# Calcium Metabolism, Serum Thyroid-Stimulating Hormone, Prolactin, and Growth Hormone in Spontaneously Hypercholesterolemic Rats (42853)

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*Abstract.* **We have shown previously that spontaneously hypercholesterolemic (SHC) rats exhibit abnormal bone metabolism with advanced bone resorption, which develops with age. In this study, we measured serum levels of growth hormone, thyroid-stimulating hormone, and prolactin in addition to several parameters of calcium metabolism and renal function in young (6-week) and old (24-week) SHC rats and compared these with age-matched Sprague-Dawley rats. In young SHC rats, urinary excretion of hydroxyproline and serum levels of calcium were significantly elevated and excretion of protein into urine and urea nitrogen in the serum were normal, suggesting that calcium metabolism was abnormal without kidney dysfunction at this age. Serum growth hormone and thyroid-stimulating hormone levels were markedly higher (20- to 30-fold and 4- to 5-fold, respectively) in young and old SHC rats, whereas serum prolactin levels were similar. A high level of serum thyroid-stimulating hormone was associated with elevated levels of thyroxine and triiodothyronine in young SHC rats, but not old ones. These results demonstrate that the rat exhibits abnormalities in endocrine function as well as calcium metabolism preceding the occurrence of renal dysfunction.**

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Spontaneously hypercholesterolemic (SHC) rats were originally selected based on their high plasma cholesterol levels from Sprague-Dawley rats and established as a model for hypercholesterolemia by Imai and colleagues (1, 2). We have recently found that the SHC rat exhibits abnormal bone metabolism characterized by either higher excretion of hydroxyproline (OH-P) and calcium into urine, a high level of serum calcium, or a decrease in bone weight and calcium content in the ash (3; manuscript in preparation). Models such as rats with abnormal calcium metabolism have been used to study the metabolism of bone (4). In SHC rats, abnormal bone metabolism develops with age, and the older rats have severe bone resorption associated with spontaneous glomerulonephritis. Old SHC rats therefore provide a useful model for renal osteodystrophy. The abnormal bone metabolism in SHC rats may be caused initially by undefined factor(s) and then may develop following the renal dysfunction in older animals.

This study made use of young (6-week) and old (24-week) SHC rats to measure the serum levels of growth hormone (GH), thyroid-stimulating hormone (TSH), and prolactin (PRL), because these hormones are known to play basic roles in calcium metabolism (5–7).

## Materials and Methods

Male SHC rats were bred in the laboratory at Takeda Chemical Industries Ltd. The SHC rats (4-week old) and Sprague-Dawley (SD) rats (4-week old), purchased from Charles River were maintained in a controlled environment at 24 + 1°C in a 14-hr light:10-hr dark cycle (lights on at 0730 hr) and had free access to water and laboratory chow (CE-2; Clea). The chow contained 1.25 g of calcium, 1.06 g of phosphate, and 200 IU of vitamin $D_3$/100 g.

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At 6 or 24 weeks of age, the rats were killed at 1000–1200 hr by decapitation, and blood was collected. Urine samples were collected by placing rats in a metabolic cage for 24 hr before killing. Serum and urine were stored at −20°C for subsequent measurement of hormones and other parameters. The weights of the rats, kidneys, thyroids, pituitaries, adrenals, and testes were measured.

PRL, GH, and TSH in the serum were assayed by radioimmunoassay using the antisera provided by the NIADDK Rat Pituitary Hormone Distribution Program, and the results were expressed in terms of NIAMDD-rat PRL-RP-2, NIAMDD-rat GH-RP-1, and NIADDK-rat TSH RP-2, respectively, according to the method described earlier (8, 9). Thyroxine ($T_4$) and triiodothyronine ($T_3$) in the serum were assayed using kits (Mallinckrodt Inc., St. Louis, MO). Hydroxyproline in the urine was determined according to the method of Blumenkrantz and Asboe-Hansen (10). Calcium and phosphate in the serum and urine and urea nitrogen in the serum were measured according to the methods previously described (11, 12) using commercial kits (Wako Pure Chemicals). Protein in the urine was determined by the method of Bradford (13) using BioRad protein assay reagents.

Differences among hormones and other parameters between SHC and SD rats were evaluated by Student's *t* test. Probability values of less than 0.05 were considered significant unless otherwise described.

## Results

Body and tissue weights in SHC and control age-matched SD rats are shown in Table I. At 6 weeks, there was no significant difference in body weight between SHC and SD rats, and thyroid weight was significantly lower and pituitary weight was significantly higher in SHC rats than in SD rats. At 24 weeks, kidney weight was 2-fold higher in SHC rats. The weight of body increased to more than 500 g in SHC rats at 18 weeks (data not shown) and then decreased to 425 g at 24 weeks, confirming the previous report (3). The weights of thyroid and pituitary were significantly lower in SHC rats, although their relative values (mg/100 g body wt) were similar (SD vs SHC: 4.28 vs 4.31 for thyroid and 2.25 vs 2.35 for pituitary).

Some parameters of bone metabolism and renal function in 6- and 24-week-old SHC rats were compared with age-matched control SD rats (Table II). At 6 weeks, urinary excretion of OH-P, a marker of bone resorption, was significantly higher in SHC rats. There was a tendency for a higher excretion rate of calcium and phosphate into urine, although this was not significant. Serum concentrations of calcium and phosphate were significantly higher in SHC rats than in SD rats. The serum concentration of urea nitrogen and urinary excretion of protein, parameters of renal function, were similar in the two strains. At 24 weeks, on the other hand, urea nitrogen in the serum and urinary excretion of protein were markedly increased in SHC rats. Urinary excretion of calcium and OH-P became significantly elevated in the older SHC rats. Thus, we confirmed and expanded our previous observations that abnormal calcium metabolism develops with the advance of renal dysfunction in SHC rats (3) and suggest that the abnormality occurred at a younger age (6 weeks), preceding the expression of renal dysfunction.

Serum levels of PRL, GH, and TSH are shown in Table III. PRL levels were similar between SHC and SD rats at 6 and 24 weeks. However, GH and TSH levels were markedly elevated in SHC rats. At 6 weeks, GH levels were about 30-fold higher in SHC than in SD rats. At 24 weeks, the GH level was still high, but the difference was not significant due to the large variation. The TSH level was about 7-fold higher in 6-week-old SHC rats and 5-fold higher in 24-week-old SHC rats compared with the values of age-matched SD rats. Thus, GH and TSH levels were unexpectedly higher in young and old SHC rats compared with those of SD rats.

The high level of serum TSH may cause hyperthyroidism, or, on the contrary, it may occur as a result of hypothyroidism. To clarify this point, serum levels of $T_4$ and $T_3$ were measured (Table IV). At 6 weeks, $T_4$ and $T_3$ were significantly higher in SHC rats than in SD rats. At 24 weeks, these hormones were lower in SHC rats. Thus, high levels of serum TSH were associated with elevation of thyroid hormones in young SHC rats.

## Discussion

We confirmed the previous finding that SHC rats show an abnormal calcium metabolism, which develops

**Table I.** Body and Organ Weights in Male SHC and SD Rats

| Rats | Age (week) | Body weight (g) | Kidney[a] (g) | Thyroid (mg) | Pituitary (mg) | Adrenal[a] (mg) | Testes (g) |
|---|---|---|---|---|---|---|---|
| SD | 6 | 214 ± 4 | 1.04 ± 0.03 | 13.9 ± 1.0 | 6.7 ± 0.3 | 17.1 ± 0.5 | 1.92 ± 0.05 |
| SHC | 6 | 207 ± 5 | 1.10 ± 0.03 | 11.3 ± 0.4[b] | 8.7 ± 0.4[b] | 16.3 ± 0.5 | 1.84 ± 0.05 |
| SD | 24 | 533 ± 17 | 1.69 ± 0.03 | 22.9 ± 1.6 | 12.0 ± 0.5 | 28.4 ± 1.4 | 3.24 ± 0.13 |
| SHC | 24 | 425 ± 33[b] | 3.28 ± 0.15[b] | 18.3 ± 0.7[b] | 10.0 ± 0.6[b] | 30.5 ± 0.6 | 2.92 ± 0.17 |

[a] The weight of left side is shown.
[b] Significant difference ($P < 0.05$), mean ± SE ($n = 6$).

**Table II.** Markers for Bone Metabolism and Renal Function in Male SHC and SD Rats

| Rats | Age (week) | Urinary | | | | | Serum | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Urine volume (ml/day) | Calcium (mg/day) | Phosphate (mg/day) | OH-P (μg/day) | Protein (mg/day) | Urea nitrogen (mg/dl) | Calcium (mg/dl) | Phosphate (mg/dl) |
| SD | 6 | 9.8 ± 0.5 | 0.91 ± 0.12 | 18.10 ± 1.17 | 1547.7 ± 72.9 | 7.6 ± 0.5 | 14.7 ± 0.9 | 9.50 ± 0.10 | 11.43 ± 0.12 |
| SHC | 6 | 13.4 ± 0.7 | 1.17 ± 0.07 | 20.31 ± 1.19 | 2339.0 ± 98.1[a] | 8.1 ± 0.6 | 15.7 ± 0.9 | 10.09 ± 0.11[a] | 11.84 ± 0.13[a] |
| SD | 24 | 8.9 ± 0.8 | 0.70 ± 0.05 | 13.40 ± 1.50 | 507.1 ± 42.8 | 11.0 ± 0.9 | 17.9 ± 0.5 | 9.55 ± 0.13[a] | 6.78 ± 0.29 |
| SHC | 24 | 35.2 ± 4.8[a] | 2.70 ± 0.60[a] | 16.65 ± 1.74 | 1273.0 ± 166.0[a] | 229.1 ± 35.5 | 146.4 ± 30.1[a] | 9.43 ± 0.28 | 13.24 ± 3.40 |

[a] Significant difference ($P < 0.05$) between SD and SHC rats, mean ± SE ($n = 6$).

**Table III.** Serum Levels of PRL, GH, and TSH in Male SHC and SD Rats

| Rats | Age (week) | PRL (ng/ml) | GH (ng/ml) | TSH (pg/ml) |
|---|---|---|---|---|
| SD | 6 | 11.5 ± 3.8 | 2.4 ± 1.6 | 437 ± 107 |
| SHC | 6 | 19.5 ± 2.7 | 73.3 ± 11.1[a] | 2656 ± 242[a] |
| SD | 24 | 30.1 ± 5.3 | 16.9 ± 9.3 | 1143 ± 238 |
| SHC | 24 | 21.3 ± 3.1 | 271.0 ± 128.4 | 4767 ± 277[a] |

[a] Significant difference ($P > 0.05$) between SD and SHC rats, mean ± SE ($n = 6$).

**Table IV.** Serum Levels of $T_4$ and $T_3$ in SHC and SD Rats

| Rats | Age (week) | $T_4$ (μg/dl) | $T_3$ (ng/dl) |
|---|---|---|---|
| SD | 6 | 3.70 ± 0.47 | 49.3 ± 3.1 |
| SHC | 6 | 7.66 ± 0.15[a] | 93.9 ± 4.2[a] |
| SD | 24 | 5.32 ± 0.32 | 52.8 ± 5.8 |
| SHC | 24 | 1.96 ± 0.59[a] | 43.8 ± 12.6 |

[a] Significant difference ($P < 0.05$) between SD and SHC rats, mean ± SE ($n = 6$).

with aging (3). We found that GH and TSH levels were markedly higher in SHC rats compared with those of age-matched control SD rats, whereas the PRL level was similar between them. Since these anterior pituitary hormones participate in calcium metabolism (5–7), this finding seems to be important to describe the nature of SHC rats as a model for abnormal bone metabolism.

In young SHC rats, the high level of serum TSH is consistent with high levels of $T_4$ and $T_3$. Hyperthyroidism may be responsible for the pathogenesis of advanced bone resorption in young SHC rats. Thyroid hormones have been shown to increase the degradation of collagen and to stimulate bone resorption in cultured calvariae (14–16). Thyroid hormones reportedly also decrease 1,25-dihydroxyvitamin $D_3$ and increase 24,25-dihydroxyvitamin $D_3$ *in vivo* and *in vitro* (17, 18). Hyperthyroidism is known to cause an increase of bone turnover, osteoporosis, and hypercalcemia in human patients (19–21). Therefore, SHC rats may be a useful model for hyperthyroidism-associated osteoporosis and hypercalcemia.

Imai *et al.* (1) showed by histologic examination that glomerulonephritis developed in the kidney of SHC rats by 9 months. In the present study, renal dysfunction was confirmed at 24 weeks in SHC rats. Although the details of pathogenesis of glomerulonephritis are still unclear, participation of oxygen radicals in acute nephrotoxic nephritis is reported (22). Since generation of superoxide is increased in the liver in hyperthyroid states (23), hyperthyroidism may also be related to the development of renal dysfunction observed in old SHC rats.

Growth hormone is known as a regulator of skeletal growth (5, 7). Harris *et al.* (24, 25) reported that growth hormone deficiency was associated with a decrease in cartilage cell proliferation and a subsequent decline in linear growth, and excess growth hormone produced an increase in the skeletal growth *in vivo* in the adult dog. Since it has been reported that GH enhances the formation of 1,25-hydroxyvitamin $D_3$ (26, 27), increases the intestinal content of vitamin D-dependent calcium-binding protein (28), and stimulates the intestinal absorption of calcium (29), the elevated level of serum GH in SHC rats may contribute to maintain the serum levels of calcium but not to cause bone resorption. GH is thought to exert its stimulatory effects through the production of somatomedin (30, 31). For the synthesis of somatomedin by the liver, thyroid hormone is required (32). The smaller extent of bone resorption in young than old SHC rats may be explained partly by an indirect effect of thyroid hormones on somatomedin production. High levels of thyroid hormones in young SHC rats may exert dual effects on bone metabolism, a stimulatory action on bone formation by increasing somatomedin and promoting bone resorption by their direct action as described above.

In the morphologic study by Tsukuda (3), the thyroidal follicles were extremely small and poor in colloid in SHC rats irrespective of age from 6 to 26 weeks, suggesting the hyperfunctional state in the gland in SHC rats. Indeed, thyroid hormones were higher in young SHC rats in the present study. However, thyroid hormones decreased in spite of high levels of serum TSH in old SHC rats. There was no morphologic evidence indicating inflammatory thyroiditis in old SHC

rats (3; A. Shino, personal communication). This phenomenon may be related to the high level of serum urea nitrogen due to renal dysfunction. It is reported that uremic patients exhibit hypothyroidism due to failures at multiple levels of the hypothalamopituitary thyroid axis (33).

Secretion of GH is positively regulated by thyroid hormones (34–36). Therefore, an increase in thyroid hormones may partially contribute to the GH levels in young SHC rats. However this is not the whole reason, because the GH level was still high in older SHC rats in which thyroid hormone levels were reduced. Hypersecretion of GH in addition to TSH may be due to the intrinsic nature of the hypothalamopituitary function of SHC rats. Secretion of TSH is known to be under negative feedback control by thyroid hormones at the levels of the hypothalamus and pituitary (9, 37, 38). The fact that a high level of serum TSH was associated with the levels of thyroidal hormones suggests that the set point of thyroid hormone levels at the hypothalamohypophysial system for negative feedback control is high in SHC rats.

SHC rats exhibited hypersecretions of TSH and GH, and these abnormalities in the endocrine milieu preceded significant pathologic changes such as glomerulonephritis (1), hypercholesterolemia (1, 2), and bone resorption.

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