NOTE

Biochemical Analysis of the Bone of SHC Rats with Spontaneous Hypercholesterolemia and Renal Dysfunction

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Abstract. Spontaneously hypercholesterolemic (SHC) rats exhibit severe abnormalities in renal function and bone metabolism at old ages, in addition to hypercholesterolemia. SHC rats were also found to show endocrine abnormalities such as hyperthyroidism from young ages. In this study, biochemical and microdensitometric analyses were carried out using femurs to characterize further the abnormality in bone metabolism: whether biochemical markers of the bone may be affected by these abnormalities. At 6 weeks of age, the ashed weight and calcium content of the dried femurs were slightly lower in SHC rats than in age-matched Sprague-Dawley (SD) rats. None of the markers of microdensitometric analysis was changed. At 24 weeks of age, the ashed weight of dried femurs and the density of the marrow region of femurs were lower in the SHC rats. The results indicate that SHC rats exhibit severe abnormality in bone metabolism leading to biochemical changes in the bone at old ages whereas changes in bone markers were minimal at young ages before the onset of severe renal dysfunction.

Key words: Spontaneously hypercholesterolemic (SHC) rats, Bone, Renal dysfunction.

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SHC RATS, established from SD rats, spontaneously develop focal glomerular sclerosis besides hypercholesterolemia [1, 2, 3]. In 26-week-old SHC rats, marked hypertrophy of the parathyroid gland and severe calcification in soft tissues were noted [3]. The double fluorescence labeling method and contact microradiography demonstrated impaired mineralization of osteoid tissue in the tibial trabecular bone of old SHC rats [4]. The results seemed to indicate that the trabecular bone lesion was characterized by osteitis fibrosa accompanied by osteomalacia [4]. Mild increase in bone resorption in association with endocrine abnormalities such as significant increases in GH, TSH and thyroid hormones was

also noted in the young SHC rats [5]. The mild increase in bone resorption in the young SHC rats was suggested to be due to the endocrine abnormalities. Thus, SHC rats may serve as a good model for understanding a mutual, causal relationship between these abnormalities giving useful information about knowledge of diseases related with these abnormalities.

In this study, we determined the mineral contents and bone density of femurs, as the endpoints of abnormality in bone metabolism, to confirm and extend previous observations for further clarification of the bone metabolism of SHC rats.

Materials and Methods

Animals

Male SHC rats bred in Takeda Chemical Ind.

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Ltd., and age-matched SD rats purchased from Charles River, Japan were maintained under the same conditions for at least 2 weeks before the experiments were started. Rats were fed a laboratory chow (CE-2, CLEA Japan) *ad libitum*. The chow contains 1.25 g of calcium, 1.06 g of phosphate, and 200 IU of vitamin D₃ per 100 g.

Examination of femurs

Right femurs stripped of muscle and soft tissues were weighed (wet weight) and kept at -20°C until analyzed by soft X ray. After the radiological analysis, femurs were washed with 10 ml of water, and delipidated by keeping femurs in 9 ml of $\text{CHCl}_3\text{-CH}_3\text{OH}$ (2:1) for 2 days. The delipidation was repeated once more as described above except that femurs were kept in the organic solvent for 3 days. Then, they were dried at 70°C for 3 days and weighed (dry weight). The mineral content was determined by weighing ashed femurs after heating the dry bones at 600°C for 24 h. The calcium [6] and phosphate [7] contents were determined by dissolving ashed femurs in 7 (for 6-week-old rats) or 10 ml (for 24-week-old rats) of 2N HCl and taking aliquots of the solution.

Femurs were roentgenographed using a soft X-ray machine (Type CSM, Softex Co., Ltd., Japan) and a soft X-ray film (FG, Fuji Film Co., Ltd., Japan). The bone density of femurs in the roentgenograph was measured at points 0.18/1 (distal metaphysis) and 0.43/1 (diaphysis) from the

edge of the distal and using the Interactive Image Analyzer System (IBAS II; Carl Zeiss, West Germany) as reported previously [8]. The outer diameter (D) and bone density ($\sum \text{GS/D}$) in the distal metaphysis; outer diameter (D), inner diameter (d), cortical width (D-d), cortical thickness index (CTI)=(D-d)/D, mean density of both cortical regions ($\Delta \text{GS}_{\text{max}}$)=($\Delta \text{GS}_1 + \Delta \text{GS}_2$)/2, density of marrow region ($\Delta \text{GS}_{\text{min}}$), and bone density ($\sum \text{GS/D}$, mean density integrated area/outer diameter) in the diaphysis were measured following the microdensitometry of Inoue *et al.* [8].

Statistical Analysis

Differences between SHC and SD rats were analyzed by *t*-test.

Results and Discussion

At 6 weeks of age, the SHC and the SD rats did not differ with respect to the wet and dry weights, and the total mineral, calcium, and phosphate contents of femurs (Table 1). However, the mineral and calcium contents of femurs based on the dry weight of the bone were slightly lower with statistical significance in SHC rats.

At 24 weeks of age, the SHC and the SD rats did not differ with respect to the wet and dry weights, and the total mineral, calcium, and phosphate contents of femurs. Based on the wet weight of

Table 1. Mineral contents of femurs of SD and SHC rats at ages of 6 and 24 weeks

Rats	Age	Femur weight (mg)			Ca (mg/femur)	P (mg/femur)	ashed wt. /wet wt. (%)	Ca (mg/g wet wt.)	P (mg/g wet wt.)
		wet wt.	dry wt.	ashed wt. (mg/femur)					
SD	6 week	612±13	295±6	171±4	60.5±1.3	30.5±0.6	27.9±0.3	98.9±1.5	49.9±0.6
SHC	6 week	603±18	289±10	164±6	57.8±2.0	29.7±1.0	27.2±0.4	95.7±1.1	49.2±0.5
SD	24 week	1223±36	866±29	588±21	213.0±6.0	101.1±3.2	48.0±0.6	174.4±2.5	82.7±1.3
SHC	24 week	1327±33	880±31	583±21	207.3±8.0	102.7±3.3	43.9±0.5**	156.0±2.4**	77.4±0.7**

Rats	Age	ashed wt./dry wt. (%)		Ca (mg/g dry wt.)	P (mg/g dry wt.)	Ca (mg/g ashed wt.)	P (mg/g ashed wt.)
SD	6 week	58.0±0.2		205.5±1.3	103.6±0.5	354±2	179±1
SHC	6 week	56.8±0.3*		199.6±0.4**	102.5±0.3	352±2	181±1
SD	24 week	67.9±0.3		246.4±2.4	116.9±1.1	363±3	172±1
SHC	24 week	66.3±0.1**		235.6±1.9**	116.8±0.6	355±3	176±1*

Mean±SEM (n=6), *, $P<0.05$; **, $P<0.001$ (*t*-test, compared with SD rats).

Fig. 1. Roentgenographs of femurs of SD and SHC rats at ages 6 and 24 weeks ($\times 6$ for 6 weeks and $\times 2$ for 24 weeks). At the age of 24 weeks, the bone density in the medullary canal is slightly lower in SHC than in SD rats.

Table 2. Microdensitometric analysis of femurs of SD and SHC rats at ages of 6 and 24 weeks

Rats	Age	Femoral length (mm)	Metaphysis		Diaphysis					
			D ^a (mm)	Σ GS/D ^b	D (mm)	d ^c (mm)	Δ GS _{min} ^d	Δ GS _{max} ^e	CTI ^f	Σ GS/D
SD	6 week	28.8 $\pm$ 0.2	5.74 $\pm$ 0.15	0.58 $\pm$ 0.04	3.79 $\pm$ 0.10	3.16 $\pm$ 0.09	0.28 $\pm$ 0.02	1.56 $\pm$ 0.06	0.167 $\pm$ 0.006	0.64 $\pm$ 0.01
SHC	6 week	28.1 $\pm$ 0.2	5.48 $\pm$ 0.12	0.50 $\pm$ 0.03	3.76 $\pm$ 0.05	3.17 $\pm$ 0.06	0.26 $\pm$ 0.03	1.42 $\pm$ 0.01	0.157 $\pm$ 0.006	0.66 $\pm$ 0.04
SD	24 week	40.8 $\pm$ 0.2	5.88 $\pm$ 0.10	0.81 $\pm$ 0.07	4.42 $\pm$ 0.11	2.97 $\pm$ 0.11	0.93 $\pm$ 0.05	2.02 $\pm$ 0.07	0.328 $\pm$ 0.010	1.14 $\pm$ 0.14
SHC	24 week	40.6 $\pm$ 0.3	5.88 $\pm$ 0.07	0.73 $\pm$ 0.04	4.63 $\pm$ 0.08	3.21 $\pm$ 0.05	0.72 $\pm$ 0.07*	2.07 $\pm$ 0.07	0.305 $\pm$ 0.014	1.09 $\pm$ 0.05

Mean $\pm$ SEM (n=6). *, $P < 0.05$ (*t*-test, compared with SD rats).

a, outer diameter; b, bone density; c, inner diameter; d, density of marrow region; e, mean density of both cortical regions; f, cortical thickness index ((D-d)/D).

femurs, however, the mineral, calcium, and phosphate contents of femurs of the SHC rats were significantly lower. The mineral and calcium contents of femurs on a dry weight-basis were also significantly lower in SHC rats. The phosphate content in ashed bones was also significantly lower in SHC rats at this age.

Microdensitometric analysis of the soft X-ray patterns of femurs showed that none of the morphometric markers of the SHC rats was

significantly different from those of the SD rats at 6 weeks of age (Table 2, Fig. 1).

At 24 weeks of age, the density of the marrow region (Δ GS_{min}) of femurs significantly decreased in the SHC rats. Some of the other morphometric variables, such as Σ GS/D (bone density) and CTI (cortical thickness index), tended to be slightly lower, and d (inner diameter) tended to be slightly higher, in the SHC at 24 weeks of age. The results are compatible with the previous findings, by

fluorescent light microscopy and contact micro-radiography that mineralization defects and increased bone turnover were present in old SHC rats, and confirm and extend these findings [4]. Biochemical markers such as calcium and phosphate contents of the bone may be used as much better markers for bone abnormality than those by histomorphological analyses from the quantitative point of view and from simplicity of assays.

These results indicate that severe abnormality in bone metabolism enough to induce changes in biochemical markers of the bone was observed after onset of severe renal dysfunction which became marked at ages over 12 weeks [3, 4]. Abnormalities in endocrine function such as elevation in serum levels of GH, TSH, T_4 and T_3 were noted in the SHC rats at the age of 6 weeks [5]. These endocrine abnormalities alone may not be sufficient to induce changes in biochemical markers of the bone. Another possibility is that these abnormalities may compensate each other, balancing bone formation and resorption at young ages, since thyroid hormones and growth hormone act in the opposite directions; the former stimulate bone resorption and turnover [9–12] and the latter enhances 1, 25-hydroxyvitamin D_3 formation and vitamin D-dependent calcium binding protein [13–15]. Examination of age-associated changes in vitamin D metabolism and PTH secretion in detail would help to characterize the abnormality in bone metabolism of SHC rats. At present, it may be worth mentioning about marked hypertrophy of the parathyroid gland [3] and increased serum

level of immunoreactive PTH (human PTH kit for C terminal peptide, EIKEN, Japan; data not shown) in old SHC rats. Thus, hyperthyroidism may be involved in decrease in mineral content of the bone in old SHC rats. Reduction in vitamin D activation due to severe renal dysfunction is also likely related with decrease in ashed weight of the bone.

The abnormality in bone markers at 24 weeks of age is compatible with the quantitative histomorphometric analyses of the tibia of 27-week-old male SHC rats demonstrating the existence of bone changes characterized by osteitis fibrosa accompanied by osteomalacia, changes frequently observed in patients with severe renal dysfunction [16]. Although the exact mechanism of the abnormality in bone metabolism in old SHC rats still remains to be clarified, SHC rats seem to be unique and useful rodents to spontaneously develop abnormalities in endocrine, lipid and bone metabolism as well as renal dysfunction. Therefore, SHC rats may serve as good models for studies on causal relationships between these abnormalities.

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