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Plasma 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol concentrations are decreased in hind limb unloaded Dahl salt-sensitive female rats

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Abstract

Plasma 1,25-dihydroxyvitamin D (1,25-(OH)₂D) concentration was shown to decrease during bed rest in several studies when baseline plasma 25-hydroxyvitamin D (25-OHD) concentration was sub-optimal. Dahl salt-sensitive female (S) rats, but not Dahl salt-resistant female (R) rats, demonstrated a 50% decrease in plasma 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃) concentration after 28 days of hind limb unloading (HU, disuse model) during low salt intake (0.3%). We tested the vitamin D endocrine system response of female S rats to hind limb unloading during high salt intake (2%, twice that of standard rat chow to mimic salt intake in the USA). Hind limb unloading resulted in lower plasma 25-OHD₃ concentrations in S-HU rats than in R-HU rats ($P < 0.05$) and greater urinary loss of 25-OHD₃ by S-HU rats than by S rats ($P < 0.05$). Plasma 1,25-(OH)₂D₃ concentration of S-HU rats was half that of S rats, but was unchanged in R-HU rats. The association of low plasma 25-OHD concentration with decrease in plasma 1,25-(OH)₂D concentration of hind limb unloaded rats and of bed rest participants (published studies) suggests that low vitamin D status might be a risk factor for decrease in plasma vitamin D hormone concentration during long-term immobilization or bed rest.

Keywords

25-Hydroxyvitamin D; 1,25-Dihydroxyvitamin D; Salt sensitivity; Dahl rats; Immobilization

1. Introduction

Bed rest has been associated with changes in the vitamin D endocrine system. Serum concentrations of vitamin D hormone (1,25-dihydroxyvitamin D, 1,25-(OH)₂D) were decreased within one week of bed rest [1–3], after long-term bed rest [4–6], and after long-term space flight [7–8]. The Dahl salt-sensitive (S) female rat, but not the Dahl salt-resistant (R) rat, also demonstrated a decrease in plasma 1,25-(OH)₂D concentration when hind limb unloaded (a disuse and space flight model) for 28 days [9].

The Dahl S rat is a widely studied model of salt-induced hypertension [10–11]. The Dahl S rat, but not the Dahl R rat, develops hypertension, accompanied by hypovitaminosis D, when fed a high salt diet [10–14]. Plasma concentrations of the liver metabolite of cholecalciferol,

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25-hydroxycholecalciferol (25-OHD₃), are similar in young S and R rats when the rats are fed a low salt diet (0.3% sodium chloride). High salt intake, however, causes significant decreases in plasma 25-OHD₃ concentrations of S rats, but not R rats [12,14–16]. Blood pressure was shown to be directly correlated and plasma 25-OHD₃ concentration indirectly correlated with the number of days that young S rats were fed an 8% salt diet [12,14]. We demonstrated that Dahl S rats lose protein-bound cholecalciferol metabolites into urine and that this loss is markedly accelerated during high salt intake [17], resulting in low plasma 25-OHD₃, marker of vitamin D status, and 24,25-dihydroxycholecalciferol (24,25-(OH)₂D₃) concentrations [12–16]. High salt intake, thus, creates a vitamin D deficiency state in Dahl S rats in the presence of standard amounts of dietary vitamin D [12–16]. Plasma concentrations of the vitamin D hormone, 1,25-(OH)₂D₃, were shown to be higher in young female S rats during high salt intake than during low salt intake [14].

Female S rats fed a low salt diet (0.3% salt) and hind limb unloaded (a disuse and space flight model) for 28 days exhibited 50% lower plasma 1,25-(OH)₂D₃ concentration than loaded female S rats, whereas loaded and unloaded female R rats exhibited similar plasma 1,25-(OH)₂D₃ concentrations [9]. Plasma 25-OHD₃ concentrations of unloaded S rats were significantly lower than that of unloaded R rats [9]. We report here on the effect of a disuse model on the vitamin D endocrine system of female S rats during 2% salt intake (double that of a standard rat diet and designed to mimic the salt intake of the American population).

2. Materials and methods

2.1. Animals and diets

The rats were hind limb unloaded at the NASA Ames Research Center. The Animal Care and Use Committee at NASA Ames Research Center approved the protocols involving the use of the rats. Guidelines followed were those of the Public Health Service and the revised animal welfare act as regulated by USDA.

Four to five week old Dahl salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) female rats (100–110 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and housed at NASA Ames Research Center in a room with 12 h light - dark cycles. The rats were divided into four groups (S, loaded and hind limb unloaded; R, loaded and hind limb unloaded) and fed a high salt diet (20 g sodium chloride/kg, TD 95078, Harlan Teklad, Madison, WI) throughout the experimental period. Food was weighed daily in order to adjust the amount to be given to control rats to maintain body weights identical to tail-suspended rats. The diet in this study provided 5 IU cholecalciferol/gram or approximately 100 IU/day for each rat, compared with 2 IU cholecalciferol/gram in a commonly used diet (Global Rodent Diet, Harlan-Teklad).

Rats were killed by decapitation, after anesthesia with isoflurane (3%) and cardiac puncture, at day 28. Heparinized plasma was obtained by centrifuging the blood at 1,500 g for 10 min and was stored at –80°C until analysis. The adrenals were excised and weighed.

2.2. Hind limb unloading

The hind limb unloaded (tail-suspended, HU) rat was used as a disuse model [18–22]. This model utilizes a special cage with an overhead pulley system to which the tails are attached by paper clip through a loop of orthopedic tape made by attaching the cut ends of the tape firmly to two-thirds the length of the tail. The rats were suspended in a 30 degree head-down tilt for 28 days in previously described [20] space flight model cages modified for 24 h urine collection. Urine (24 h) collected at day 21 was analyzed to provide comparisons with hind limb unloaded Dahl rats fed a low salt (0.3% sodium chloride) diet [9].

2.3 Cholecalciferol metabolite concentrations

25-Hydroxy[26(27)-methyl-³H]cholecalciferol, 24R,25-dihydroxy[26,27-methyl-³H]cholecalciferol, and 1,25-dihydroxy[26,27-methyl-³H]cholecalciferol (Amersham Corp., Arlington Heights, IL) were separately purified on a Zorbax-CN column (Agilent Technologies, Wilmington, DE) with hexane-isopropanol-methanol (94/5/1, v/v) at a flow rate of 1.3 ml [23]. Synthetic 25-hydroxycholecalciferol (25-OHD₃), 24,25-dihydroxycholecalciferol (24,25-(OH)₂D₃), and 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃), purchased from ICN Pharmaceuticals (Irving, CA), served as standards. For the assay of 25-OHD₃ and 24,25-(OH)₂D₃, 800 dpm of each radio-labeled metabolite were added to the plasma and urine samples prior to purification to determine recovery.

Plasma (0.5 ml) and urine (3 ml) samples were purified using a dichloromethane/methanol liquid-liquid extraction [12], followed by the solid-phase extraction method of Reinhardt and Hollis [24]. The procedure has been previously described by us [9,41]. The limits of detection in the urine samples were 0.078 pmol/ml for 25-OHD₃ and 0.030 pmol/ml for 24,25-(OH)₂D₃. In this study, 25-OHD₃ and 24,25-(OH)₂D₃ were measured in separate aliquots of urine, because the decision to measure urinary 24,25-(OH)₂D₃ was made later. The manufacturer's (DiaSorin) instructions were used to measure 1,25-(OH)₂D₃ concentration, by radioimmunoassay, in a separate aliquot of plasma. Samples were measured in duplicate.

2.4 25-Hydroxycholecalciferol binding activity and protein

Urine and plasma were tested for specific binding to 25-OHD₃ (DBA) by previously published methods [9,13,17,41]. Aliquots of diluted urine and plasma samples (0.5 ml) were incubated with radiolabeled 25-OHD₃ (1.16 nM, 20,000 dpm) in the presence of ethanol or 200-fold molar excess unlabeled 25-OHD₃. Binding in the presence of 200-fold excess unlabeled 25-OHD₃ (non-specific binding) was subtracted from total binding to obtain specific binding (DBA). Binding activity (pmol/l x h) was multiplied by the 24 h urine volume to obtain pmol/h.

Urinary and plasma protein were measured by the bicinchoninic acid protein assay kit (Sigma-Aldrich, St. Louis, MO.), using an automated plate reader (Spectra Max 250, Molecular Devices, Sunnyvale, CA).

2.5 Parathyroid hormone, calcium, and sodium

Plasma parathyroid hormone (PTH) concentrations were measured with a rat intact PTH ELISA kit (Immutopics, San Clemente, CA), using an automated plate reader (Spectra Max 250, Molecular Devices, Sunnyvale, CA). Total plasma calcium and urinary calcium and sodium were measured with an Electrolyte 10+ Analyzer (Nova Biomedical, Waltham, MA). Calcium and sodium concentrations of the urine were multiplied by the 24 h urine volume to derive UCaV and UCaV/UNaV ratios.

2.6 Statistical methods

A mean ± SEM was calculated for each group. Statistical significance ($P < 0.05$) was evaluated using two-way ANOVA followed by Tukey's multiple comparisons test (SigmaStat, SPSS Inc., Chicago, IL.). Effects tested were S vs. R, S vs. S-HU, R vs. R-HU, and S-HU vs. R-HU. The Mann-Whitney rank sum test was used to determine the effect of hind limb unloading on urinary 25-OHD₃ and urinary 24,25-(OH)₂D₃ of S rats, since these metabolites were non-detectable in the urine of R rats. Linear regression analyses were conducted with SigmaStat software (SPSS, Inc.).

3. Results

Loaded and unloaded pairs were weight-matched experimentally by controlling the amount of food eaten. Average body weights of S rats were greater ($P < 0.001$) than that of R rats (S vs. S-HU, 233 ± 5 vs. 235 ± 3 g; R vs. R-HU, 197 ± 12 vs. 196 ± 6 g). Adrenal to body weight ratios were similar in loaded and unloaded S (28 ± 2 vs. 29 ± 1 mg/100 g) and R (26 ± 1 vs. 29 ± 1 mg/100 g) rats, suggesting absence of stress in the hind limb unloaded rats.

Plasma 25-OHD₃ and 24,25-(OH)₂D₃ concentrations were significantly lower in S rats than in R rats overall and significantly lower in S-HU rats than in R-HU rats (Fig. 1). S rats lost 25-OHD₃ and 24,25-(OH)₂D₃ into urine, but these metabolites were non-detectable in the urine of R rats (Fig. 2). Hind limb unloading significantly increased urinary loss of 25-OHD₃ and 24,25-(OH)₂D₃ by S rats. Protein and DBA were significantly higher in the urine of S rats, compared with R rats, and were highest in S-HU rats (Fig. 3). Urinary protein was significantly increased overall by hind limb unloading (Fig. 3).

Plasma 25-OHD₃ concentrations of S and R rats were inversely correlated with urinary DBA ($r = -0.50$, $P = 0.03$, $n = 19$) and urinary protein ($r = -0.56$, $P = 0.003$, $n = 26$). Plasma 24,25-(OH)₂D₃ concentrations of S and R rats were inversely correlated with urinary DBA ($r = -0.65$, $P = 0.003$, $n = 19$) and urinary protein ($r = -0.76$, $P < 0.001$, $n = 26$). Plasma 24,25-(OH)₂D₃ concentrations of S rats were inversely correlated with urinary 24,25-(OH)₂D₃ ($r = -0.84$, $P < 0.001$, $n = 11$). Urinary 24,25-(OH)₂D₃ of S rats, but not urinary 25-OHD₃, was directly correlated with urinary DBA ($r = 0.73$, $P = 0.04$, $n = 8$) and urinary protein ($r = 0.96$, $P < 0.001$, $n = 9$). The inverse associations above are for day 21 urinary components and day 28 plasma components.

Plasma 25-OHD₃ and 24,25-(OH)₂D₃ concentrations of S and R rats were directly correlated ($r = 0.67$, $P < 0.001$, $n = 29$). Mean plasma DBA of S-HU rats was greater than that of R-HU rats (S-HU vs. R-HU, 70 ± 3 vs. 52 ± 7 pmol/ml $\times$ h, $P = 0.004$, $n = 6-8$). Loaded S and R rats exhibited similar plasma DBA (S vs. R, 61 ± 2 vs. 63 ± 5 pmol/ml $\times$ h; $n = 7-8$). Plasma protein concentration (range: 55 ± 2 to 61 ± 6 mg/ml; $n = 6-8$) did not significantly differ between the four groups of rats.

Mean plasma 1,25-(OH)₂D₃ concentration was significantly higher in loaded S rats than in loaded R rats (Fig. 4). Mean plasma 1,25-(OH)₂D₃ concentration of S-HU rats was less than half that of S rats, but plasma 1,25-(OH)₂D₃ concentration of R rats was not affected by hind limb unloading. Plasma PTH concentrations were similar in the four groups (Table 1). Plasma calcium did not significantly differ among the four groups, but urinary calcium was significantly greater for S than for R rats (Table 1). Urinary calcium/urinary sodium ratios were significantly higher for S rats than for R rats (Table 1).

4. Discussion

This study was conducted to determine the effect of hind limb unloading (disuse model) on the vitamin D endocrine responses of Dahl S rats fed a diet with twice the salt content of Purina chow (1%), to mimic high salt intake in humans. Studies indicate that the salt intake of the USA population is two-three times recommended [3,25]. Prevalence of salt sensitivity was determined to be 18% in Caucasian-Americans and 37% in African-Americans 18–25 year-old [26] and more than 50% in the hypertensive population [27]. The use of both S and R rats in this study allows a look at the effects of salt-sensitivity. Implications of this study would be relevant to bed rest patients who are salt-sensitive.

Effect of salt sensitivity

The significantly greater body weights of S rats, compared with R rats, have been previously demonstrated [9,12]. The two most abundant vitamin D metabolites in plasma, 25-OHD₃ and 24,25-(OH)₂D₃, were detectable in the urine of S rats, but not R rats. A consequence was significantly lower plasma 25-OHD₃ and 24,25-(OH)₂D₃ concentrations in S rats overall. Vitamin D metabolites are transported in blood bound to vitamin D binding protein (DBP). Urinary DBA and protein were also significantly higher for S rats than for R rats. Plasma 25-OHD₃ and 24,25-(OH)₂D₃ concentrations of S and R rats in this and another study [13] were inversely correlated with urinary DBA and protein. We previously concluded that loss of protein, bound to vitamin D metabolites, is the pacemaker for the sensitivity of the vitamin D endocrine system to salt [13]. Plasma 1,25-(OH)₂D₃ concentrations of S rats were significantly higher than that of R rats in the present study. Female S rats have previously been demonstrated to significantly increase plasma 1,25-(OH)₂D₃ concentration in response to high salt intake [14].

Plasma 24,25-(OH)₂D₃ concentrations of S rats were inversely correlated with urinary 24,25-(OH)₂D₃. Plasma 24,25-(OH)₂D₃ concentrations of loaded and unloaded S and R rats were directly correlated with plasma 25-OHD₃ concentrations. Plasma 24,25-(OH)₂D₃ concentrations of S rats would thus be determined by urinary 24,25-(OH)₂D₃ loss and by substrate concentration. In the case of S rats, 24,25-(OH)₂D₃ replacement would be limited by diminishing 25-OHD₃ concentrations. Salt caused a greater percentage decrease in plasma 24,25-(OH)₂D₃ concentrations than in plasma 25-OHD₃ concentrations of S rats. 24,25-Dihydroxycholecalciferol inhibits the stimulatory actions of 1,25-(OH)₂D₃ on membrane-initiated phosphate and calcium absorption in intestines [28]. The two metabolites also regulate endochondral ossification via membrane-mediated mechanisms; 24,25-(OH)₂D₃ stimulates protein kinase C activity in chondrocytes from the growth plate resting zone, but 1,25-(OH)₂D₃ stimulates protein kinase C activity in chondrocytes from the growth zone [29]. Since plasma 1,25-(OH)₂D₃ concentration is increased and plasma 24,25-(OH)₂D₃ concentration is decreased in female S rats in response to high salt intake [14], high salt intake increases the plasma 1,25-(OH)₂D₃/24,25-(OH)₂D₃ ratio of these rats.

Effect of hind limb unloading

Plasma 25-OHD₃ and 24,25-(OH)₂D₃ concentrations were significantly lower in S-HU rats than in R-HU rats. Hind limb unloading during 2% salt intake increased the loss of 25-OHD₃ and 24,25-(OH)₂D₃ into urine by S rats, a phenomenon not seen in a previous study of hind limb unloaded S rats fed 0.3% salt. Both urinary protein and DBA were higher for S-HU rats, compared with S rats. There was also a tendency toward increased protein loss and decreased plasma 24,25-(OH)₂D₃ concentration by R-HU rats, compared with R rats.

Plasma 1,25-(OH)₂D concentration decreased during bed rest [1–6] and spaceflight [7–8]. Plasma 1,25-(OH)₂D₃ concentration in S-HU rats was less than 50% of that in S rats after 28 days, a phenomenon previously observed when S-HU rats were fed a low salt diet [9]. Plasma 1,25-(OH)₂D₃ concentration in R-HU rats was, however, similar to that in R rats, as previously observed when R-HU rats were fed a low salt diet [9]. Plasma 1,25-(OH)₂D concentrations were transiently decreased in young male hind limb unloaded Sprague-Dawley rats, but returned to normal within 5–15 days [19]. S-HU, but not R-HU or unloaded Sprague-Dawley rats, thus model the vitamin D hormone response to bed rest and space flight. In two recent bed rest studies, however, plasma 1,25-(OH)₂D concentrations were not decreased by 21 days of bed rest [30] or by 28, 60, or 90 days of bed rest [31].

Plasma 25-OHD concentration is an indicator of vitamin D status. Plasma 25-OHD concentrations < 30 nM have been suggested to be indicative of hypovitaminosis D in humans,

whereas 25-OHD concentrations ≥ 75 nM have been suggested as optimal [32–36]. Plasma 25-OHD concentrations were 55 and 62 nM at day 0 in the two recent bed rest studies cited above [30,31], but ≤ 40 nM, when indicated [2,3,5], in the bed rest studies in which plasma 1,25-(OH) $_2$ D concentrations were decreased. Perhaps low plasma 25-OHD concentrations contribute, in part, to the decrease in plasma 1,25-(OH) $_2$ D concentrations during bed rest. Decrease in plasma 1,25-(OH) $_2$ D $_3$ concentration of S-HU rats, and not R-HU rats, might have resulted from the significantly lower plasma 25-OHD $_3$ concentration of S-HU rats, compared with that of R-HU rats. Plasma 25-OHD concentrations were not decreased, compared with baseline, in bed rest subjects [2–3,5,30–31], in hind limb unloaded Sprague-Dawley rats [21], and in R-HU rats in this and a previous report [9].

Plasma PTH concentrations were high in this experiment, perhaps a response to high salt intake [14]. Plasma PTH concentrations were not significantly different in S and R rats and were unaffected by hind limb unloading, as was previously reported for S and R rats fed 0.3% salt [9]. Plasma PTH concentrations have been reported to decrease [1,5–6,30–31] and to remain unchanged [2,4,37] as a result of bed rest. Plasma PTH concentrations were decreased, compared with baseline, in African-American men at day 7 of bed rest when salt intake was high, but not when salt intake was low [3]. Urinary calcium of S and S-HU rats was more than twice that of R and R-HU rats in this study on the effect of high salt intake. Loaded and unloaded S and R rats fed a low salt diet, however, excreted similar amounts of calcium [9]. We have previously demonstrated that female S rats excrete more calcium than female R rats during high salt intake [14].

Low vitamin D status is associated with increased risk for osteoporosis, muscle weakness, prostate and colorectal cancer, autoimmune diseases, cardiac hypertrophy, and hypertension [34,36,38]. The prevalence of sub-optimal vitamin D status in hospitalized patients is high [39,40]. Lower plasma 25-OHD $_3$ concentrations in S-HU rats, compared with R-HU rats, suggest that long-term bed rest or immobilization might be a risk factor for low vitamin D status in salt-sensitive individuals. R rats are also affected by salt, but to a lesser degree. Urinary 25-OHD $_3$ of 12.75 month-old R rats was one-third that of S rats when the rats were raised on a standard 1% salt diet [13]. We have recently demonstrated that feeding S rats cholecalciferol at a level five times that in the Harlan, Inc. diet optimized plasma 25-OHD $_3$ concentration during 3% salt intake [41], but did not ameliorate urinary loss of vitamin D metabolites. Vitamin D metabolites were detectable in the urine of 20 of 21 black and white female adolescents participating in a calcium balance study and urinary DBA was correlated with urinary sodium [42]. Clinical studies are needed to determine whether urinary loss of vitamin D metabolites is further increased by bed rest when participants ingest a typical diet, thus necessitating higher vitamin D intake and/or lower salt intake.

In summary, we have examined the response of Dahl S and R female rats to 28 days of hind limb unloading, while they were fed a 2% salt diet to mimic salt intake in the USA. Plasma 25-OHD $_3$ and 24,25-(OH) $_2$ D $_3$ concentrations of S-HU rats were significantly lower than that of R-HU rats. Urinary 25-OHD $_3$ and 24,25-(OH) $_2$ D $_3$ of S-HU rats were greater than that of S rats, but these metabolites were undetectable in the urine of R and R-HU rats. Plasma 1,25-(OH) $_2$ D $_3$ concentration of S-HU rats was half that of S rats, but was unchanged in R-HU rats, compared with R rats. The association of low plasma 25-OHD concentration with decrease in plasma 1,25-(OH) $_2$ D concentration of hind limb unloaded rats and of bed rest participants (published studies) suggests that low vitamin D status might be a risk factor for decrease in plasma vitamin D hormone concentration during long-term immobilization or bed rest.

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Fig. 1.

Plasma 25-hydroxycholecalciferol and 24,25-dihydroxycholecalciferol concentrations are decreased in hind limb unloaded Dahl salt-sensitive female rats fed 2% salt. Dahl salt-sensitive (S) and salt-resistant (R) female rats were fed a 2% salt diet and hind limb unloaded (HU) for 28 days as described in Section 2.2. Loaded S and R rats served as controls. Plasma (A) 25-hydroxycholecalciferol (25-OHD₃) and (B) 24,25-dihydroxycholecalciferol (24,25-(OH)₂D₃) concentrations at day 28 were measured by radioimmunoassay after pre-purification [9,12,24,41]. Values are means $\pm$ SEM, n = 6–8 rats per group. Statistical significance was evaluated by two-way ANOVA. Salt sensitivity effect on plasma 25-OHD₃, $P = 0.01$, and 24,25-(OH)₂D₃, $P < 0.001$, concentrations. HU effect on plasma 24,25-(OH)₂D₃, $P = 0.012$. ^bS-HU vs. R-HU, $P < 0.05$. ^dS vs. R, $P < 0.01$.

Fig. 2.

Urinary 25-hydroxycholecalciferol and 24,25-dihydroxycholecalciferol are increased by hind limb unloading of Dahl salt-sensitive female rats fed 2% salt. Dahl salt-sensitive (S) and salt-resistant (R) female rats were fed a 2% salt diet and hind limb unloaded (HU) for 28 days as described in Section 2.2. Loaded S and R rats served as controls. Urinary 25-hydroxycholecalciferol (25-OHD₃) and 24,25-dihydroxycholecalciferol (24,25-(OH)₂D₃) at day 21 (non-detectable for R rats) were measured by radioimmunoassay after pre-purification [9,12,24,41]. Values are means $\pm$ SEM, $n = 5-7$ rats per group. Statistical significance was evaluated by the Mann-Whitney rank sum test. ^a S vs. S-HU, $P < 0.05$.

Fig. 3.

Urinary 25-hydroxycholecalciferol binding activity and protein are increased by hind limb unloading of Dahl salt-sensitive and salt-resistant female rats fed 2% salt. Dahl salt-sensitive (S) and salt-resistant (R) female rats were fed a 2% salt diet and hind limb unloaded (HU) for 28 days as described in Section 2.2. Urinary (A) 25-hydroxycholecalciferol binding activity (DBA) and (B) protein at day 21 were measured as described in Section 2.4. Values are means $\pm$ SEM, $n = 6-8$ (protein) and $n = 3-7$ (binding activity). Statistical significance was evaluated by two-way ANOVA. Salt sensitivity effect on urinary binding activity and protein, $P < 0.001$. HU effect on urinary protein, $P < 0.001$. ^aS vs. S-HU, $P < 0.05$. ^bS-HU vs. R-HU, $P < 0.001$. ^dS vs. R, $P < 0.001$.

Fig. 4.

Plasma 1,25-dihydroxycholecalciferol concentrations are decreased in hind limb unloaded Dahl salt-sensitive female rats fed 2% salt. Dahl salt-sensitive (S) and salt-resistant (R) rats were fed a 2% salt diet and hind limb unloaded (HU) for 28 days as described in Section 2.2. Plasma 1,25-dihydroxycholecalciferol concentrations at day 28 were measured by radioimmunoassay as described in Section 2.3. Values are means $\pm$ SEM, $n = 4-6$ rats per group. Statistical significance was evaluated by two-way ANOVA. Interaction between salt sensitivity and HU, $P = 0.02$. ^aS vs. S-HU, $P < 0.01$. ^dS vs. R, $P = 0.02$.

Table 1

Dahl salt-sensitive female rats excrete more calcium into urine than Dahl salt-resistant female rats during 2% salt intake

Parameters	Loaded	Hind limb unloaded
Salt-Sensitive		
Plasma calcium (mM)	2.22 ± 0.05	2.28 ± 0.03
Plasma parathyroid hormone (pg/ml)	219 ± 54	287 ± 56
Urinary calcium (μmol/24 h)	45 ± 7 ^d	61 ± 6 ^b
Urinary sodium (mmol/24 h)	2.9 ± 0.3	3.8 ± 0.4
Urinary calcium/sodium (mmol/100 mmol)	1.6 ± 0.2	1.7 ± 0.2 ^b
Salt-Resistant		
Plasma calcium (mM)	2.28 ± 0.03	2.21 ± 0.05
Plasma parathyroid hormone (pg/ml)	350 ± 89	281 ± 66
Urinary calcium (μmol/24 h)	16 ± 2	23 ± 9
Urinary sodium (mmol/24 h)	2.9 ± 0.4	2.3 ± 0.7
Urinary calcium/sodium (mmol/100 mmol)	0.7 ± 0.1	0.9 ± 0.2

Dahl salt-sensitive (S) and salt-resistant (R) rats were fed a 2% salt diet and hind limb unloaded (HU) for 28 days as described in Section 2.2. Loaded S and R rats served as controls. Plasma parathyroid hormone (PTH) at day 28 was measured with a rat intact PTH ELISA kit as described in Section 2.5. Plasma calcium concentrations at day 28 and urinary calcium and sodium concentrations at day 21 were determined with an automated analyzer as described in Section 2.5. Values are means ± SEM, n = 5–8 rats per group. Statistical significance was evaluated by two-way ANOVA. Salt sensitivity effect on urinary calcium, $P < 0.001$, and urinary calcium/sodium, $P = 0.006$.

^b S-HU vs. R-HU, $P < 0.05$.

^d S vs. R, $P = 0.01$.