Evidence for a Difference in Vitamin D Metabolism Between Spontaneously Hypertensive and Wistar-Kyoto Rats

THEODORE W. KURTZ, ANTHONY A. PORTALE, AND R. CURTIS MORRIS, JR.

SUMMARY It has been contended that the metabolism of vitamin D in spontaneously hypertensive rats (SHR) is different from that in Wistar-Kyoto rats (WKY). To investigate this possibility, the plasma concentration of 1,25-dihydroxycholecalciferol (1,25(OH)2D) and several known determinants of its production rate were measured in SHR and WKY given normal and restricted amounts of dietary phosphorus. In 12-week-old male SHR given a normal amount of dietary phosphorus, the mean plasma concentration of 1,25(OH)2D (72 ± 5 pg/ml) was significantly lower than that in age-matched WKY (129 ± 6 pg/ml; p < 0.001). The lower plasma concentration of 1,25(OH)2D in the SHR could not be attributed to higher circulating levels of inorganic phosphorus or ionized calcium, lower plasma concentrations of 25-hydroxycholecalciferol, or acidosis. However, in the SHR, urinary excretion of cyclic adenosine 3',5'-monophosphate (12.5 ± 0.4 nmol/mg creatinine) was significantly lower than that in WKY (15.2 ± 0.3 nmol/mg creatinine; p < 0.001). In both SHR and WKY, restriction of dietary phosphorus for 1 week induced an increase in the plasma concentration of 1,25(OH)2D without affecting blood pressure. The current findings indicate that in 12-week-old male SHR, 1,25(OH)2D metabolism is different from that in age-matched WKY. The activity of 25-hydroxyvitamin D-1α-hydroxylase, however, appears to be at least partially responsive to short-term restriction of dietary phosphorus. In SHR, the activity of 25-hydroxyvitamin D-1α-hydroxylase may be lower than that in WKY, perhaps due in part to some impairment in the renal metabolism of, or responsiveness to, cyclic adenosine 3',5'-monophosphate. (Hypertension 8: 1015-1020, 1986)

KEY WORDS • 1,25-dihydroxycholecalciferol • hypertension • spontaneously hypertensive rats • phosphorus
Materials and Methods

Virus-antibody–free 11-week-old male SHR and WKY were obtained from Charles River Laboratories (Wilmington, MA, USA) and given either a normal phosphorus diet or a phosphorus-deficient diet. In the experiments with the normal phosphorus diet, the rats were fed a semipurified diet prepared with egg white solids, sucrose, corn oil, a vitamin mix that provided (per kilogram of diet) 2205 U of vitamin D₃, and a mineral mix that contained phosphorus in the form of dibasic and monobasic (4:1) sodium phosphate and dibasic and monobasic (4:1) potassium phosphate. The diet contained 0.63% calcium and 0.56% phosphorus. These amounts of calcium and phosphorus are more than adequate to sustain maximal bone mineralization in growing rats. In the experiments with the phosphorus-deficient diet, the rats were given the same basic diet as in the normal phosphorus experiment except that sodium bicarbonate and potassium chloride were added to the mineral mix instead of sodium phosphate and potassium phosphate. This addition controlled for the extra sodium and potassium provided by sodium phosphate and potassium phosphate in the normal phosphorus diet. The phosphorus-deficient diet contained 0.63% calcium and less than 0.034% phosphorus.

The rats were housed individually in metabolic cages to provide for the control of food intake and the collection of 24-hour urine samples. All rats were kept in one room and studied over the same 1-week period. A 12-hour light cycle (0600-1800) was maintained throughout the experiment. The daily amount of food given to each spontaneously hypertensive rat was determined by the daily amount of food consumed by a Wistar-Kyoto partner. The SHR consumed all of the food provided.

After 6 days, 24-hour urine collections were obtained for measurements of urinary excretion of calcium, phosphorus, and cyclic adenosine 3',5'-monophosphate (cyclic AMP). After 7 days, measurements of blood pressure were performed in the unanesthetized, unrestrained state through indwelling catheters (polyethylene tubing, PE-50) implanted in the femoral artery. The catheters were tunneled subcutaneously to an exit in the nape of the neck and filled with a heparinized solution of 5% dextrose. Throughout all surgical procedures, the animals were anesthetized with ether (administered by inhalation). Blood pressure was measured 4 hours after stopping the ether anesthesia. To measure blood pressure, the arterial catheter was connected to a low-volume pressure transducer (Statham p23Gb; Oxnard, CA, USA) and the pressure tracing was recorded on a Gould recorder. Mean arterial pressure was measured 20 to 30 minutes after the arterial catheter was connected to the transducer. Blood pressure was calculated from the average of six readings obtained while each animal was resting and motionless but alert in a metabolic cage. Blood pressures were measured in the SHR and WKY at the same time of day. After the measurement of blood pressure, heparinized blood samples were obtained through the arterial catheter for measurement of pH, ionized calcium, inorganic phosphorus, total calcium, 25-hydroxyvitamin D (25-OH D), and 1,25(OH)₂D.

Plasma and urinary concentrations of total calcium and inorganic phosphorus were measured as previously described. Arterial pH and ionized calcium were measured in whole blood with an ionized calcium/pH analyzer (Model 8; NOVA Biomedical, Newton, MA, USA). Measurements of the plasma concentrations of 1,25(OH)₂D and 25-OH D and urinary concentrations of cyclic AMP were performed by the Nichols Institute Reference Laboratories, San Juan Capistrano, CA, USA. The measurement of 1,25(OH)₂D was performed with a modified version of the calf thymus receptor assay reported by Reinhardt et al. In this assay, the interassay coefficient of variation ranges from 9.5 to 12% for a low level control and 11 to 12% for a high level control (David Endres, Nichols Institute, personal communication, 1986). The measurement of 25-OH D was performed with a competitive protein binding assay that has an interassay coefficient of variation of 12%. The measurement of cyclic AMP was performed by radioimmunoassay.

Statistical analysis was performed by Student’s t test. Statistical significance was defined as a p level less than 0.05.

Results

Normal Phosphorus Diet

In the SHR given the normal phosphorus diet, mean arterial pressure was significantly greater than that in the WKY (Table 1). Mean body weight in the SHR was also significantly greater than that in the WKY. This greater body weight is characteristic of virus-antibody–free SHR and WKY raised by Charles River Laboratories (Charles River price list, March 1, 1985). In SHR and WKY supplied by the University of Iowa Cardiovascular Center, Schedl et al. also observed greater body weight in the SHR than in the WKY.

In the SHR, the mean plasma concentration of 1,25(OH)₂D (72 ± 5 pg/ml) was significantly lower than that in the WKY (129 ± 6 pg/ml; p < 0.001). The mean plasma concentration of 25-OH D was significantly greater in the SHR than in the WKY (31 ± 1 vs 19 ± 1 ng/ml, respectively; p < 0.001), confirming the previous report of Schedl et al. The individual data points for the plasma concentrations of 1,25(OH)₂D and 25-OH D are presented in Figures 1A and 2A.

The mean blood level of ionized calcium was significantly lower in the SHR than in the WKY (see Table 1), a finding consistent with previous reports. The plasma levels of inorganic phosphorus and total calcium were also significantly lower in the SHR than in the WKY (see Table 1). In the SHR, mean arterial blood pH was slightly, but not significantly, greater than in the WKY. Urinary excretions of calcium, phosphorus, and cyclic AMP were lower in the SHR than in the WKY (see Table 1), consistent with previous studies in SHR less than 18 weeks of age.
TABLE 1. Metabolic and Physiological Characteristics of SHR and WKY Given a Normal Dietary Intake of Phosphorus

| Group   | Weight (g) | MAP (mm Hg) | Ion Ca (mg/dl) | pH | Plasma (mg/dl) | Cyclic AMP (nmol/mg cr) | Urine Ca (mg/mg cr) | P
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<tr>
<td>WKY (n = 10)</td>
<td>223 ± 3</td>
<td>113 ± 3</td>
<td>4.53 ± 0.06</td>
<td>7.45 ± 0.01</td>
<td>7.5 ± 0.3</td>
<td>10.9 ± 0.2</td>
<td>15.2 ± 0.3</td>
<td>0.40 ± 0.03</td>
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<td>SHR (n = 9)</td>
<td>285 ± 3*</td>
<td>162 ± 3*</td>
<td>4.32 ± 0.07*</td>
<td>7.48 ± 0.02</td>
<td>5.7 ± 0.4*</td>
<td>10.2 ± 0.2*</td>
<td>12.5 ± 0.4*</td>
<td>0.08 ± 0.01*</td>
</tr>
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Values are means ± SE. MAP = mean arterial pressure; Ion Ca = ionized Ca; P = inorganic phosphorus; cr = creatinine. *p < 0.001, t < 0.025, Xp < 0.01, compared with values in WKY.

Phosphorus-Deficient Diet

In the SHR given the phosphorus-deficient diet, mean arterial pressure was significantly greater than that in the WKY (Table 2). In SHR and WKY given the phosphorus-deficient diet, blood pressures did not appear to be different than those in the SHR and WKY given the normal phosphorus diet. Other investigators have found that severe restriction of dietary phosphorus over a 4- to 6-week period will lower blood pressure in Sprague-Dawley rats. In the SHR and WKY, restriction of dietary phosphorus for 1 week induced a doubling in the mean plasma concentrations of 1,25(OH)2D. The mean plasma concentration of 1,25(OH)2D (141 ± 13 pg/ml) was significantly lower in SHR than in WKY (307 ± 33 pg/ml; p < 0.001). In rats and in humans, restriction of dietary phosphorus for 7 to 10 days has previously been reported to induce twofold to threefold increases in plasma concentrations of 1,25(OH)2D. Restriction of dietary phosphorus did not appear to affect the plasma concentration of 25-OHD in either the SHR or the WKY; mean 25-OHD concentration remained greater in the SHR. The individual data points for the plasma concentrations of 1,25(OH)2D and 25-OHD are presented in Figures 1B and 2B.

In the SHR given the phosphorus-deficient diet, the mean plasma concentrations of total calcium and phosphorus were significantly lower than those in the WKY given the phosphorus-deficient diet (see Table 2). Differences in the mean levels of blood ionized calcium and arterial pH between the SHR and WKY were not statistically significant.

In the SHR given the phosphorus-deficient diet, the plasma concentration of phosphorus was lower than that in the SHR given the normal phosphorus diet. However, in the WKY given the phosphorus-deficient diet, the plasma concentration of phosphorus was not lower than that in WKY given the normal phosphorus diet. This observation was unexpected given that short-term restriction of dietary phosphorus predictably induces a decrease in plasma phosphorus in Sprague-Dawley rats.

FIGURE 1. Plasma concentrations of 1,25-dihydroxycholecalciferol (1,25(OH)2D) in SHR and WKY given a normal dietary intake of phosphorus (A) or a phosphorus-deficient diet (B) for 1 week.

FIGURE 2. Plasma concentrations of 25-hydroxyvitamin D (25-OHD) in SHR and WKY given a normal dietary intake of phosphorus (A) or a phosphorus-deficient diet (B) for 1 week.
In the SHR given the phosphorus-deficient diet, urinary excretion rates of phosphorus, calcium, and cyclic AMP were lower than those in the WKY given the phosphorus-deficient diet. With phosphorus restriction, 1,25(OH)₂D levels were increasing at a time when the levels of cyclic AMP (and presumably therefore PTH) were decreasing. Under normal circumstances and particularly when administered acutely, PTH stimulates 1α-hydroxylase and the production of 1,25(OH)₂D; however, with restriction or supplementation of dietary phosphorus, changes in plasma levels of 1,25(OH)₂D can vary inversely with those of PTH. As expected, restriction of dietary phosphorus was also associated with decreased urinary excretion of phosphorus and increased urinary excretion of calcium in both SHR and WKY.

**Discussion**

In the current study, in 12-week-old male SHR given a normal dietary intake of phosphorus, the mean plasma concentration of 1,25(OH)₂D was significantly lower than that in age-matched WKY, confirming the findings of Lucas et al.² in 12-week-old SHR given a normal dietary intake of phosphorus. Indeed, the absolute levels of 1,25(OH)₂D in the current study are almost identical to those in the study of Lucas et al.,³ in which a different technique (radioimmunoassay) was used to measure 1,25(OH)₂D. In contrast, several investigators have reported that the plasma concentration of 1,25(OH)₂D in SHR is not different from that in WKY.⁴⁻⁶ In those studies in which the animals were pair-fed, however, the plasma concentrations of 1,25(OH)₂D tended to be lower in the SHR.⁴⁻⁶ In some cases, the failure to find a statistically lower mean value of 1,25(OH)₂D in SHR could have been a consequence of the use of small sample sizes. Differences in the dietary amounts of calcium, phosphorus, and vitamin D provided in the different studies might also contribute to the inconsistent findings with respect to plasma concentrations of 1,25(OH)₂D in SHR and WKY.

The current findings provide support for the concept that metabolism of 1,25(OH)₂D in SHR is different from that in WKY. The decreased plasma levels of 1,25(OH)₂D in the SHR could reflect either a decrease in production rate of 1,25(OH)₂D or an increase in its metabolic clearance rate, or both. In the current study, we measured some of the major determinants of 1,25(OH)₂D production in the same animals in which we measured plasma levels of 1,25(OH)₂D. In the SHR, circulating levels of ionized calcium and inorganic phosphorus were decreased compared with those in the WKY, the arterial pH being slightly higher in the SHR. Thus, in the SHR, the decreased plasma concentrations of 1,25(OH)₂D cannot be attributed to suppression of 1,25(OH)₂D production by greater circulating levels of calcium or phosphorus or by acidoses. Indeed, decreased circulating levels of ionized calcium and phosphorus would be expected to stimulate production of 1,25(OH)₂D.²⁰

In the SHR, the mean plasma concentration of 25-OHD was significantly greater than that in the WKY, confirming the finding of Schell et al.⁶ Decreased feedback inhibition of vitamin D-25-hydroxylase by decreased circulating levels of 1,25(OH)₂D²⁰ might account for the increased circulating levels of 25-OHD in the currently studied SHR.

Given the finding of increased plasma levels of 25-OHD in the SHR, one cannot attribute the lower plasma concentrations of 1,25(OH)₂D to an inadequate amount of substrate available for conversion to 1,25(OH)₂D. Nevertheless, in SHR, the production of 1,25(OH)₂D might be particularly dependent on the amount of circulating 25-OHD. In a study in which SHR and WKY were fed a diet that contained 4500 U/kg vitamin D₃, twice the amount provided in the present study, Hsu et al. did not detect any difference in the serum concentrations of 1,25(OH)₂D between the two strains. However, these investigators found that provision of a vitamin D-deficient diet for 11 weeks induced a decrease in the serum concentration of 1,25(OH)₂D in SHR, but not in WKY. In both the SHR and WKY given the vitamin D-deficient diet, the plasma concentrations of 25-OHD were below the detection limit of the assay.⁷

Parathyroid hormone is an important determinant of the renal synthesis of 1,25(OH)₂D.²⁸ Circulating levels of 1,25(OH)₂D are increased in primary hyperparathyroidism and decreased in hypoparathyroidism.²⁹ Although Schell et al. did not find a statistically significant difference between the SHR and WKY with respect to serum concentrations of 1,25(OH)₂D, they argued that in the SHR, the serum concentrations of 1,25(OH)₂D were inappropriately low given the reported increases in circulating levels of PTH in SHR. It appears, however, that cyclic AMP mediates PTH-induced stimulation of 1,25(OH)₂D production,²¹ and in the SHR, we and others have found that urinary...

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**Table 2. Metabolic and Physiological Characteristics of SHR and WKY Given a Phosphorus-Deficient Diet for 1 Week**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (g)</th>
<th>MAP (mm Hg)</th>
<th>Ion Ca (mg/dl)</th>
<th>pH</th>
<th>Plasma (mg/dl)</th>
<th>Cyclic AMP (nmol/mg ct)</th>
<th>Ca (mg/mg ct)</th>
<th>P₁, Ca</th>
<th>P₁, P₀</th>
<th>P₂, P₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n = 8)</td>
<td>204 ± 3</td>
<td>117 ± 2</td>
<td>4.66 ± 0.08</td>
<td>7.46 ± 0.01</td>
<td>7.7 ± 0.4</td>
<td>11.7 ± 0.3</td>
<td>9.7 ± 0.6</td>
<td>3.2 ± 0.05</td>
<td>0.026 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>SHR (n = 9)</td>
<td>268 ± 2</td>
<td>161 ± 5</td>
<td>4.51 ± 0.07</td>
<td>7.47 ± 0.01</td>
<td>4.8 ± 0.3</td>
<td>10.9 ± 0.2</td>
<td>6.7 ± 0.7</td>
<td>1.4 ± 0.08*</td>
<td>0.016 ± 0.001*</td>
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</table>

Values are means ± SE. See Table 1 for key to abbreviations.

* p < 0.001, †p < 0.01, ‡p < 0.05, §p < 0.005, compared with values in WKY.
excretion of cyclic AMP is decreased relative to that in WKY. Thus, in the SHR, decreased production of cyclic AMP might be a determinant of decreased circulating levels of 1,25(OH)₂D. In a preliminary communication, Kawashima reported that in 12-week-old SHR, the increase in renal mitochondrial production of 1,25(OH)₂D induced by PTH administration was one third that induced in 12-week-old WKY. This finding could reflect either impaired production of cyclic AMP in response to PTH or impaired responsiveness of 25-OHD-1α-hydroxylase to cyclic AMP, or both.

In both SHR and WKY, restriction of dietary phosphorus, a maneuver known to increase the production rate of 1,25(OH)₂D, induced a doubling in the mean plasma concentrations of 1,25(OH)₂D. This similar response of SHR and WKY to restriction of dietary phosphorus may suggest that, at least in some circumstances in the SHR, the enzyme responsible for the conversion of 25-OHD to 1,25(OH)₂D, 25-OHD-1α-hydroxylase, can be normally stimulated. In the SHR, however, the absolute magnitude of the increase in the plasma concentration of 1,25(OH)₂D appeared to be less than that in the WKY despite the fact that restriction of dietary phosphorus induced a greater decrease in the plasma concentration of phosphorus in the SHR. Thus, in the SHR, the responsiveness of 25-OHD-1α-hydroxylase to reduced plasma concentrations of phosphorus might be lower than that in the WKY. In the current study, we restricted dietary phosphorus for 1 week. Had we restricted phosphorus longer, we might have observed even greater differences in the plasma levels of 1,25(OH)₂D between the SHR and WKY.

In both the SHR and WKY given the phosphorus-deficient diet, the circulating levels of total and ionized calcium appeared to regulate at higher levels, consistent with a physiological effect of the increased plasma concentrations of 1,25(OH)₂D induced by phosphorus restriction. As expected, in both the SHR and WKY, restriction of dietary phosphorus induced increases in the urinary excretion of calcium. In the SHR, the absolute increase in calcium excretion induced by phosphorus restriction was less than that in the WKY, consistent with the induction of a smaller absolute increase in plasma 1,25(OH)₂D in the SHR.

In virus-antibody–free SHR raised by Charles River Laboratories, body weights are reported to be greater than in age-matched, virus-antibody–free WKY (Charles River price list, March 1, 1985). In the current study in virus-antibody–free SHR raised by Charles River Laboratories, we also found mean body weight in SHR to be greater than that in age-matched WKY. Thus, it is possible that in the current study, the difference in 1,25(OH)₂D metabolism between the SHR and WKY was a consequence of the difference in body weights between the two strains. In studies in 12-week-old SHR from the breeder colonies of the University of Iowa Cardiovascular Center, Schedl et al. also found mean body weight to be significantly greater than that in age-matched WKY. These investigators, however, did not find a statistically significant difference in mean serum concentrations of 1,25(OH)₂D between SHR and WKY. In the study of Lucas et al., in which circulating levels of 1,25(OH)₂D in 12-week-old SHR were found to be lower than those in age-matched WKY, the mean body weight of the SHR was not different from that of the WKY (David McCarron, personal communication, 1986). Thus, the occurrence of a difference in body weight between the SHR and WKY may not be sufficient, or necessary, for the occurrence of a difference in circulating levels of 1,25(OH)₂D between the two strains. The fact that some investigators, but not all, find SHR to weigh more than age-matched WKY and that some, but not all, find circulating levels of 1,25(OH)₂D to be lower in 12-week-old SHR than in age-matched WKY could be a consequence of differences among the various research environments (e.g., diet composition and exposure to ultraviolet light) or differences among the genetic characteristics of the various sublines of SHR and WKY studied, or both.

To address these possibilities, it would be necessary to study vitamin D metabolism in SHR and WKY under a variety of different environmental conditions, as well as to study a variety of sublines of SHR and WKY under fixed environmental conditions.

In most studies of the SHR, the WKY are used as control animals. Thus, one might interpret the current results as showing an abnormality in vitamin D metabolism in SHR. However, it is possible that the WKY is not normal with respect to the control of 1,25(OH)₂D metabolism. To address this possibility, it would be necessary to measure plasma levels of 1,25(OH)₂D in a number of rat strains (e.g., Fischer-344, Lewis) given the same diet and exposure to ultraviolet light.

The finding of decreased plasma concentrations of 1,25(OH)₂D in 12-week-old SHR is consistent with reports of decreased intestinal absorption of calcium in young, 10- to 12-week-old SHR. Both of these findings are consistent with the concept that an abnormality in calcium balance might be a pathogenetic determinant of hypertension. However, the role of calcium deficiency in the pathogenesis of genetic hypertension is highly controversial. In 3- to 4-week-old "prhypertensive" SHR, Lau and co-workers have found increased circulating levels of 1,25(OH)₂D and increased intestinal absorption of calcium, suggesting that calcium deficiency is not a determinant of increased blood pressure in SHR. (It might be noted that several investigators have found blood pressure to be increased in SHR less than 4 weeks of age.) An alternative hypothesis is that in 3- to 4-week-old SHR, increased circulating levels of 1,25(OH)₂D are somehow determining of subsequent increases in blood pressure. Resnick et al. have reported that in humans with low renin essential hypertension, oral administration of NaCl induces increases in blood pressure that are positively correlated with NaCl-induced increases in the plasma concentration of 1,25(OH)₂D. In 12-week-old SHR in which blood pressure is clearly increased, the decreased levels of 1,25(OH)₂D may be a consequence of, or a protective response to, established hypertension.
In the current study in SHR, we did not specifically attempt to determine the role of vitamin D metabolism in the pathogenesis of hypertension. We did note, however, that in the SHR, increased plasma levels of 1,25(OH)₂D induced by restriction of dietary phosphorus for 1 week were not accompanied by detectable changes in blood pressure. This finding may suggest that in 12-week-old SHR, a decreased circulating concentration of 1,25(OH)₂D is not an important determinant of blood pressure. It is possible, however, that with a more sustained increase in the plasma concentration of 1,25(OH)₂D, changes in blood pressure might have been observed. Whatever the possible relationship of vitamin D metabolism to hypertension in SHR, the current findings suggest that studied together, SHR and WKY may be useful for the investigation of genetically determined variability in vitamin D metabolism.

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References
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