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High dietary cholecalciferol increases plasma 25-hydroxycholecalciferol concentration, but does not attenuate the hypertension of Dahl salt-sensitive rats fed a high salt diet

Myrtle Thierry-Palmer^{*}, Stacy Cephas, Farah F. Muttardy, and Ahmad Al-Mahmoud^a

Department of Microbiology, Immunology, and Biochemistry, 720 Westview Drive SW, Atlanta, GA 30310, USA

a Clinical Research Center, Morehouse School of Medicine, 720 Westview Drive SW, Atlanta, GA 30310, USA

Abstract

The Dahl salt-sensitive rat, a model for salt-induced hypertension, develops hypovitaminosis D during high salt intake, which is caused by loss of protein-bound vitamin D metabolites into urine. We tested the hypothesis that high dietary cholecalciferol (5- and 10-fold standard) would increase plasma 25-hydroxycholecalciferol (25-OHD₃) concentration (indicator of vitamin D status) of salt-sensitive rats during high salt intake. Salt-sensitive rats were fed 0.3% salt (low salt, LS), 3% salt (high salt, HS), 3% salt and 7.5 µg cholecalciferol/d (HS-D5), or 3% salt and 15 µg cholecalciferol/d (HS-D10) and sacrificed at week 4. Plasma 25-OHD₃ concentrations of the two groups of HS-D rats were similar to that of LS rats and more than twice that of HS rats. Urinary cholecalciferol metabolite content of HS-D rats was more than seven times that of HS rats. Systolic blood pressures of the hypertensive HS and HS-D rats did not significantly differ, whereas LS rats were not hypertensive. We conclude that high dietary cholecalciferol increases plasma 25-OHD₃ concentration, but does not attenuate the hypertension of salt-sensitive rats during high salt intake. Low salt intake may be necessary to both maintain optimal vitamin D status and prevent hypertension in salt-sensitive individuals.

Keywords

Salt sensitivity; 25-hydroxycholecalciferol; dietary cholecalciferol; hypertension; vitamin D

1. Introduction

The Dahl salt-sensitive rat is a widely studied genetic model of hypertension [1–2] that develops hypertension, renal hypertrophy, and hypovitaminosis D when fed a high salt diet [1–7]. Plasma 25-hydroxyvitamin D (25-OHD) concentration is an indicator of vitamin D status. Plasma 25-OHD concentrations of young salt-sensitive and Dahl salt-resistant rats are similar when the rats are fed a low salt diet (0.3% sodium chloride). High salt intake, however,

^{*}Corresponding author: Myrtle Thierry-Palmer, Ph.D., Professor of Biochemistry, Morehouse School of Medicine, 720 Westview Drive, SW, Atlanta, GA 30310-1495, Telephone: 404-752-1505, Fax: 404-752-1179, E-mail: mthierry-palmer@msm.edu.

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causes significant decreases in plasma 25-OHD concentration of salt-sensitive rats [3–7]. Blood pressure was shown to be directly correlated and plasma 25-OHD concentration indirectly correlated with the number of days that young salt-sensitive rats were fed an 8% salt diet [3,6] and an inverse correlation was demonstrated between blood pressure and plasma 25-OHD concentration [3,6,7]. A high salt diet, however, had no significant effect on blood pressure or plasma 25-OHD concentration of young salt-resistant rats. The low plasma 25-OHD concentrations of salt-sensitive rats during high salt intake were demonstrated to be caused in large part by loss of protein-bound 25-OHD into urine [8,9].

Elderly hypertensive females with low plasma renin activity, characteristic of salt-induced hypertension, have been demonstrated to have lower plasma 25-OHD and 24,25-dihydroxyvitamin D (24,25-(OH)₂D) concentrations, compared with normotensive elderly and young females [10]. African-Americans have been demonstrated to have significantly lower mean plasma 25-OHD concentrations compared with Caucasian-Americans in several studies involving both males and females and in two reports [11,12] based on the third National Health and Nutrition Examination Survey. Decreased synthesis of cholecalciferol from 7-dehydrocholesterol in the skin is thought to be one mechanism for lower mean plasma 25-OHD concentrations in darkly pigmented individuals [13,14]. Salt sensitivity and hypertension are more prevalent in the African-American population than in the Caucasian-American population [15,16]. Thierry-Palmer et al. [8,9] have suggested that low vitamin D status in salt-sensitive individuals may result from loss of vitamin D metabolites into urine, in addition to low dietary intake and/or decreased skin synthesis. Studies indicate that salt intake in the USA is high [17,18] and can be as much as three times the recommended maximum of 100 mmol/d [18]. Additionally, recent studies have indicated that for the general population the dietary vitamin D intake necessary to maintain optimal plasma 25-OHD concentration is considerably higher than that currently recommended [19–24].

An important question is whether high dietary vitamin D might compensate for loss of vitamin D metabolites into the urine of salt-sensitive rats during high salt intake. Previously, exogenous 25-OHD₃, administered via osmotic pumps for 15 days, did not increase plasma 25-OHD₃ concentration or decrease the blood pressure of young salt-sensitive rats fed 8% salt [7]. An 8% salt diet is excessively high and salt-sensitive rats fed this amount of salt begin to die at week 4. We have tested the hypothesis that high dietary vitamin D would compensate for the vitamin D metabolite loss of salt-sensitive rats fed 3% salt (three times standard, designed to mimic high salt intake in humans).

2. Materials and Methods

2.1. Animals and diets

The Animal Care and Use Committee at Morehouse School of Medicine 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.

Dahl salt-sensitive (SS/Jr) female rats (four to five week old, 100–110 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and housed in a room with 12 h light - dark cycles. The rats were fed diets prepared by Harlan Teklad (Madison, WI). All rats were fed a diet containing 0.3 % salt (3 g salt/kg food) during one week of acclimation. They were then divided into groups and fed 0.3% salt (low salt, LS), 3% salt (high salt, HS), 3% salt and 7.5 µg cholecalciferol/d (HS-D5), or 3% salt and 15 µg cholecalciferol/d (HS-D10) for four weeks and sacrificed. The 0.3% and 3% salt diets in this study provided 0.125 µg (5 IU) cholecalciferol/g food, a value established by the vendor. In comparison, Purina LabDiet 5001 (PMI Nutrition International, Inc., Brentwood, MO) contains 0.112 µg (4.5 IU) cholecalciferol/g food.

The rats were placed in metabolic cages 24 h prior to the start of the dietary regimen and on day 7 of each week for 24 h urine collection. Indirect blood pressure was determined prior to the initiation of the diets and weekly by tail-cuff plethysmography (Harvard Apparatus, Holliston, MA), using the median of three or four successive measurements. Body weights were determined before the diets (basal) and weekly thereafter. At sacrifice, the rats were anaesthetized with ketamine:xylazine (90:10 mg/100 g body weight) and blood was drawn, by heart puncture, into heparinized tubes. Plasma was obtained by centrifuging the blood at 1,500 g for 10 min and was stored at -80°C until analysis.

2.2. Cholecalciferol metabolite concentrations

25-Hydroxy[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 [25]. Synthetic 25-hydroxycholecalciferol (25-OHD₃) and 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃), purchased from ICN Pharmaceuticals (Irving, CA), served as standards. For the assay of 25-OHD₃, the radio-labeled metabolite (800 dpm) was added to the plasma and urine samples prior to purification to determine recovery.

Plasma (0.5 ml) and urine (1 ml) were purified using a dichloromethane/methanol liquid-liquid extraction [3], followed by solid-phase extraction on silica cartridges [26]. Fractions 1 and 2 from the solid phase extraction of the plasma samples were combined and purified by HPLC (0.46 × 25 cm Zorbax-Sil column with 95/5 v/v dichloromethane-isopropanol, 1 ml/min) for the collection of the 25-OHD₃ peaks. Radioimmunoassay kits (DiaSorin, Stillwater, MN) were used to assay the plasma 25-OHD₃ peaks. Standard curves were constructed using 25-OHD₃ (4.7, 9.4, 18.8, 37.6, 75, 150, and 300 pg/tube). The combined fractions 1 and 2 from the solid phase extraction of the urine samples, containing 25-OHD₃ and 24,25-(OH)₂D₃, were measured by radioimmunoassay kits for 25-OHD (DiaSorin), as above for plasma. In a study of female S rats fed 2% salt (unpublished), urinary 25-OHD₃ content was twice urinary 24,25-(OH)₂D₃ content, reflective of the 25-OHD₃/24,25-(OH)₂D₃ ratio in the plasma of these rats. 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.

2.3. Urinary 25-hydroxycholecalciferol binding activity and protein

Urine was tested for specific binding to 25-OHD₃ by previously published methods [8,9]. Urine samples were diluted with 0.1 M boric acid buffer, pH 8.6, containing γ -globulin. Aliquots of the diluted urine 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. A dextran-coated charcoal preparation (0.2 ml) was used to separate bound and free cholecalciferol metabolites. Duplicate incubations were made for each rat. Binding in the presence of 200-fold excess unlabeled 25-OHD₃ (non-specific binding) was subtracted from total binding to obtain specific binding. Binding activity (nmol/l × h) was multiplied by the 24 h urine volume to obtain nmol/h. Urinary protein was measured by a bicinchoninic acid protein assay kit (Sigma-Aldrich, St. Louis, MO.), using an automated plate reader (Spectra Max 250, Molecular Devices, Sunnyvale, CA).

2.4. Parathyroid hormone, calcium, and sodium

Plasma parathyroid hormone (PTH) concentration was measured with a rat intact PTH ELISA kit (Immutopics, San Clemente, CA), using an automated plate reader (Spectra Max 250, Molecular Devices, Sunnyvale, CA). 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 excretion/24 h.

2.5. Statistical analyses

A mean $\pm$ SEM was calculated for each group. Statistical significance ($P < 0.05$) was evaluated for weekly determinations (body weight, blood pressure, urinary calcium) by a four (diets) by four (weeks) mixed ANOVA test and the Greenhouse-Geisser corrected F or Tukey test. Statistical significance for parameters examined at week 4 was determined by one way ANOVA and the Holm-Sidak or Tukey method. Linear regression analyses were conducted with SigmaStat software (SPSS, Inc.).

3. Results

Body weights of the four groups of salt-sensitive rats, determined weekly, ranged from 214 ± 3 g to 222 ± 2 g at week 4. Feeding the high salt diet to salt-sensitive rats for 28 days lowered plasma 25-OHD₃ concentration by 36%, compared with feeding the low salt diet (Fig. 1A). Mean plasma 25-OHD₃ concentrations of HS-D5 and HS-D10 rats were more than twice that of HS rats. This comparison, however, excludes 31% of the HS-D rats, with plasma 25-OHD₃ concentrations more than twice the mean. Urinary cholecalciferol metabolite content of HS rats was approximately twice that of LS rats. Mean urinary cholecalciferol metabolite content of the two HS-D groups was more than seven times that of HS rats (Fig. 1B).

Protein and urinary 25-OHD₃ binding activity were significantly higher in the urine of HS rats than in that of LS rats (Table 1). Urinary 25-OHD₃ binding activity was significantly higher in the urine of HS-D5 rats than in that of HS and HS-D10 rats. Urinary cholecalciferol metabolite content was directly associated with urinary protein ($r = 0.83$, $P < 0.001$, $n = 16$) of LS and HS rats. Addition of the two HS-D groups to the plot decreased the correlation ($r = 0.41$, $P = 0.02$, $n = 31$). Urinary cholecalciferol metabolite content was directly associated with urinary 25-OHD₃ binding activity ($r = 0.65$, $P < 0.001$, $n = 32$) of LS, HS, and HS-D rats.

Plasma 1,25-(OH)₂D₃ and PTH concentrations did not significantly differ between the four dietary regimens, but there was a tendency toward lower concentrations of the two hormones in HS-D rats, compared with HS rats (Fig. 2). High salt intake caused an increase in urinary calcium, compared with low salt intake (Fig. 3). HS-D rats excreted significantly more calcium overall than HS rats. HS-D10 rats excreted significantly more calcium than HS-D5 rats. At week 4, urinary calcium of the HS-D rats was similar to that of the HS rats.

Urinary sodium (Table 1) increased in response to high salt intake and was unaffected by high dietary cholecalciferol during high salt intake. Blood pressure of HS rats was significantly higher than that of LS rats during the four week study ($P < 0.001$) and similar to that of HS-D rats. Blood pressure was significantly increased by time ($P < 0.001$). Week 4 data are shown for blood pressure (Table 1). Plasma 25-OHD concentration was inversely correlated with blood pressure ($r = -0.76$, $P = 0.047$, $n = 7$) of LS and HS rats. The relationship was lost when values for HS-D rats were added to the plot.

4. Discussion

Dahl salt-sensitive and salt-resistant rats exhibit similar plasma 25-OHD, 1,25-(OH)₂D, and 24,25-(OH)₂D concentrations when fed a low salt diet, suggesting that vitamin D metabolism is normal in salt-sensitive rats [3,5,6]. We demonstrated that loss of protein-bound vitamin D metabolites into urine is a primary cause of hypovitaminosis D when salt-sensitive rats are fed a high salt diet [8,9]. We report here that high dietary cholecalciferol increased plasma 25-OHD₃ concentrations of young Dahl salt-sensitive rats fed 3% salt. The salt content in this experiment was three-fold that of a standard diet to mimic salt intake of humans. In a recent study, the salt intake of African-American men, assessed by urinary sodium, was as high as three times the recommended maximum [18]. The urine of HS-D rats had more than seven

times the cholecalciferol metabolite content of that of HS rats, suggesting that high dietary cholecalciferol increased synthesis of 25-OHD₃, without attenuating the pathology affecting vitamin D metabolite loss. Since plasma and urinary cholecalciferol metabolite content were similar in HS-D5 and HS-D10 rats, we conclude that the cholecalciferol 25-hydroxylases were saturated at $\geq 7.5 \mu\text{g}$ cholecalciferol/d in the majority of these rats. There was, however, variability in plasma 25-OHD₃ concentration; 31% of the rats had plasma 25-OHD₃ concentrations (not included) greater than twice the mean.

We identified both 25-OHD and 25-OHD binding activity in the urine of Dahl S rats [8,9] and concluded that protein-bound vitamin D metabolites were lost into the urine. The urine was shown to have primarily albumin and also vitamin D binding protein (DBP). In this study, urinary protein content was similar for HS and HS-D rats, but binding activity was higher in the urine of HS-D5 rats than in the urine of HS and HS-D10 rats. The greater loss of cholecalciferol metabolites by HS-D rats (seven times that of HS rats) appears to result in large part from a greater amount of cholecalciferol metabolites bound to excreted protein. The inability of 25-OHD₃, administered via osmotic pumps, to increase plasma 25-OHD₃ concentration in an earlier study [7] may have been due to its greater loss into urine, compared with dietary cholecalciferol in this experiment, as a result of greater binding to plasma proteins and higher salt intake.

We found no attenuating effects of high dietary cholecalciferol -- and normal plasma 25-OHD₃ concentrations -- on the proteinuria of salt-sensitive rats fed a high salt diet. Johnson et al. [27] have suggested that kidney damage precedes salt-induced hypertension. In nephrotic syndrome, urinary 25-OHD is accompanied by urinary DBP, proteinuria, and decreased plasma 25-OHD concentration [28–30]. Saha [31] demonstrated an inverse relationship between serum 25-OHD and protein in patients with heavy proteinuria. In this study, we demonstrate a direct correlation between urinary cholecalciferol metabolite content and urinary protein or urinary 25-OHD₃ binding activity of LS, HS, and HS-D rats. A megalin-cubilin complex provides an endocytic pathway for reabsorption of 25-OHD-DBP by kidney proximal tubules [32,33]. Although the status of the megalin-cubilin system of salt-sensitive rats has not yet been determined, glomerulosclerosis was demonstrated (assessed by kidney histology) in the rats fed 3% salt (unpublished), suggesting renal damage as a primary cause for the loss of 25-OHD₃ binding protein(s) and protein in general. Nagase et al. [34] have indicated that podocyte injury underlies the glomerulopathy of Dahl salt-hypertensive rats. Podocytes are epithelial cells covering the glomerular capillaries and are involved in filtering proteins.

High dietary cholecalciferol during high salt intake did not attenuate the hypertension of salt-sensitive rats. We have previously demonstrated an inverse relationship between plasma 25-OHD₃ concentration and blood pressure in young salt-sensitive rats [3,6,7] fed 8% salt and standard vitamin D₃ (1.5 μg cholecalciferol/d). We demonstrate this relationship again for LS and HS rats fed standard cholecalciferol. This inverse relationship between plasma 25-OHD concentration and blood pressure was perhaps caused by the inability of salt-sensitive rats to synthesize sufficient 25-OHD₃ to compensate for urinary loss. The amount of 25-OHD₃ in the 24 h urine (assumed to be two-thirds of the urinary cholecalciferol metabolite content) of HS rats at week 4 was approximately 5% of the amount in plasma. This study indicates that five times the standard cholecalciferol intake is sufficient to compensate for 25-OHD₃ loss when rats are fed 3% salt (three-times the standard salt intake) for 28 days. Spontaneously hypertensive rats have normal plasma 25-OHD concentrations [3,35,36], so decreased plasma 25-OHD concentration may be specific to salt-induced hypertension. Studies on humans have indicated an inverse relationship between plasma 25-OHD concentration or UVB light exposure and blood pressure [14,37]. The data presented here suggest that in the case of salt-induced hypertension, low vitamin D status may be secondary to hypertension and protein loss.

We previously demonstrated that vitamin D-depleted and vitamin D-replete salt-sensitive rats had similar blood pressures when fed a high salt diet [7].

Hyperparathyroidism is secondary to low vitamin D status [5,9,38]. Vieth et al. [38] showed that vitamin D supplementation to Wistar rats resulted in higher serum 25-OHD and lower serum PTH and 1,25-(OH)₂D concentrations. Plasma 1,25-(OH)₂D₃ and PTH concentrations of HS-D rats were slightly, but not significantly, lower than that of HS rats. High salt intake (8%) has been shown to significantly increase plasma PTH and 1,25-(OH)₂D concentrations of female salt-sensitive rats [5] and may have attenuated the lowering of plasma PTH and 1,25-(OH)₂D₃ concentrations by high dietary cholecalciferol in this study. Additionally, mean plasma 25-OHD₃ concentrations of the four dietary groups were not markedly different, in contrast to 8% salt intake [5].

The calciuric effect of sodium, exhibited by salt-sensitive rats in this and other studies [5,39], is well known. Nordin et al. [40] have reported a urinary calcium/sodium ratio of 1 mmol/100 mmol for human subjects, when sodium is the determinant of calcium excretion. HS-D rats excreted significantly more calcium than HS rats. Urinary calcium of HS-D rats was dose dependent, although plasma 1,25-(OH)₂D₃ and PTH concentrations were not increased in these rats. HS-D rats ceased to excrete excess calcium at week 4, compared with HS rats, suggesting some degree of adaptation to high dietary cholecalciferol with regard to calcium excretion.

The prevalence of salt sensitivity in 18–23 year-old Caucasian-Americans was 18%, compared with 37% in age-matched African-Americans [15]. The prevalence of salt sensitivity in the hypertensive USA population has been estimated to be approximately 50% [16]. Since we have demonstrated 25-OHD binding activity and vitamin D metabolites in the urine of African-American and Caucasian-American female adolescents [41] and in that of healthy young African-American men (unpublished work), urinary 25-OHD loss is another potential mechanism for low vitamin D status. Low vitamin D status is associated with osteoporosis, decreased muscle strength, prostate cancer, cardiac hypertrophy, and immune dysfunction [19–20]. Studies have indicated that the dietary vitamin D intake necessary to maintain optimal plasma 25-OHD concentration (approximately 75 nM) in humans can be as high as 5–10 times that currently recommended [19–24]. Saadi et al. [42] provided 2,000 IU/d ergocalciferol (vitamin D₂) for 3 months to young nulliparous Arab and East Indian women residing in the United Arab Emirates. This level of supplementation (10-times the current RDA for persons under 50 years-old) raised serum 25-OHD concentration to ≥50 nM in only 36% of the women and in only 50% of the women with baseline serum 25-OHD concentrations ≥20 nM. Perhaps different levels of vitamin D metabolite excretion might explain the heterogeneity. In this study of Dahl S rats fed a high salt diet, 10-times the standard cholecalciferol intake increased mean plasma 25-OHD₃ concentrations one-fold, but increased mean urinary cholecalciferol metabolite content six-fold.

In summary, we demonstrate that dietary cholecalciferol levels five and 10 times standard increased plasma 25-OHD₃ concentration, but did not attenuate the hypertension of Dahl salt-sensitive rats fed a 3% salt diet for 4 weeks. Salt-sensitive rats fed high dietary cholecalciferol and high salt excreted more calcium during the first 3 weeks of supplementation than salt-sensitive rats fed high salt only. Although high dietary vitamin D during high salt intake can improve vitamin D status, these data suggest that low salt intake may be necessary to both maintain optimal vitamin D status and prevent hypertension in persons who are salt-sensitive.

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References

1. Dahl LK. Salt and hypertension. *Am J Clin Nutr* 1972;25:231–244. [PubMed: 5009786]
2. Rapp JP. Development of inbred Dahl salt-sensitive and inbred Dahl salt-resistant rats. *Hypertension* 1987;9(suppl 1):I21–I23.
3. Thierry-Palmer M, Carlyle KS, Williams MD, Tewolde T, Caines-McKenzie S, Bayorh MA, Emmett N, Harris-Hooker SA, Sanford GL, Williams EF. Plasma 25-hydroxyvitamin D concentrations are inversely associated with blood pressure of Dahl salt-sensitive rats. *J Steroid Biochem Mol Biol* 1998;66:255–261. [PubMed: 9744523]
4. Wu X, Vieth R, Milojevic S, Sonnenberg H, Melo LG. Regulation of sodium, calcium and vitamin D metabolism in Dahl rats on a high-salt/low potassium diet: genetic and neural influences. *Clin Exper Pharm Physiol* 2000;27:378–383.
5. Faqi AS, Sherman DD, Wang M, Pasquali M, Bayorh MA, Thierry-Palmer M. The calciuric response to dietary salt of Dahl salt-sensitive and salt-resistant female rats. *Am J Med Sci* 2001;322:333–338. [PubMed: 11780690]
6. Thierry-Palmer M, Tewolde TK, Forté C, Wang M, Bayorh MA, Emmett NL, White J, Griffin K. Plasma 24, 25-dihydroxyvitamin D concentration of Dahl salt-sensitive rats decreases during high salt intake. *J Steroid Biochem Mol Biol* 2002;80:315–321. [PubMed: 11948016]
7. Thierry-Palmer M, Tewolde TK, Wang M, Carlyle KS, Forté C, Bayorh MA, Emmett NL, Williams EF. Exogenous 25-hydroxycholecalciferol does not attenuate salt-induced hypertension. *J Steroid Biochem Mol Biol* 1998;67:193–199. [PubMed: 9879978]
8. Thierry-Palmer M, Doherty A, Bayorh MA, Griffin K. Dahl salt-sensitive rats excrete 25-hydroxyvitamin D into urine. *J Nutr* 2003;133:187–190. [PubMed: 12514288]
9. Thierry-Palmer M, Cephas S, Sayavongsa P, Doherty A, Arnaud SB. Dahl salt-sensitive rats develop hypovitaminosis D and hyperparathyroidism when fed a standard diet. *Bone* 2005;36:645–653. [PubMed: 15784187]
10. Imaoka M, Morimoto S, Kitano S, Fukuo F, Ogihara T. Calcium metabolism in elderly hypertensive patients: possible participation of exaggerated sodium, calcium and phosphate excretion. *Clin Exp Pharmacol Physiol* 1991;18:631–641. [PubMed: 1835682]
11. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone* 2002;30:771–777. [PubMed: 11996918]
12. Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, Allen C, Dougherty C, Gunter EW, Bowman BA. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr* 2002;76:187–192. [PubMed: 12081833]
13. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesize vitamin D₃. *Lancet* 1982;1:74–76. [PubMed: 6119494]
14. Rostand SG. Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension* 1997;30:150–156. [PubMed: 9260973]
15. Falkner B, Kushner H. Effect of chronic sodium loading on cardiovascular response in young blacks and whites. *Hypertension* 1990;15:36–43. [PubMed: 2295513]
16. Weinberger MH, Miller JZ, Luft FC, Grim CE, Fineberg NS. Definitions and characteristics of sodium sensitivity and blood pressure resistance. *Hypertension* 1986;8(suppl II):II127–II134. [PubMed: 3522418]
17. Sacks FM, Svetkey LP, Vollmer VM, Appel LJ, Brey GA, Harsha D. DASH Sodium Collaborative Research Group. Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (DASH) diet. *N Engl J Med* 2001;344:3–10. [PubMed: 11136953]
18. Thierry-Palmer M, Cephas S, Pasquali M, Schwartz E, Sayavongsa P, Umeakunne K, Lapu-Bula R, Ofili E. The vitamin D endocrine system of African-American men during head-down tilt bed rest. *J Grav Physiol* 2005;12:1–9.
19. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 2004;79:362–371. [PubMed: 14985208]

20. Vieth R, Bischoff-Ferrari H, Boucher BJ, Dawson-Hughes B, Garland CF, Heaney RP, Holick MF, Hollis BW, Lamberg-Allardt C, McGrath JJ, Norman AW, Scragg R, Whiting SJ, Willett WC, Zitterman A. The urgent need to recommend an intake of vitamin D that is effective. *Am J Clin Nutr* 2007;85:649–650. [PubMed: 17344484]
21. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr* 2003;77:204–210. [PubMed: 12499343]
22. Weaver CM, Fleet JC. Vitamin D requirements: current and future. *Am J Clin Nutr* 2004;80(suppl 6):1735S–1739S. [PubMed: 15585797]
23. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006;84:18–28. [PubMed: 16825677]
24. Hollis BW, Wagner CL. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. *Am J Clin Nutr* 2004;80(suppl 6):1752S–1758S. [PubMed: 15585800]
25. Jones G. Chromatographic separation of 24(R), 25-dihydroxyvitamin D₃ and 25-hydroxyvitamin D₃-26, 23-lactone using a cyano-bonded phase packing. *J Chromatogr* 1983;276:69–75.
26. Reinhardt TA, Hollis BW. 1,25-dihydroxyvitamin D microassay employing radioreceptor techniques. *Methods in Enzymol* 1986;123:176–185. [PubMed: 3010038]
27. Johnson RJ, Herrera-Acosta GF, Schreiner GF, Rodriguez-Iturbe B. Subtle acquired renal injury as a mechanism of salt-sensitive hypertension. *N Engl J Med* 2002;346:913–923. [PubMed: 11907292]
28. Freundlich M, Bourgoignie JJ, Zilleruelo G, Abitbol C, Canterbury JM, Strauss J. Calcium and vitamin D metabolism in children with nephrotic syndrome. *J Pediatr* 1986;108:383–387. [PubMed: 3485195]
29. Khamiseh G, Vaziri ND, Oveisi F, Ahmadnia MR, Ahmadnia L. Vitamin D absorption, plasma concentration and urinary excretion of 25-hydroxyvitamin D in nephrotic syndrome. *Proc Soc Expt Biol Med* 1991;196:210–213.
30. Schmidt-Gayk H, Grawunder C, Tschöpe W, Schmitt W, Ritz E, Pietsch V, Andrassy K, Bouillon R. 25-Hydroxy-vitamin-D in nephrotic syndrome. *Lancet* 1997;2:105–108. [PubMed: 69193]
31. Saha H. Calcium and vitamin D homeostasis in patients with heavy proteinuria. *Clin Nephrol* 1994;41:290–296. [PubMed: 8050209]
32. Nykjaer A, Dragun D, Walther D, Vorum H, Jacobsen C, Herz J, Melsen F, Christensen EI, Willnow TE. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH)vitamin D₃. *Cell* 1999;96:507–515. [PubMed: 10052453]
33. Nykjaer A, Fyfe JC, Kozyraki R, Leheste JR, Jacobsen C, Nielsen MS, Verroust PJ, Aminoff M, de la Chapelle A, Moestrup SK, Ray R, Gliemann J, Willnow TE, Christensen EI. Cubilin dysfunction causes abnormal metabolism of the steroid hormone 25(OH) vitamin D₃. *Proc Natl Acad Sci USA* 2001;98:13895–13900. [PubMed: 11717447]
34. Nagase M, Shibata S, Yoshida S, Nagase T, Gotoda T, Fujita T. Podocyte injury underlies the glomerulopathy of Dahl salt-hypertensive rats and is reversed by an aldosterone blocker. *Hypertension* 2006;47:1084–1093. [PubMed: 16636193]
35. Kurtz TW, Portale AA, Morris RC Jr. Evidence for a difference in vitamin D metabolism between spontaneously hypertensive and Wistar-Kyoto rats. *Hypertension* 1986;8:1015–1020. [PubMed: 3770864]
36. Lucas PA, Brown BC, Drueke T, Lacour B, Mertz JA, McCarron DA. Abnormal vitamin D metabolism, intestinal calcium transport, and bone calcium status in the spontaneously hypertensive rat compared with its genetic control. *J Clin Invest* 1986;78:221–227. [PubMed: 3755141]
37. Krause R, Buhning M, Hoffenmuller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. *Lancet* 1998;352:709–710. [PubMed: 9728997]
38. Vieth R, Milojevic S, Peltekova V. Improved cholecalciferol nutrition in rats is noncalcemic, suppresses parathyroid hormone and increases responsiveness to 1, 25-dihydroxycholecalciferol. *J Nutr* 2000;130:578–584. [PubMed: 10702588]
39. Thierry-Palmer M, Sherman DD, Emmett NL, Wang M, Bayorh MA, Donkoh N. The calciuric response to dietary salt of Dahl salt-sensitive and salt-resistant male rats. *Am J Med Sci* 2001;321:342–347. [PubMed: 11370798]

40. Nordin BE, Need AG, Morris HA, Horowitz M. The nature and significance of the relationship between urinary sodium and urinary calcium in women. *J Nutr* 1993;123:1615–1622. [PubMed: 8360790]
41. Thierry-Palmer M, Henderson V, El Hammali R, Cephas S, Palacios C, Martin B, Weaver CM. Black and white female adolescents lose vitamin D metabolites into urine. *Am J Med Sci* 2008;335:278–283. [PubMed: 18414066]
42. Saadi HF, Dawodu A, Afandi BO, Zayed R, Benedict S, Nagelkerke N. Efficacy of daily and monthly high-dose calciferol in vitamin D-deficient nulliparous and lactating women. *Am J Clin Nutr* 2007;85:1565–1571. [PubMed: 17556694]

Figure 1.

High dietary cholecalciferol during high salt intake increases plasma 25-hydroxycholecalciferol (25-OHD₃) and urinary cholecalciferol metabolite content of Dahl salt-sensitive rats. Values are means $\pm$ SEM, $n = 4 - 7$ (plasma 25-OHD₃), $n = 8$ (urinary cholecalciferol metabolite content). Cholecalciferol: 7.5 μ g (D5) or 15 μ g (D10)/d. Overall effect of diet on plasma 25-OHD₃, $P = 0.02$, and urinary cholecalciferol metabolite content, $P < 0.001$. ^cLow salt vs. high salt-D, $P < 0.05$. ^bHigh salt vs. high salt-D5, $P < 0.05$. ^dHigh salt vs. high salt-D10, $P < 0.05$. Plasma 25-OHD₃ concentrations that were greater than twice the mean have been excluded from the plotted data (4 HS-D5, 1 HS-D10).

Figure 2. Plasma 1,25-dihydroxycholecalciferol and parathyroid hormone concentrations of Dahl salt-sensitive rats fed high dietary cholecalciferol during high salt intake. Values are means $\pm$ SEM, $n = 6 - 8$. Cholecalciferol: 7.5 μ g (D5) or 15 μ g (D10)/d.

Figure 3.

High dietary cholecalciferol during high salt intake increases urinary calcium of Dahl salt-sensitive rats. Values are means $\pm$ SEM, $n = 7 - 8$. Cholecalciferol: 7.5 μg (D5) or 15 μg (D10)/d. Overall effect of diet, $P < 0.001$. Difference between any two diets, $P < 0.001$. ^bHigh salt vs. high salt-D5, $P < 0.01$. ^dHigh salt vs. high salt-D10, $P < 0.001$.

Systolic blood pressure and urinary sodium, protein, and 25-OHD₃ binding activity of Dahl salt-sensitive rats fed low salt (LS), high salt (HS), and high salt plus cholecalciferol (HS-D) at week 4

Parameters	LS	HS	HS-D5	HS-D10
Systolic blood pressure (mm Hg)	125 ± 2 ^a (4)	168 ± 3 (4)	180 ± 4	166 ± 3 (4)
Urinary sodium (mmol/24 h)	0.69 ± 0.08 ^a	5.7 ± 0.2	5.0 ± 0.4	5.7 ± 0.3
Urinary protein (g/24 h)	0.15 ± 0.02 ^a	0.29 ± 0.03	0.31 ± 0.03	0.31 ± 0.02 (7)
Urinary 25-OHD ₃ binding (nmol/h)	0.15 ± 0.02 ^a	0.45 ± 0.05	0.75 ± 0.08 ^{b,e}	0.53 ± 0.1

Values are means ± SEM, n = 8, except when otherwise indicated in parentheses. Vitamin D₃ (cholecalciferol): 7.5 µg (D5) or 15 µg (D10)/d.

^aLS vs. HS or HS-D, *P* < 0.01.
^bHS vs. HS-D5, *P* < 0.05.
^eHS-D5 vs. HS-D10, *P* < 0.05.