Selective Knockout of the Vitamin D Receptor in the Heart Results in Cardiac Hypertrophy

Is the Heart a Drugable Target for Vitamin D Receptor Agonists?

Robert U. Simpson, PhD

The report by Dr Chen and colleagues, published in this issue of Circulation, “Cardiomyocyte-Specific Deletion of the Vitamin D Receptor Gene Results In Cardiac Hypertrophy,” is important and timely. This study extends and supports other studies that have demonstrated that vitamin D and the vitamin D receptor (VDR) signaling system possess anti-hypertrophic activity in the heart. The study also furthers our knowledge of this action by demonstrating an involvement of the pro-hypertrophic calcineurin/nuclear factor of activated T-cells/modulatory calcineurin-interacting protein-1 pathway. Thereby, they identify a potential mechanism to account for the reported beneficial effects of vitamin D on the cardiovascular system. To respond to the question posed in the title of this editorial, “Is the heart a drugable target for vitamin D receptor agonists?” Chen et al’s report makes an affirmative response more plausible.

The first studies to demonstrate a connection between cardiovascular homeostasis and vitamin D status used the established rat model of vitamin D deficiency. The notion of an involvement of the vitamin D endocrine system with cardiovascular function began >25 years ago by our identification of the VDR in rat cardiac myocytes, and this led us to the physiological studies to establish relevance. These animal studies established a connection between vitamin D deficiency and cardiovascular dysfunctions, including cardiac hypertrophy, fibrosis, hypertension, and the elevation of serum calcium, parathyroid hormone, and renin levels. These reports supported a role for vitamin D in maintaining cardiovascular homeostasis through both the direct action of 1,25-dihydroxyvitamin D on cardiomyocyte VDR and indirect actions on circulating hormones and calcium. Our studies in rats maintained on a vitamin D-deficient diet revealed that ventricular muscle mass and contractile function were both markedly enhanced. Increased systolic blood pressure, renin levels, hydroxyproline, and serum creatine phosphokinase were reported, and coincided with a reduction in serum calcium. Subsequently, more recently, the VDR was identified in the human heart cells.

Other important preclinical studies demonstrating the involvement of the VDR in the cardiovascular system came with the creation of the global VDR knockout (VDRKO) mouse. As with the vitamin D-deficient rat, these VDRKO mice were hypertensive, and their heart weight/body weight ratios were significantly increased. In addition, the renal renin mRNA levels of adult VDRKO mice were higher than those of wild-type mice. The size of left ventricular cardiomyocytes in VDRKO mice was markedly increased in comparison with wild-type mice. In addition, levels of atrial natriuretic peptide mRNA and circulating atrial natriuretic peptide and the cardiac renin mRNA level were significantly increased in the VDRKO mice. These data strongly supported VDR involvement in the regulation of cardiovascular functions, at least in part, through modulation of the local cardiac renin-angiotensin system and expression of natriuretic peptides. Similar observations were made in mice lacking CYP27B1, the key enzyme involved in the synthesis of 1,25-dihydroxyvitamin D or calcitriol. As a result of the ablation of calcitriol synthesis, vehicle-treated CYP27B1 knockout mice developed hypertension, cardiac hypertrophy, and impaired cardiac function, along with an upregulation of the renin-angiotensin system in both renal and cardiac tissues. These abnormalities were normalized by calcitriol treatment. Rahman et al confirmed the cardiac hypertrophic phenotype of the VDRKO mice and showed that tissue inhibitors of metalloproteinase types 1 and 3 were significantly underexpressed, whereas metalloproteinases such as matrix metalloproteinase types 2 and 9 were upregulated in VDRKO mice.

Extracellular matrix remodeling mediated by matrix metalloproteinases is known to contribute to progressive left ventricular remodeling, dilation, and heart failure. The data suggest that the expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases may be regulated by VDR, and that modulation of heart extracellular matrix metabolism may be one of the mechanisms involved in vitamin D’s cardiovascular activities. Fibroblasts are principally responsible for deposition of the excessive fibrotic extracellular matrix (ECM), and activated fibroblasts may directly cause hypertrophy of cardiomyocytes via paracrine mechanisms, further contributing to impaired cardiac function. The fibrotic ECM causes increased stiffness and induces pathological signaling within cardiomyocytes, resulting in progressive cardiac failure. Also, the excessive ECM impairs mechanolectric coupling of cardiomyocytes and increases the risk of arrhythmias. The data presented in the

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association. From the Department of Pharmacology, University of Michigan Medical School, and Cardiavent Inc., Ann Arbor, MI. Correspondence to Robert U. Simpson, PhD, 1301 MSRB III, 1150 West Medical Center Drive, Ann Arbor, MI 48109. E-mail robsim@umich.edu

(Circulation. 2011;124:1808-1810.) © 2011 American Heart Association, Inc. Circulation is available at http://circ.ahajournals.org DOI: 10.1161/CIRCULATIONAHA.111.061234

1808
The mechanisms involved in cardiac ECM changes resulting from vitamin D signaling ablation are not solely a result of direct effects on cardiomyocyte VDR. According to their report, as might be expected, fibrosis may involve other cells such as the cardiac fibroblast.

Preclinical intervention studies of cardiovascular dyshomeostasis in rodent models have also been reported. Paricalcitol, an analog of calcitriol, was tested in the Dahl salt-sensitive rat model of hypertension and heart failure. The Dahl salt-sensitive rat is an established animal model in which a high-salt diet induces hypertension, cardiac hypertrophy, and heart failure. Dahl salt-sensitive rats became vitamin D-deficient during the development of cardiac dysfunction. Paricalcitol therapy prevented the appearance of both pathological and echocardiographic evidence of cardiac hypertrophy and cardiac dysfunction. In addition, serum brain natriuretic peptide and cardiac atrial natriuretic factor mRNA expression levels were normalized after paricalcitol treatment. One relevant observation made in this study was that the effect of paricalcitol in the Dahl salt-sensitive rat was independent of blood pressure control. Recently, calcitriol treatment was studied in spontaneously hypertensive heart failure rats that possess 1 or 2 copies of the corpulent gene (cp), a mutant form of the leptin receptor. Calcitriol treatment of the spontaneously hypertensive heart failure rats fed a high-salt diet resulted in a reduction in heart weight, myocardial collagen levels, left ventricular diameter, and cardiac output despite higher serum leptin levels.

Direct evidence that human vitamin D deficiency could lead to cardiovascular disease initially came from patients with end-stage renal disease. In end-stage renal disease, the damaged kidney fails to convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, which results in a severe calcitriol deficiency. In the absence of adequate calcitriol levels, secondary hyperparathyroidism develops, resulting in elevated levels of circulating parathyroid hormone. The sustained stress on myocardial tissue leads to cardiac hypertrophy, myocardial fibrosis, and heart failure. In addition, studies have also linked elevated levels of parathyroid hormone with hyperlipidemia and an increased risk of atherosclerosis and vascular disease. Administration of activated forms of vitamin D to patients with end-stage renal disease and secondary hyperparathyroidism has resulted in decreased left ventricular hypertrophy along with a decrease in cardiovascular mortality.

With respect to human health, it is important to note that vitamin D itself does 1 thing, and it does this perfectly: it treats and can cure vitamin D deficiency. The Figure depicts the activation of vitamin D to form the steroid hormone 1,25-dihydroxyvitamin D or calcitriol. This activation of vitamin D to a hormone is similar to the synthesis of other steroid hormones in that P450 enzymes metabolize precursor forms to the active hormone. However, unlike all other steroid hormones that use plentiful cholesterol for substrate, vitamin D (cholecalciferol) deficiency occurs in humans, and disease states exist because insufficient calcitriol is then produced. Diseases linked with vitamin D deficiency include cardiovascular disease, stroke, osteoporosis, osteomalacia, several forms of cancer, autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and type 1 diabetes mellitus, type 2 diabetes mellitus, and depression. The study reported in this volume demonstrates that the cardiomyocyte VDR, a classic member of the steroid/thyroid/retinoid hormone receptor family, has a physiological function, and that it can control cardiac hypertrophy. This study furthers our understanding of the cardiac fibrosis associated with preclinical study models of vitamin D deficiency or VDR global knockout mice by demonstrating that ablation of just the cardiomyocyte is insufficient to yield ECM changes. Thus, cardiac fibroblasts, as discussed above, may be regulated by the VDR that affects fibrosis in the heart.

As shown in Figure, 25-hydroxyvitamin D metabolites are further hydroxylated, primarily in the kidneys, to form

![Figure](http://circ.ahajournals.org/)

**Figure.** Vitamin D metabolism and activation to the steroidal hormone calcitriol (1,25-dihydroxyvitamin D). VDR indicates vitamin D receptor.
1,25-dihydroxyvitamin D$_3$ and 1,25-dihydroxyvitamin D$_{3s}$, which are the active forms of the hormone that bind to specific vitamin D receptors in target tissues. Formation of 1,25-dihydroxyvitamin D is tightly regulated and has a short half-life, making it unsuitable for assessing vitamin D status. In vitamin D-deficient states, there is in fact excess production of parathyroid hormone (secondary hyperparathyroidism), stimulating the kidneys to produce even more 1,25-dihydroxyvitamin D, such that levels can appear to be normal or even elevated. The 25-hydroxyvitamin D metabolite, which reflects total body bioavailability of the prohormone, is therefore the commonly accepted measure of vitamin D status. The serum concentration of 25-hydroxyvitamin D is typically used to determine vitamin D status. It reflects vitamin D produced in the skin, and that acquired from the diet, as well, and has a fairly long circulating half-life of 15 days. The level of serum 1,25-dihydroxyvitamin D is not usually used to determine vitamin D status, because it has a short half-life of several hours and is tightly regulated by parathyroid hormone, calcium, and phosphate, such that it does not decrease significantly until vitamin D deficiency is already well advanced. Interest in the preclinical studies of VDR-dependent regulation of cardiovascular function and morphology has led to several clinical reports and studies. The majority of these studies are retrospective and epidemiological, but intervention studies are now being reported. It is critical that the above discussion of the biology of vitamin D be kept in mind. Clinical studies in which vitamin D status, as assessed by 25-hydroxyvitamin D levels, is not measured or insignificantly affected are simply not relevant.

Currently, my research has focused on developing an analog of calcitriol, a selective VDR agonist that has selectivity and high efficacy to treat the heart failure phenotype and high-renin–associated dysfunctions. The hormone calcitriol has toxic hypercalcemic actions, and this limits its therapeutic utility. Our preclinical pharmacology and toxicology studies have yielded a selective VDR agonist drug candidate, CARD-024, with efficacy and safety sufficient to obtain Food and Drug Administration Investigational New Drug status. We will begin first-in-human phase I studies in the coming weeks. A wonderful part of focusing one’s research on vitamin D is its great history of doing translation research long before the term was used; it is just what we have been doing. The report by Chen and colleagues strongly supports the notion that cardiovascular diseases are targets for VDR agonist therapeutics. I am confident that clear and unambiguous studies will soon determine whether this notion is accurate.

Disclosures
Dr Simpson is President of Cardiavent, Inc and has received funding from the National Institutes of Health (HL074894), The American Heart Association, The American Dairy Council, The Michigan Institute of Clinical and Health Research, Abbott Laboratories, and Health and Human Services: Qualified Therapeutics Discovery Program.

References

Key Words: Editorsials [ ] heart failure [ ] vitamin D
Selective Knockout of the Vitamin D Receptor in the Heart Results in Cardiac Hypertrophy: Is the Heart a Drugable Target for Vitamin D Receptor Agonists?

Robert U. Simpson

Circulation. 2011;124:1808-1810
doi: 10.1161/CIRCULATIONAHA.111.061234

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/124/17/1808