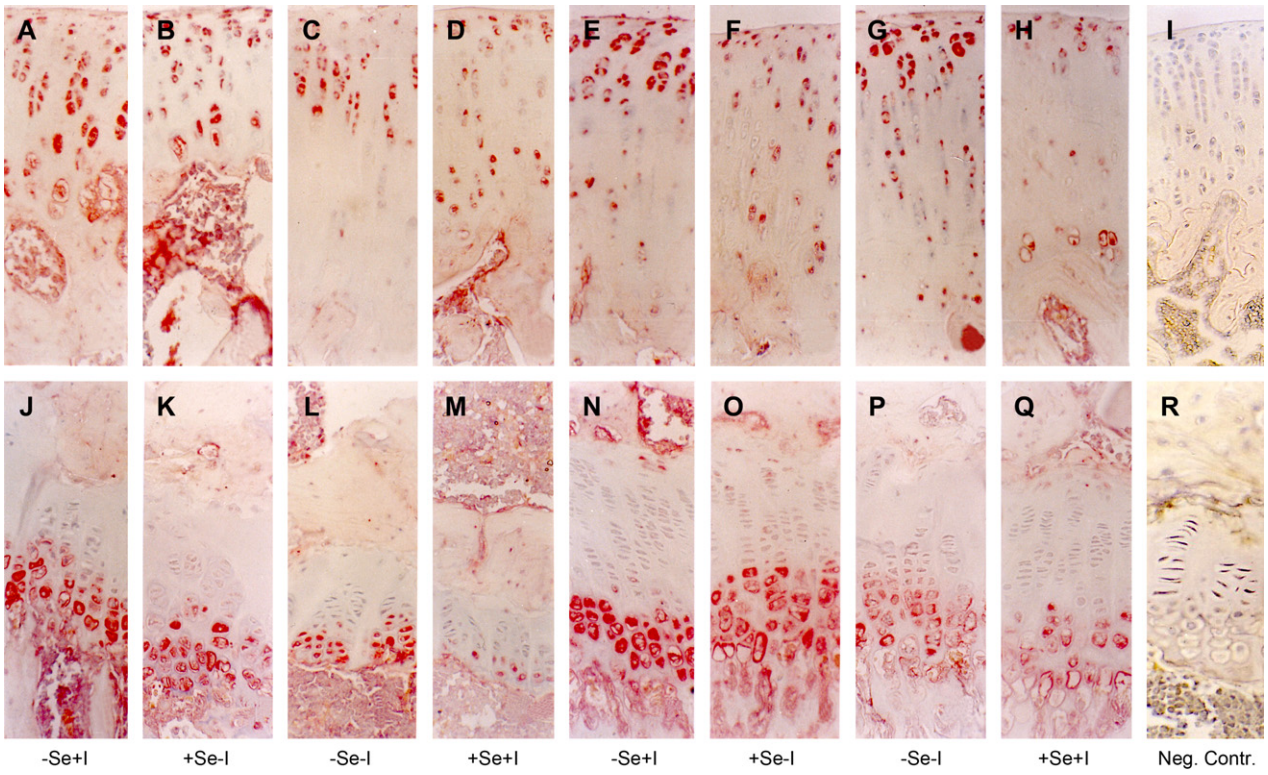

Fig. 3. Immunohistochemical staining of PTHrP in the articular cartilage and the growth plate cartilage. Intracellular staining of PTHrP in middle and deep zones of articular cartilage in F_0 rats of −Se+I (3A), +Se−I (3B), −Se−I (3C), +Se+I (3D) and in F_1 rats of −Se+I (3E), +Se−I (3F), −Se−I (3G), and +Se+I (3H) groups. Intracellular staining of PTHrP in the hypertrophic zone of growth plate cartilage in F_0 and F_1 rats of −Se+I (3J,3N), +Se−I (3K,3O), −Se−I (3L,3P) and +Se+I (3M,3Q) groups. 3I and 3R show negative controls (20×).

(F_1 rats), and in combined selenium and iodine deficiency group (F_0 rats). In the articular cartilage, ColX expression extended from deep zone to middle zone, indicating that chondrocytes became hypertrophic similar to osteoarthritic cartilage[21]. Secondly, a weaker ColX expression was observed in the hypertrophic zone of growth plate cartilage in groups with selenium or iodine deficiency alone, or in combination, as compared to the control. These findings indicate that selenium or iodine deficiency and combined deficiency mainly disturbed chondrocyte differentiation from proliferative to hypertrophic zones in growth plate cartilage, a similar finding as in KBD cartilage. In our previous study, ColX expression was decreased in the hypertrophic zone of the growth plate cartilage in KBD children, and extended from deep zone to middle zone of articular cartilage in KBD adults[32]. Selenium deficiency has an inhibitory role in the synthesis and expression of ColX in hypertrophic chondrocytes in growth plate cartilage of mini-pigs, while supplementation of the low selenium diet with additional selenium restored the signals of ColX to normal levels[33].

EFFECTS OF SELENIUM OR/AND IODINE DEFICIENCY ON PTHrP EXPRESSION

PTHrP was expressed in enhanced amounts in the middle zones of articular cartilage, and in the hypertrophic zones of the growth plate in selenium or iodine deficiency and combined deficiency groups in both F_0 and F_1 rats. The changes of PTHrP expression were consistent with KBD cartilage[32]. PTHrP has important functions in the regulation of chondrocyte proliferation and differentiation by binding to its PTH/PTHrP receptor. These PTH/PTHrP receptors are expressed at low level by proliferating chondrocytes, and at high level by prehypertrophic/early hypertrophic chondrocytes[34]. PTHrP acts primarily to keep proliferating chondrocytes in the proliferative pool. Overexpression of PTHrP in chondrocytes delays the appearance of hypertrophic chondrocytes[35]. PTHrP/PTH can revert hypertrophic chondrocytes to a prehypertrophic proliferating stage[36].

Combined selenium and iodine deficiency has a significant effect on the tissue markers of bone and cartilage remodeling (ColX and PTHrP), compatible with osteoarthrochondritis. However, the experimental protocol did not induce focal chondronecrosis in mature or deep zone as typically observed in Kashin-Beck osteoarthropathy.

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