Published in final edited form as:

Nutr Metab Cardiovasc Dis. 2010 November ; 20(9): 641–646. doi:10.1016/j.numecd.2009.07.009.

VITAMIN D DEFICIENCY IN THE SPONTANEOUSLY HYPERTENSIVE HEART FAILURE [SHHF] PRONE RAT

Robert Przybylski, Sylvia Mccune, Ph.D.², Bruce Hollis, Ph.D.³, and Robert U. Simpson, Ph.D.¹

¹Department Of Pharmacology, University Of Michigan Medical School, 1301 MSRB III, Box 632, Ann Arbor, MI 48104-0632

²Department of Integrative Physiology, University of Colorado at Boulder, 354 UCB, Clare Small 114, Boulder, CO 80309-0354

³Department of Pediatrics, Medical University of South Carolina, 173 Ashley Ave, CRI, RM 313, Charleston, SC 29425-8510

Abstract

Background and Aims—Vitamin D deficiency has been associated with the etiology and pathogenesis of heart disease including congestive heart failure. We previously observed cardiac hypertrophy in vitamin D deficient rats and vitamin D-receptor knockout mice. These studies indicate that the absence of vitamin D-mediated signal transduction and genomic activation results in increased sensitivity of the heart to ionotropic stimuli and cardiomyocyte hypertrophy. This study's aim is to investigate the relationship between vitamin D status and the heart failure phenotype in the rat.

Methods and Results—Vitamin D status was assessed by measuring 25-hydroxyvitamin D levels and related to heart weight in young, middle-aged and aging spontaneously hypertensive, heart failure-prone (SHHF) rats. We also measured the effects of the vitamin D hormone, 1,25(OH)₂D₃, on cardiac function in SHHF rats. Cardiac hypertrophy in this model of the failing heart increased with age and related to decreasing vitamin D status. Vitamin D deficiency presented after cardiac hypertrophy was first observed. Additionally, we found that 1,25(OH)₂D₃ treatment between 4.0–7.0 months of age prevented cardiac hypertrophy and permits decreased workload for the heart while allowing adequate blood perfusion and pressure, resulting in reduced cardiac index.

Conclusions—Our findings suggest that low vitamin D status is associated with the progression and final terminal phase of the heart failure phenotype and not with initial heart hypertrophy. Also, we report that in the vitamin D sufficient SHHF rat, 1,25(OH)₂D₃ treatment provided protection against the progression of the heart failure phenotype.

Keywords

Vitamin D; Heart Failure; SHHF Rat

© 2009 Elsevier B.V. All rights reserved.

Correspondence: Robert U. Simpson, 1301 MSRB III, Box 632 University of Michigan Medical School, Ann Arbor, MI 48104-0632, 734 763-3255; Fax: 734 763-4450. robsim@umich.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Recent studies have found vitamin D deficiency to be epidemic in proportion [1,2], with an estimated 50% of North America's older population having insufficient vitamin D status [3]. In addition, researchers have linked vitamin D deficiency to a number of conditions including all-cause mortality [4]. The observation that the majority of human cells contain vitamin D receptors initiated research into vitamin D and its expanding relation to normal physiology and morbidity [2]. Conditions recently linked to vitamin D deficiency include cancer [5], immune system dysfunction [6], hypertension [7], diabetes [8,9] and cardiovascular diseases [10,11] including heart failure.

Heart failure is a complex disease in which the capability of the ventricle to either eject, fill, or both is reduced at normal physiological filling pressures and can result in an inability to carry out routine activities without fatigue or dyspnea [12]. In 2005 there were approximately 5,300,000 known cases of heart failure in the US alone and nearly 300,000 US deaths were attributed to heart failure as either the underlying or secondary cause. While heart failure treatment has improved in recent years, the prognosis for heart failure patients remains grim, as 8 in 10 men and 7 in 10 women under the age of sixty-five will succumb to heart failure within eight years of being diagnosed with the disease [13].

Low vitamin D status affects heart structure and function and this relationship has clinical relevance [14–16]. One recent report found that on average congestive heart failure (CHF) patients had 34% lower 25(OH)D₃ levels than age and sex matched controls [14]. Prior to this clinical observation, studies from our lab demonstrated that vitamin D deficiency alters myocardial function, morphology, and ECM of rats [18,19]. Animal studies also revealed that 1,25(OH)₂D₃ affects two processes central to cardiomyocyte function. First, 1,25(OH)₂D₃ deficiency was shown to alter Ca²⁺ handling resulting in increased sensitivity of the heart to inotropic stimuli and second it influences remodeling of the heart by increasing heart size and collagen content [17,18]. More recently it was shown that ablation of the vitamin D receptor (VDR) results in profound changes in heart structure and that the VDR knockout (VDRKO) mouse phenotype was characterized by cardiac hypertrophy and fibrosis [19–21].

Humans obtain vitamin D₂ and vitamin D₃ through diet and can produce vitamin D₃ through exposure to sunlight via cutaneous UVB-induced synthesis [22–24]. These forms of vitamin D are hydroxylated in the liver to produce the major circulating form of vitamin D, 25-hydroxyvitamin D (25(OH)D₃), which is representative of vitamin D status [2]. 25-hydroxyvitamin D is ultimately converted by the kidney to 1,25-dihydroxyvitamin D (1,25(OH)₂D₃), which is the biologically active form [2]. It is difficult to acquire vitamin D from dietary sources and these sources account for just 10–20% of the vitamin D required by humans [10], leaving supplementation or exposure to direct sunlight as the primary source of vitamin D.

Biochemical and physiological changes observed in spontaneously hypertensive, heart failure-prone (SHHF) rats mimic changes seen in human heart failure patients, suggesting the SHHF rat makes a relevant animal model for human heart failure [25,26]. SHHF rats are hypertensive with associated cardiac hypertrophy within their first year and progress to the heart failure phenotype within twenty-four months [25–27].

In this study we examined the relationship between the progression of the heart failure phenotype and vitamin D status in SHHF rats and separately assessed the effects of 1,25(OH)₂D₃ treatment on cardiac morphology and function in SHHF rats fed a high-salt diet.

Methods

Animals, animal care and housing

The lean (+/?) SHHF rats came from Charles Rivers Laboratories (Boston, MA) and originated from mating of Koletsky and inbred SHR rats. The obese (+/+) SHHF rats came from the SHHF/Mcc-facp rat colony originally maintained by Dr. Sylvia McCune and were purchased from Charles Rivers Laboratories (Boston, MA). The Wistar rats were also purchased from Charles River Laboratories (Boston, MA). All rats were allowed access to food and water *ad libitum*. All experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health [DHHS Publication No. (NIH) 85–23, Revised 1985] and with institutional guidelines for the humane treatment of animals and approved by the ULAM animal care committee at the University of Michigan.

Heart and brain harvest

Three sets of rats were analyzed. 1) Lean (+/?) SHHF rats were sacrificed at 2.0 (n=4), 5.5 (n=4) and 15.0 (n=3) months. 2) Obese (+/+) SHHF rats (n=3) and comparative Wistar control rats (n=3) were sacrificed at 12.0 months. 3) A separate group of lean (+/?) SHHF rats were either treated with 1,25(OH)₂D₃ (n=5) or not treated (control, n=6) and control Wistar (n=6) rats were analyzed and sacrificed at 7.0 months. Rats were weighed and heparinized (1500 U/kg rat wt.) by IP injection and after 20 minutes anesthetized with sodium pentobarbital (Nembutal, Abbot Laboratories, North Chicago, IL, 162.5 U/kg rat wt., IP). Blood was collected from the IVC and the animal exsanguinated. The heart and brain were rapidly excised and weighed.

Serum and blood assays

Serum 25(OH)D₃ levels were assayed in the groups of lean (+/?) SHHF rats at 2.0, 5.5, and 15.5 months and in the obese (+/+) SHHF rats and comparative Wistar control rats at 12.0 months using the methods of Hollis et al [28].

Biochemical assays

Blood calcium (Arsenazo III), phosphate (Phosphomolybdate) and magnesium (Formazan Dye) levels were measured in the lean (+/?) SHHF rats at 2.0, 5.5, and 15.5 months and in the obese (+/+) SHHF rats and comparative Wistar control rats at 12.0 months by the Special Chemistry Clinical Laboratories, University of Michigan Hospital.

Diet

The lean (+/?) SHHF rats sacrificed at 2.0, 5.5 and 15.0 months and obese (+/+) SHHF rats and Wistar control rats sacrificed at 12.0 months were fed a standard diet (Harlan Teklad maintenance diet, TD 2014, Madison, WI). A high-salt diet was used to create a rodent model of the heart failure phenotype [29]. For this study the control SHHF rats, 1,25(OH)₂D₃-treated rats and the Wistar control rats were fed a high-salt diet (8% NaCl) (Harlan Teklad diet, TD 92012, Madison, WI) to initiate hypertrophy at weaning.

Animal dosing

Starting at 4.0 months of age and ending at 7.0 months, a separate treatment group of lean (+/?) SHHF rats was given daily (Mon-Fri) subcutaneous injections (200–250ul) of 18ng 1,25(OH)₂D₃ (Sigma-Aldrich, St. Louis, MO) per 100g-body weight. 1,2-propanediol (Sigma-Aldrich, St. Louis, MO) was used as a carrier. Control lean (+/?) SHHF and Wistar rats were dosed similarly with 1,2-propanediol (Sigma-Aldrich, St. Louis, MO).

Blood pressure measurement

Systolic and diastolic blood pressure (BP) were measured in the 1,25(OH)₂D₃-treatment group and SHHF control group rats at 7.0 months of age (11.0 weeks of treatment) using the tail cuff method (Visitech Systems, Apex, NC). Rats were habituated to the procedure and placed on the warming chamber and their blood pressure was recorded from the tail by the occlusion method.

Heart function analysis

Cardiac index was determined in the 1,25(OH)₂D₃-treatment group and SHHF and control group rats. Two-dimensional and M-mode echocardiography (ECG) images were recorded on rats anesthetized with isoflurane using a GE S10-MHz phased-array transducer, connected to a General Electric, Vivid 7 Ultrasound System and the AnonyMOUSE/rat ECG Screening System (Mouse Specifics, Boston, MA).

Statistical analysis

Differences in serum 25(OH)D₃ levels and heart weight to brain weight ratios in the lean SHHF (+/?) rats at 2.0, 5.5, and 15.5 months were evaluated using a one-way analysis of variance (ANOVA). Differences in heart weight to brain weight ratios between the SHHF and Wistar rats were evaluated by an unpaired Student's *t*-test. BP data, heart function data and heart weight to brain weight ratios in 1,25(OH)₂D₃-treatment group and SHHF and Wistar control group rats were analyzed similarly.

Results

At 12.0 months obese (+/+) SHHF rats had lower serum 25(OH)D₃ levels than Wistar control rats and had a higher average heart weight to brain weight ratio than Wistar control rats

Figure 1a shows a significant decrease in serum 25(OH)D₃ levels ($P < .03$) in the 12.0-month-old obese (+/+) SHHF rats compared to control 12.0-month-old Wistar rats.

Figure 1b illustrates that the average heart weight to brain weight ratio ($P < .001$) in 12.0-month old obese (+/+) SHHF rats was significantly higher than the average heart weight to brain weight ratio in Wistar control rats. Thus, the obese (+/+) SHHF rats presented with cardiac hypertrophy at 12.0 months.

Serum calcium, phosphate, magnesium were not significantly different in the obese (+/+) SHHF and Wistar animals at 12.0 months.

Lean (+/?) SHHF rats became vitamin D deficient between 5.5 months and 15.5 months and the heart weight to brain weight ratios for lean (+/?) SHHF rats increased over time

Figure 2a examines 25(OH)D₃ serum levels in lean (+/?) SHHF rats at 2.0, 5.5 and 15.5 month of age. Serum 25(OH)D₃ levels show significant differences between the three age groups (ANOVA, $P < .05$). Average serum levels of 25(OH)D₃ in the lean SHHF rats decreased from 2.0 months to 5.5 months and decreased significantly from 5.5 months to 15.5 months. Thus, the data reveal that the lean SHHF rat becomes vitamin D deficient between 5.5 and 15.5 months.

Figure 2b examines average heart weight to brain weight ratios for lean SHHF rats at 2.0, 5.5 and 15.5 months of age. The measured ratios revealed highly significant differences between the three groups (ANOVA, $P < .0001$). The average ratio increased significantly from 2.0 months to 5.5 months and increased further at 15.5 months, indicating that cardiac hypertrophy presented in lean (+/?) SHHF rats at 5.5 months and increased to 15.5 months.

1,25(OH)₂D₃ treatment attenuated cardiac hypertrophy and reduced cardiac index in lean SHHF rats fed high-salt diet

After 13.0 weeks of 1,25(OH)₂D₃ treatment we measured heart weight to brain weight ratios in the Wistar control rats, lean SHHF control rats and 1,25(OH)₂D₃ treated lean SHHF rats fed a high-salt diet. As illustrated in figure 3, the mean heart weight to brain weight ratio measured in the 1,25(OH)₂D₃ treatment group was significantly lower ($P < .02$) than the same ratio measured in the SHHF control group. The data indicate that 1,25(OH)₂D₃ treatment attenuated cardiac hypertrophy in this relevant rodent model of heart failure. As shown in figure 4, 1,25(OH)₂D₃ treatment also resulted in significantly lower ($P < .02$) cardiac index, relative to the lean SHHF control group, in rats fed the high-salt diet.

At 6.0 months, SHHF control rat systolic BP was 273 ± 12 mmHg, diastolic BP was 155 ± 58 mmHg and mean BP was 194 ± 40 mmHg (mean $\pm$ SEM). For the 1,25(OH)₂D₃-treated (11.0 weeks) rats systolic BP was 254 ± 10 mmHg, diastolic BP was 203 ± 10 mmHg and mean BP was 220 ± 8 mmHg (mean $\pm$ SEM). There were no significant ($P > 0.1$) differences in these measurements between the control and treatment groups. These results are similar to other experiments in our laboratory with the SHHF rat that do not see a significant effect of 1,25(OH)₂D₃ treatment on BP.

Discussion

Obese (+/+) SHHF rats compared to their lineage control (Wistar) rats at 12.0 months of age were vitamin D deficient and had significant cardiac hypertrophy. This observation suggests a relationship between low vitamin D status and the heart failure phenotype. These results are consistent with our previous study of vitamin D depletion in the Sprague-Dawley rat that showed low vitamin D status related to morphological changes and cardiovascular dysfunction [16].

Cardiac hypertrophy in lean (+/?) SHHF rats was observed at 5.5 months and further increased by 15.5 months. This suggests that the lean SHHF rat progressed to the heart failure phenotype by 5.5 months and that the disease advanced to 15.5 months (Fig. 2). However, lean SHHF rats are not vitamin D deficient at 5.5 months and vitamin D deficiency is not observed until 15.5 months. These findings indicate that low vitamin D status is not associated with the early stages of the heart failure phenotype in these rats; rather it is associated with the exacerbation and final terminal phase of the heart failure phenotype.

After 13.0 weeks of 1,25-dihydroxyvitamin D treatment, we found that lean SHHF rats had significantly lower heart weight to brain weight ratios as compared to lean SHHF controls (Fig. 3). Both groups were fed a high-salt (8%NaCl) diet known to cause hypertension and accelerate the development of the heart failure phenotype [29]. Our heart function analysis of cardiac index after 1,25-dihydroxyvitamin D treatment of SHHF rats suggests that the action of 1,25-dihydroxyvitamin D is to permit decreased workload for the heart and yet allow adequate blood perfusion and pressure. Thus, our results suggest that 1,25-dihydroxyvitamin D treatment attenuates cardiac workload and thereby provides protection against the progression of cardiac hypertrophy and heart failure phenotype in the SHHF rat.

Our finding that decreasing vitamin D status is associated with the progression of the heart failure phenotype and that 1,25-dihydroxyvitamin D treatment provides protection against the progression of this phenotype suggests that the same might be true in human CHF. Human studies performed using CHF patients to investigate the relationship between vitamin D status and heart failure have been largely consistent with this finding.

One study conducted on patients undergoing evaluation for cardiac transplantation found that patients with New York Heart Association (NYHA) class III or IV heart failure had low levels of serum 25(OH)D₃ and 1,25(OH)₂D₃. Furthermore, decreasing vitamin D status correlated with increasing severity of heart failure [30]. More recently, researchers evaluating end-stage heart failure patients awaiting cardiac transplantation found that 47% of patients awaiting urgent transplantation had low 1,25(OH)₂D₃ levels compared to 31% of patients awaiting elective transplantation [31]. Additionally, a randomized trial investigated the effects of vitamin D supplementation in ambulatory patients with NYHA class II or greater heart failure. Patients were randomly given calcium 500 mg and cholecalciferol 2000 IU or calcium 500 mg and placebo daily for 9 months. The vitamin D supplementation group showed a significant decrease in TNF- α and a significant increase in anti-inflammatory cytokine IL-10 [32].

Vitamin D deficiency in heart failure patients may result from a number of factors. The debilitating effects of CHF result in a sedentary lifestyle and diminished exposure to sunlight [33,34], which contribute to low vitamin D levels in CHF patients [35] and may subsequently exacerbate CHF [10]. CHF is also associated with intestinal, hepatic and renal congestion, which decrease the ability to absorb vitamin D, produce the major circulating form of vitamin D and produce the biologically active form of vitamin D respectively [2]. Additionally, the ability to produce vitamin D cutaneously decreases with age and this ability is reduced at northern latitudes, by the use of sunscreen and by dark skin tone [2].

In summary, we observed that cardiac hypertrophy increased with age and was associated with declining vitamin D status in a relevant animal model of the failing heart. This report provides evidence of the clear association of vitamin D deficiency with the heart failure phenotype in both the obese (+/+) and lean (+/?) SHHF rat. Also, 25-hydroxyvitamin D deficiency presented after cardiac hypertrophy was initially observed. These findings suggest that low vitamin D status is associated with the exacerbation and final terminal phase of the heart failure phenotype. This report reveals that vitamin D hormone treatment protects against the advancement of cardiac hypertrophy and dysfunction in lean SHHF rats. These results, coupled with the findings that vitamin D deficiency is pervasive within the subset of the population afflicted with heart failure, suggests the potential for clinical utilization of vitamin D analogs for treatment of human heart failure. Lastly, our study supports the importance of monitoring human vitamin D status for the prevention of disease, in particular heart disease.

Acknowledgments

Support: NIH grant (HL-074894, RUS), Abbott Laboratories grant-in-aid (RUS) and MICHR pilot grant (RUS).

References

1. Vieth R, Bischoff-Ferrari H, Boucher BJ, et al. The urgent need to recommend an intake of vitamin D that is effective. *Am J Clin Nutr* 2007;85:649–650. [PubMed: 17344484]
2. Holick M. Vitamin D deficiency. *N Engl J Med* 2007;357(3):266–281. [PubMed: 17634462]
3. Norman A, Bouillon R, Whiting S, Vieth R, Lips P. 13th workshop consensus for vitamin D nutritional guidelines. *J Steroid Biochem Mol Biol* 2007;103(3–5):204–205. [PubMed: 17234402]
4. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. *Arch Intern Med* 2008;168:1340–1349. [PubMed: 18574092]
5. Garland C, Garland F, Gorham E, et al. The role of vitamin D in cancer prevention. *Am J Public Health* 2006;96:252–261. [PubMed: 16380576]
6. Liu P, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006;311:1770–1773. [PubMed: 16497887]

7. Forman J, Giovannucci E, Holmes M, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 2007;49(5):1063–1069. [PubMed: 17372031]
8. Hyppönen E, Laara E, Reunanen A, Järvelin M, Virtanen S. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 2001;358:1500–1503. [PubMed: 11705562]
9. Chiu K, Chu A, Go V, Saad M. Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. *Am J Clin Nutr* 2004;79:820–825. [PubMed: 15113720]
10. Zittermann A. Vitamin D and disease prevention with special reference to cardiovascular disease. *Prog Biophys Mol Biol* 2006;92(1):39–48. [PubMed: 16600341]
11. Martins D, Wolf M, Pan D, et al. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 2007;167:1159–1165. [PubMed: 17563024]
12. Adams K, Zannad F. Clinical definition and epidemiology of advanced heart failure. *Am Heart J* 1998;135:S204–S215. [PubMed: 9630086]
13. American Heart Association. Heart Disease and Stroke Statistics — 2008 Update. Dallas, Texas: American Heart Association; 2008. ©2008, American Heart Association.
14. Zittermann A, Schleithoff S, Tenderich G, Berthold H, Körfer R, Stehle P. Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure? *J Am Coll Cardiol* 2003;41:105–112. [PubMed: 12570952]
15. Weishaar R, Simpson R. Vitamin D₃ and cardiovascular function in rats. *J Clin Invest* 1987;79:1706–1712. [PubMed: 3034981]
16. Weishaar R, Simpson R. The involvement of vitamin D₃ with cardiovascular function II: direct and indirect effects. *Am J Physiol* 1987;253:E675–E683. [PubMed: 2827502]
17. Weishaar R, Kim S, Saunders D, Simpson R. Involvement of vitamin D in regulating cardiovascular function III. Effects on physical and morphological properties of heart muscle. *Am J Physiol* 1990;258:E134–E142. [PubMed: 2154115]
18. Simpson R, Weishaar R. 1,25 Dihydroxyvitamin D₃ and intracellular calcium in the myocardium. *Cell Calcium* 1988;9:285–292. [PubMed: 3066493]
19. Simpson R, Hershey S, Nibelink K. Characterization of heart size and blood pressure in the vitamin D receptor knockout mouse. *J Steroid Biochem Mol Biol* 2007;103(3–5):521–524. [PubMed: 17275289]
20. Nibelink K, Tishkoff D, Hershey S, Rahman A, Simpson R. 1,25(OH)₂Vitamin D₃ actions on cell proliferation, size, gene expression, and receptor localization, in the HL-1 cardiac myocyte. *J Steroid Biochem Mol Biol* 2007;103(3–5):533–537. [PubMed: 17276054]
21. Rahman A, Hershey S, Ahmed A, Nibelink K, Simpson R. Heart extracellular matrix gene expression profile in the Vitamin D receptor knockout mice. *J Steroid Biochem Mol Biol* 2007;103:416–419. [PubMed: 17275288]
22. Holick M. Resurrection of vitamin D deficiency and rickets. *J Clin Invest* 2006;116:2062–2072. [PubMed: 16886050]
23. Holick, M.; Garabedian, M. Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In: Favus, M., editor. *Primer on the metabolic bone diseases and disorders of mineral metabolism*. 6th ed.. Washington, DC: American Society for Bone and Mineral Research; 2006. p. 129–137.
24. DeLuca H. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004;80:S1689–S1696.
25. Gerdes A, Onodera T, Wang X, McCune S. Myocyte remodeling during the progression to failure in rats with hypertension. *Hypertension* 1996;28:609–614. [PubMed: 8843886]
26. Heyen J, Blasi E, Nikula K, Rocha R, Daust H, Friedrich G. Structural, functional, and molecular characterization of the SHHF model of heart failure. *Am J Physiol* 2002;283:H1775–H1784.
27. Johnsen D, Kacimi R, Anderson B, Thomas T, Said S, Gerdes A. Protein kinase C isozymes in hypertension and hypertrophy: Insight from SHHF rat hearts. *Mol Cell Biochem* 2005;270(1–2):63–69. [PubMed: 15792354]
28. Hollis B, Wagner C, Drezner M, Binkley N. Circulating Vitamin D₃ and 25-hydroxyvitamin D in Humans: An Important Tool to Define Adequate Nutritional Vitamin D Status. *J Steroid Biochem Mol Biol* 2007;103(3–5):631–634. [PubMed: 17218096]

29. Inoko M, Kihara Y, Morii I, Fujiwara H, Sasayama S. Transition from compensatory hypertrophy to dilated, failing left ventricles in Dahl salt-sensitive rats. *Am J Physiol* 1994;H2471–H2482. [PubMed: 7810745]
30. Shane E, Mancini D, Aaronson K, et al. Bone mass, vitamin D deficiency, and hyperparathyroidism in congestive heart failure. *Am J Med* 1997;103:197–207. [PubMed: 9316552]
31. Zittermann A, Schleithoff S, Gotting C, et al. Poor outcome in end-stage heart failure patients with low circulating calcitriol levels. *Eur J Heart Fail* 2008;10(3):321–327. [PubMed: 18304873]
32. Schleithoff S, Zittermann A, Tenderich G, Berthold H, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr* 2006;83:754–759. [PubMed: 16600924]
33. Kriegsman D, Deeg D, van Eijk J, Penninx B, Boeke A. Do disease characteristics add to the explanation of mobility limitations in patients with different chronic diseases? A study in The Netherlands. *J Epidemiol Community Health* 1997;51(6):676–685. [PubMed: 9519132]
34. Albanese M, Plewka M, Gregori D, et al. Use of medical resources and quality of life of patients with chronic heart failure: A prospective survey in a large Italian community hospital. *Eur J Heart Fail* 1999;1:411–417. [PubMed: 10937956]
35. Zittermann A, Sabatschus O, Jantzen S, et al. Exercise-trained young men have higher calcium absorption rates and plasma calcitriol levels in comparison to age-matched sedentary controls. *Calcif Tissue Int* 2000;67:215–219. [PubMed: 10954775]

Figure 1.

Serum 25(OH)D₃ and heart weight to body weight ratios in obese SHHF rats -(A) Average serum of 25(OH)D₃ in Wistar control rats and obese SHHF (+/+) rats at 12.0 months of age. (B) Average heart weight to brain weight ratios for Wistar control rats and obese SHHF (+/+) rats at 12.0 months of age. Bars represent the mean $\pm$ SEM.

Figure 2.

Serum 25(OH)D₃ and heart weight to body weight ratios in lean SHHF rats -(A) Average serum of 25(OH)D₃ in lean SHHF (+/?) rats at 2.0, 5.5, and 15.5 months of age. (B) Average heart weight to brain weight ratios for lean SHHF (+/?) rats at 2.0, 5.5, and 15.5 months of age. Bars represent the mean $\pm$ SEM.

Figure 3.

1,25(OH)₂D₃ treatment in lean SHHF rats –Heart weight to body weight ratios in 30-week old Wistar control rats, lean SHHF control rats and lean SHHF rats treated with 1,25(OH)₂D₃ for 13 weeks. Bars represent mean ± SEM.

Figure 4.

Cardiac index in $1,25(\text{OH})_2\text{D}_3$ treated SHHF rats -Effect of $1,25(\text{OH})_2\text{D}_3$ treatment on cardiac index in SHHF (cp/+) rats. Cardiac index was determined using echocardiography in rats fed a high-salt diet (8% NaCl) and treated with or without $1,25(\text{OH})_2\text{D}_3$ for 13 weeks. Bars represent mean $\pm$ SEM.