

Vitamin D₃ and Cardiovascular Function in Rats

Ronald E. Weishaar and Robert U. Simpson

Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48109

Abstract

We have previously identified a receptor for 1,25-dihydroxyvitamin D₃ in myocardial cells (Simpson, R. U. 1983. *Circulation* 68:239). To establish the relevance of this observation, we evaluated the role of the prohormone vitamin D₃ in regulating cardiovascular function. In rats maintained on a vitamin D₃-deficient diet for nine weeks, increases in systolic blood pressure (BP) and serum creatine phosphokinase (CPK) were observed. These increases coincided with a reduction of serum calcium from 10.3 to 5.6 mg/dl. However, while serum calcium remained depressed throughout the study, increases in BP and serum CPK were transient. After nine weeks of vitamin D₃-depletion, but not after six weeks, ventricular and vascular muscle contractile function were also markedly enhanced. The increase in ventricular contractile function could not be prevented by maintaining serum calcium at 9.0 mg/dl during the period of D₃-depletion. These observations suggest a primary role for the vitamin D₃-endocrine system in regulating cardiovascular function.

Introduction

The importance of vitamin D₃ for maintaining plasma levels of calcium is well known and is due to the effect that the vitamin D₃ metabolite 1,25-dihydroxyvitamin D₃ (1,25[OH]₂D₃)¹ exerts on calcium metabolism in the bone, kidney, and intestine (1–3). A number of recent studies have indicated that 1,25(OH)₂D₃ may also play a direct role in regulating metabolic events in other tissues and cells, including the pancreas (4), brain (5), pituitary gland (4, 6), and cancer cells (7). This direct role has been suggested by the identification of a specific receptor for 1,25(OH)₂D₃ in these cells, as well as the presence of 1,25(OH)₂D₃-dependent calcium-binding proteins (8–10).

In previous reports we demonstrated that a specific receptor for 1,25(OH)₂D₃ is present in cultured heart and skeletal muscle (11, 12). Subsequently, Walters et al. identified a receptor for 1,25(OH)₂D₃ in a low-salt chromatin preparation from normal rat hearts (13), and Thomasset and co-workers have shown that

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Address reprint requests to Robert U. Simpson, Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109.

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1. *Abbreviations used in this paper:* CPK, creatine phosphokinase; HR, heart rate; LVSP, left ventricular systolic pressure; 25(OH)D₃, 25-hydroxyvitamin D₃; 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; PSS, physiological salt solution.

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myocardial tissue contains a vitamin D₃-dependent calcium-binding protein (10). These observations suggest a role for 1,25(OH)₂D₃ in regulating cardiac metabolism.

Although administration of toxic quantities of vitamin D₃ have been shown to produce heart failure (14), presumably through an indirect elevation of plasma calcium and subsequent myocardial calcinosis, a direct role for 1,25(OH)₂D₃ in contributing to the regulation of myocardial metabolism has not been described. There is considerable evidence, however, to indicate that changes in myocardial calcium homeostasis are important in mediating certain aspects of cardiovascular dysfunction (15–17).

In the present study, the role of vitamin D₃ in regulating cardiovascular function was evaluated by examining the response of rats maintained on a vitamin D₃-deficient diet for either 6 or 9 wk. The results of this study clearly demonstrate that vitamin D deficiency produces significant changes in normal cardiovascular function.

Methods

Male weanling Sprague-Dawley rats (Holtzman Co., Madison, WI) were obtained from mothers maintained on a low vitamin D₃ diet. Immediately upon arrival the rats were housed in an area removed from ultraviolet light, including fluorescent light. The rats were maintained on a vitamin D₃-deficient diet that contained 0.4% calcium and 0.4% phosphorus (diet TD84475, Teklad Laboratories, Madison, WI). In a subsequent study rats were also maintained for 9 wk on a vitamin D₃-deficient diet containing 2.5% calcium and 1.5% phosphate (diet TD86029, Teklad Laboratories). After 1 wk the rats were weighed and measurements of systolic blood pressure (BP) and heart rate (HR) were made. Blood samples were also taken from an incision in the tail for measurements of serum Ca, PO₄, and creatine phosphokinase (CPK). Measurements of BP, HR, and serum Ca, PO₄, and CPK were made at weekly or biweekly intervals throughout the remainder of the study. Following these initial measurements the rats were randomly divided into two groups. The D⁺ group was maintained on the 0.4% Ca/0.4% PO₄ diet, which was supplemented with 2 IU vitamin D₃/g diet. The vitamin D₃ was dissolved in corn oil and added directly to the diet. Rats in the D⁻ group received the same diet, containing an equal amount of corn oil. Both groups received an equal amount of the diet (15 g/rat/d). Changes in body weight were recorded weekly or biweekly.

After 6 wk half the animals in each group were sacrificed for evaluation of in vitro cardiac and vascular muscle contractility. At 9 wk the remainder of the animals were sacrificed and measurements of in vitro contractility repeated.

Analysis of serum. Blood samples (2–3 ml) were collected under vacuum into side arm test tubes previously rinsed with deionized water. The blood was allowed to clot for 30 min, centrifuged, and the serum was decanted and stored at –20°C until analysis (usually within 1 mo). Serum calcium was measured using atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer, model 2380, Perkin-Elmer Corp., Norwalk, CT). The spectrophotometer was equipped with a single-element hollow cathode lamp (excitation wavelength of 422.7 nm). A nitrous oxide-acetylene flame was employed to reduce interference from serum proteins and increase sensitivity. 0.1% lanthanum chloride was included in all samples to control ionization interference. Serum phosphate was measured by the method of Fiske and SubbaRow (18), using

a commercial kit (Kit 670-C, Sigma Chemical Co., St. Louis, MO). Serum CPK was measured using the colorimetric method described by Hughes (19), also using a commercial kit (Kit 520, Sigma Chemical Co.). Serum levels of 25-hydroxyvitamin D₃ (25(OH)D₃) were measured using radioimmunoassay after extraction of 25(OH)D₃ and other hydroxylated metabolites with acetonitrile (20), using a commercial kit (Catalogue No. 5800, Immuno Nuclear Corp., Stillwater, MN). Serum 1,25(OH)₂D₃ was measured by the method of Reinhardt et al. (21), also using a commercial kit (Immuno Nuclear Corp.). For this measurement vitamin D₃ metabolites were extracted using acetonitrile and then partially purified using a C₁₈ cartridge. Further purification was achieved using a silica cartridge. Percent recovery averaged 50–55%. Quantification was achieved using a 1,25(OH)₂D₃ receptor protein competitive binding assay. Dextran-coated charcoal suspension was used to separate bound from free hormone. For measurements of 25(OH)D₃ and 1,25(OH)₂D₃, blood from five pentobarbital-anesthetized rats was taken via heart puncture and pooled to obtain sufficient serum for analysis of both 25(OH)D₃ and 1,25(OH)₂D₃.

Measurement of blood pressure and heart rate. Systolic blood pressure and heart rate were measured weekly or biweekly in conscious, restrained animals by tail cuff occlusion using standard equipment and techniques (Narco Bio-Systems, Heathdyne Co., Houston, TX). An average of six recordings was made for each rat.

Measurement of in vitro contractility. Rats were injected with heparin (100 U/kg) 30 min before sacrifice via cervical dislocation. After stunning, the chest cavity was rapidly opened and the heart and thoracic aorta were quickly removed and placed in a beaker containing ice-cold saline until contractions ceased (within 20 s). The heart and aorta were then rinsed in a second beaker of ice-cold saline, after which the aorta was removed and placed in an ice-cold physiological salt solution (PSS) containing the following: 130 mM NaCl, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.17 mM MgSO₄, 4.9 mM NaHCO₃, 5.5 mM glucose, 1.6 mM CaCl₂, and 0.03 mM CaNa₂EDTA (pH 7.4). The aorta was refrigerated overnight and evaluated the following day.

The heart was then mounted via the aortic root and perfused Langendorff fashion at 37°C. The composition of the perfusate was as follows: 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25.0 mM NaHCO₃, 11.0 mM glucose, and 2.0 mM CaCl₂ (2.5 mM CaCl₂ + 0.5 mM Na₂EDTA). The perfusate was bubbled continuously with 95% O₂/5% CO₂, which maintained the pH at ~7.4. The heart was perfused in this manner for 10 min, during which the aortic perfusion pressure was adjusted to 80 mmHg. A Millar pressure transducer was also placed into the lumen of the left ventricle through a small incision in the left atria. Once properly positioned, the pressure transducer was sutured in place. This transducer was used for measuring left ventricular systolic pressure (LVSP), the first derivative of the rise and fall in left ventricular pressure ($\pm dP/dt$), and HR. After the 10-min equilibration period the concentration of calcium in the perfusate was reduced to 1.0 mM (1.5 mM CaCl₂ + 0.5 mM Na₂EDTA), and once a new steady state of contractility was achieved (within 5 min) measurements of LVSP, $\pm dP/dt$, and HR were made. The concentration of calcium in the perfusate was then incrementally increased to 1.5, 2.0, 2.5, and 3.0 mM at 5-min intervals. Measurements of contractility and HR were made at each new concentration of extracellular calcium.

The following day rings 3–4 mm in length were prepared from the thoracic aortae obtained the day before. These rings were always taken from the same location (~3 mm below the aortic arch), because preliminary studies showed that the response to different constricting agents, e.g., KCl, can vary depending upon the site of origin of the aortic ring (personal observation). The rings were mounted vertically between two stainless steel triangles in a 50-ml water-jacketed tissue bath containing PSS maintained at 37°C. The composition of this salt solution was the same as previously described. After mounting, resting tension was gradually increased to 5.0 g. This degree of resting tension resulted in a maximum contractile response to 100 mM KCl or to 1.0 μ M norepinephrine (personal observation). The aortic rings were allowed to equilibrate for 90 min, during which PSS was changed once. The rings were then constricted by increasing the concentration of KCl in the PSS to 100 mM.

After the maximum response was obtained, the rings were rinsed three times at 10-min intervals with fresh PSS. Resting tension was then readjusted to 5.0 g, and increasing concentrations of norepinephrine were added to the bath, until a maximum response was obtained. The rings were again washed three times at 10-min intervals with fresh PSS, after which resting tension was readjusted, and the rings were again challenged with increasing concentrations of norepinephrine. The response to the second challenge of norepinephrine was used for all calculations, because preliminary results showed that the first response to norepinephrine occasionally varied, whereas the second response to norepinephrine was very reproducible. Following the second challenge with norepinephrine, the rings were removed from the bath, blotted dry, and weighed.

Statistical analyses. Changes in serum calcium, phosphate, CPK, systolic BP, and HR were evaluated using an unpaired Student *t* test (22). Changes in the relationship between extracellular calcium and cardiac contractility and the relationship between increasing concentrations of norepinephrine and vascular contractile response between the two groups were evaluated using analysis of variance (22).

Results

Body weight and serum changes. Fig. 1 illustrates the rate of growth for the vitamin D₃-deficient rats maintained on a 0.4% calcium diet (D⁻ rats), and for the vitamin D₃-sufficient rats maintained in the same diet (D⁺ rats). As can be seen, the D⁻ rats gained slightly less weight during the 9-wk course of the study than did the D⁺ rats. After 9 wk, however, the difference in body weight between the two groups was < 10%.

Changes in serum Ca, PO₄, and serum CPK, which accompanied vitamin D₃-depletion are shown in Fig. 2. Within 4 wk, serum Ca levels in the D⁻ rats had fallen to roughly 50% of the level observed in the D⁺ rats (10.5 \pm 0.3 mg/dl for the D⁺ rats and 5.6 \pm 0.2 mg/dl for the D⁻ rats). Serum Ca remained at this reduced level in the D⁻ rats throughout the remainder of the study (Fig. 2).

A large increase in serum CPK was observed in the D⁻ rats, which coincided roughly with the development of hypocalcemia (Fig. 2). Within 3–4 wk after the onset of vitamin D₃-depletion, the serum CPK in the D⁻ rats was more than double that observed in the D⁺ rats (188 \pm 11 IU/dl for the D⁻ rats and 85 \pm 13 IU/dl for the D⁺ rats). However, whereas the level of serum Ca remained low throughout the study, the increase in serum CPK was transient, and after 8 wk the level of serum CPK in the D⁻ rats had returned to normal (Fig. 2). A slight increase in serum PO₄ was also observed in the D⁻ rats (Fig. 2). This increase, however, was never > 15% above the level in the D⁺ rats.

In addition to a profound reduction in serum calcium, changes in the circulating levels of 1,25(OH)₂D₃ and 25(OH)D₃ were also used to establish vitamin D₃-deficiency. As Table I

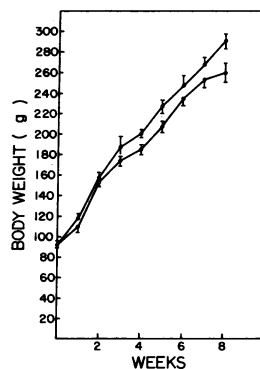


Figure 1. Representative age-dependent changes in body weight (g) for vitamin D₃-deficient rats maintained on a diet containing 0.4% calcium and 0.4% phosphate (○), and vitamin D₃-sufficient rats maintained on the same diet (●). Each symbol represents the mean \pm SEM of at least eight rats.

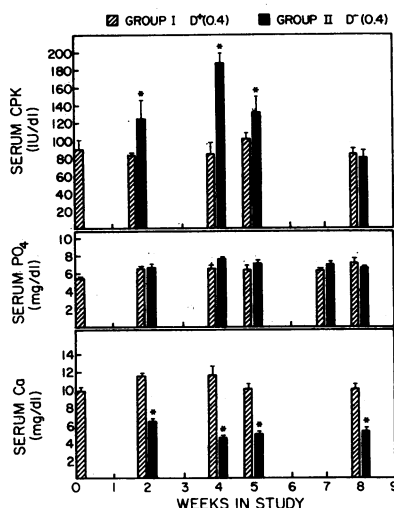


Figure 2. Age-dependent changes in serum calcium (mg/dl), serum phosphorus (mg/dl), and serum CPK (IU/dl) in the vitamin D₃-deficient rats maintained on a diet containing 0.4% calcium and 0.4% phosphate (hatched box), and vitamin D₃-sufficient rats maintained on the same diet (solid box). Bars represent the mean±SEM for 3–6 serum samples. *P < 0.05.

illustrates, serum levels of both 1,25(OH)₂D₃ and 25(OH)₂D₃ were below the level of detection in rats maintained on the vitamin D₃-deficient diet for 6 or 9 wk.

BP and HR changes. The effect of vitamin D₃-depletion on systolic blood pressure and HR is shown in Fig. 3. Removal of vitamin D₃ from the diet was associated with a significant increase in systolic BP in the vitamin D₃-deficient rats, as compared with the vitamin D₃-sufficient rats. An early increase in systolic BP was observed in both groups, which may be age related, due to equilibration of the rats to their environment or the change in diet for the weanling rats. This initial increase, however, was much greater in the D⁻ rats than in the D⁺ rats. As was the case with the increase in serum CPK, the increase in systolic BP in the vitamin D₃-deficient rats was transient and by 8 wk there was no difference in BP between the two groups (Fig. 3). Subsequent studies have shown that BP remains the same in both groups for up to 18 wk (personal observation). No difference in HR between the two groups was observed at any time during the study.

Changes in in vitro contractile function. At 6 wk and again at 9 wk half of the rats in both groups were sacrificed for evaluation of cardiac and vascular muscle contractile function in vitro. Changes in cardiac contractility were evaluated by examining the response of Langendorff-perfused hearts from the

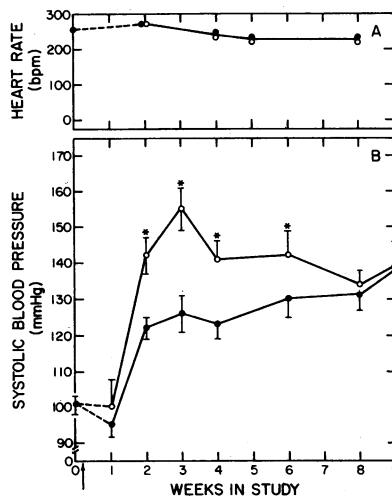


Figure 3. Age-dependent changes in systolic BP (mmHg) and HR (bpm) in the vitamin D₃-deficient rats maintained on a diet containing 0.4% calcium and 0.4% phosphate (○), and vitamin D₃-sufficient rats maintained on the same diet (●). Each symbol represents the mean±SEM for 5–8 rats. *P < 0.05.

D⁻ and D⁺ rats to changes in extracellular Ca. Changes in vascular muscle contractility were evaluated by examining the response of isolated aortic rings from the D⁻ and D⁺ rats to increasing concentrations of exogenous norepinephrine.

Changes in cardiac contractile function. After 6 wk of vitamin D₃ depletion, a slight increase in the rate of pressure development (+dP/dt) in isolated hearts from the D⁻ rats was observed (Fig. 4 A). In addition, a slight increase in the rate of relaxation (-dP/dt) was also noted (Fig. 4 B). These increases, however, were not statistically significant (P > 0.05). After 9 wk on the vitamin D₃-deficient diet, however, large and statistically significant increases in +dP/dt and -dP/dt were observed in the hearts from the D⁻ rats compared with hearts from the D⁺ rats (Fig. 5, A and B). In both groups the percent increase in +dP/dt and -dP/dt after each incremental elevation of extracellular calcium was comparable, and the shape of the calcium-contractility concentration-response curves over the same concentration range was identical for both the D⁻ and D⁺ hearts. As shown in Fig. 5, A and B, the major difference between the two groups appears to be the magnitude of the response evoked by extracellular calcium.

Changes in vascular contractile function. Fig. 6, A and B illustrate the sensitivity of aortic rings from the D⁺ and D⁻ rats to exogenous norepinephrine following 6 and 9 wk of vitamin

Table I. Levels of 1,25(OH)₂D₃ and 25(OH)₂D₃ in Serum From Vitamin D₃-Deficient and Vitamin D₃-Sufficient Rats

Group	Serum 1,25-(OH) ₂ D ₃	Serum 25-(OH) ₂ D ₃
	pgm/ml	ngm/ml
Vitamin D ₃ -deficient rats		
6 wk	*	*
9 wk	*	*
Vitamin D ₃ -sufficient rats		
6 wk	132.5	9.5
9 wk	97.0	14.1

Each value represent the level of 1,25-(OH)₂D₃ or 25-(OH)₂D₃ in pooled serum from five rats.

* Below the level of detection (10 pgm/ml for 1,25(OH)₂D₃ and 0.1 ngm/ml for 25(OH)₂D₃).

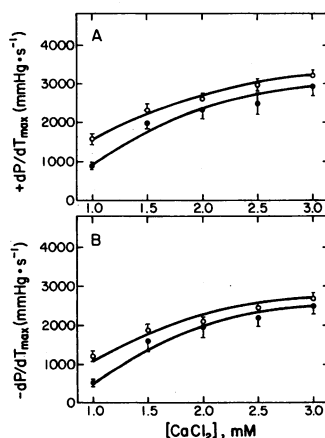


Figure 4. Changes in +dP/dt (A) and -dP/dt (B) in response to changes in extracellular calcium in isolated, Langendorff perfused hearts from vitamin D₃-deficient rats maintained on a diet containing 0.4% calcium and 0.4% phosphate (○), and vitamin D₃-sufficient rats maintained on the same diet (●) after 6 wk of study. Each symbol represents the mean±SEM of 4–6 hearts from each group. No significant differences were observed between the two groups.

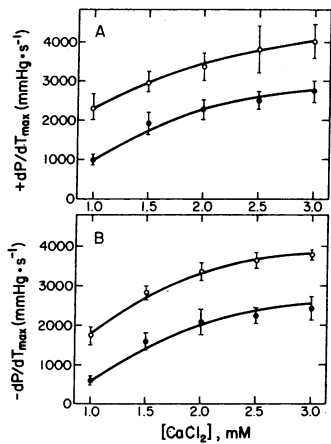


Figure 5. Changes in $+dP/dt$ (A) and $-dP/dt$ (B) in response to changes in extracellular calcium in isolated, Langendorff perfused hearts from vitamin D_3 -deficient rats maintained on a diet containing 0.4% calcium and 0.4% phosphate (\circ), and vitamin D_3 -sufficient rats maintained on the same diet (\bullet) after 9 wk of study. Each symbol represents the mean \pm SEM of 5–7 hearts from each group. A statistically significant difference ($P < 0.05$) was observed between the two groups.

D_3 depletion. As can be seen, no difference in norepinephrine sensitivity was observed between the two groups after 6 wk on the vitamin D_3 -deficient diet (Fig. 6 A). In both groups, the EC_{50} values for norepinephrine were roughly 30 nM. Because serum Ca in the D^- rats was reduced by 50% at this time, these experiments were repeated in PSS containing 0.8 mM Ca instead of the usual 1.6 mM calcium. As before, no differences in norepinephrine sensitivity were observed between the two groups (data not shown).

After 9 wk of vitamin D_3 depletion, a significant increase in the sensitivity of aortic rings from the D^- rats to exogenous norepinephrine was observed (Fig. 6 B). The EC_{50} values for norepinephrine were 25 nM for the aortae from the D^+ rats and 9 nM for aortae from the D^- rats.

Changes in the magnitude of the contractile response of aortae from the vitamin D_3 -deficient and vitamin D_3 -sufficient rats to norepinephrine are shown in Fig. 7, A and B. As can be seen, norepinephrine evoked comparable increases in tension in aortae from both groups after 6 wk of vitamin D_3 depletion (Fig. 7 A). However, after 9 wk on the vitamin D_3 -deficient diet, norepinephrine produced much greater increases in tension in the aortae from the D^- rats compared with the D^+ rats (0.542 ± 0.038

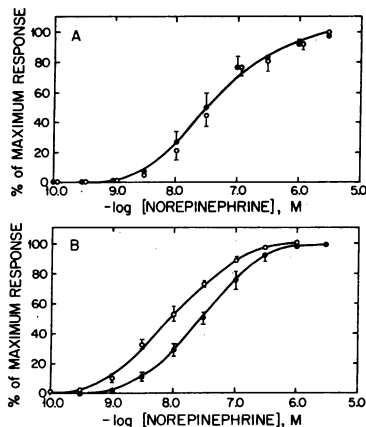


Figure 6. Changes in the sensitivity of isolated aortic rings to exogenous norepinephrine in vitamin D_3 -deficient rats maintained on a diet containing 0.4% calcium and 0.4% phosphate (\circ), and vitamin D_3 -sufficient rats maintained on the same diet (\bullet) after 6 wk (A) or 9 wk (B) of study. Each symbol represents the mean \pm SEM of 4–6 rings from each group. Statistically significant differences ($P < 0.05$) were observed after 9 wk of vitamin D_3 depletion but not after 6 wk of vitamin D_3 -depletion.

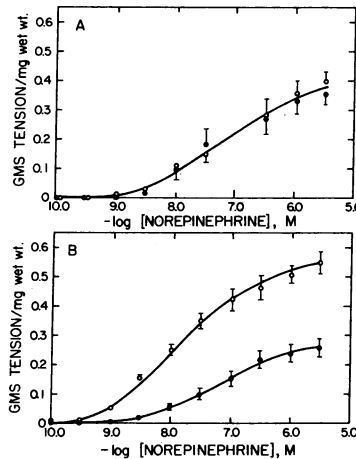


Figure 7. Changes in the magnitude of the response of isolated aortic rings to exogenous norepinephrine in vitamin D_3 -deficient rats maintained on a diet containing 0.4% calcium and 0.4% phosphate (\circ), and vitamin D_3 -sufficient rats maintained on the same diet (\bullet) after 6 wk (A) or 9 wk (B) of study. Each symbol represents the mean \pm SEM of 4–6 rings from each group. Statistically significant differences ($P < 0.05$) were observed after 9 wk of vitamin D_3 depletion but not after 6 wk of vitamin D_3 depletion.

g/mg wet wt. for the D^- rats vs. 0.258 ± 0.027 g/mg wet wt. for the D^+ rats) (Fig. 7 B).

Direct/indirect effects of vitamin D_3 on cardiac contractile function. To determine whether vitamin D_3 exerts a direct effect on cardiac contractile function or whether its effect represents a secondary response to the hypocalcemia that accompanies vitamin D_3 depletion, it was necessary to develop a vitamin D_3 -deficient diet in which serum Ca and PO_4 are maintained at normal or near-normal levels. For these studies diets containing varying amounts of Ca and PO_4 were fed to vitamin D_3 -deficient rats and serum levels of Ca and PO_4 were recorded.

Table II illustrates the steady-state levels of serum Ca and PO_4 obtained after long-term administration of the various diets to vitamin D_3 -deficient rats, compared with levels obtained in rats maintained on the vitamin D_3 -sufficient diet containing 0.4% Ca and 0.4% PO_4 . Although increasing the amount of Ca in the vitamin D_3 -deficient diet from 0.4% to 2.5% normalized serum Ca, failure to also increase the amount of PO_4 in the diet results in profound hypophosphatemia (Table II). This reduction is not significantly altered by increasing the level of PO_4 in the diet from 0.4% to 1.0%. Increasing PO_4 content to 1.5%, however, was found to prevent hypophosphatemia in the vitamin D_3 -de-

Table II. Levels of Serum Electrolytes after Chronic Administration of Diets Varying in Calcium and Phosphate Composition to Vitamin D_3 -Sufficient and Vitamin D_3 -Deficient Rats

Vitamin D_3	Diet composition		Serum electrolytes	
	% Calcium	% Phosphate	Calcium mg/dl	Phosphate mg/dl
+	0.4	0.4	10.3 ± 0.2 (47)	5.8 ± 0.2 (75)
-	0.4	0.4	5.6 ± 0.2 (32)	6.3 ± 0.2 (66)
-	2.5	0.4	9.7 ± 0.5 (16)	2.3 ± 0.3 (31)
-	2.5	1.0	9.9 ± 0.4 (15)	2.6 ± 0.2 (27)
-	2.5	1.5	9.0 ± 0.4 (14)	5.6 ± 0.2 (31)

Data represent the mean \pm SEM of the number of samples in parentheses.

ficient diet containing 2.5% Ca. This latter diet was therefore used for all subsequent studies.

To assess the possible involvement of hypocalcemia with the increases in cardiac contractile function which accompany vitamin D₃ depletion, rats were placed on the vitamin D₃-deficient diet containing high Ca (2.5%) and high PO₄ (1.5%) for 9 wk to determine whether maintenance of serum Ca during the period of vitamin D₃ depletion could prevent these changes.

After 9 wk of vitamin D₃ depletion, rats maintained on either the 0.4% Ca, 0.4% PO₄ diet (D⁻, 0.4% Ca) or the 2.5% Ca, 1.5% PO₄ diet (D⁻, 2.5% Ca) were sacrificed and the contractile response of isolated Langendorff-perfused hearts to increasing concentrations of extracellular Ca was evaluated. For comparative purposes, the response of isolated hearts from rats maintained for 9 wk on the vitamin D₃-sufficient diet (D⁺, 0.4% Ca) was also evaluated. As Fig. 8 illustrates, large and statistically significant increases in +dP/dt were again observed in hearts from the D⁻, 0.4% Ca rats compared with hearts from the D⁺, 0.4% Ca rats. Fig. 8 also illustrates that similar increases were also observed in the D⁻, 2.5% Ca rats. Because this latter diet raised serum Ca to near-normal levels in the vitamin D₃-deficient rats, these results suggest that this change represents a direct response to vitamin D₃ depletion, rather than a secondary response to the hypocalcemia that normally accompanies vitamin D₃ depletion.

Discussion

Recently, Simpson et al. identified a specific receptor for 1,25(OH)₂D₃, the metabolically active form of vitamin D₃, in heart cell preparations (11, 12). Walters and co-workers subsequently demonstrated a receptor for 1,25(OH)₂D₃ in normal rat hearts (13), and Stumpf et al., using autoradiographic techniques, demonstrated nuclear receptors for 1,25(OH)₂D₃ in rat heart cells (23). Thomasset et al. have also shown that myocardial tissue contains a 10,000 K vitamin D₃-dependent Ca-binding protein that is immunochemically similar to that found in intestine (10). Given the close relationship between changes in Ca homeostasis in cardiac muscle and changes in myocardial con-

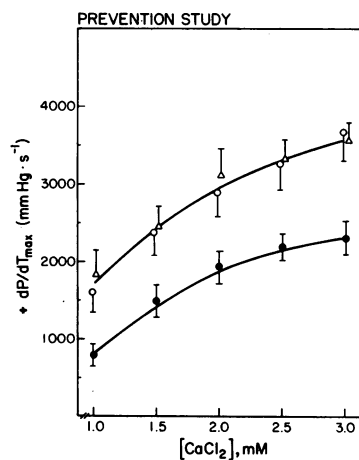


Figure 8. Changes in +dP/dt in response to changes in extracellular calcium in isolated, Langendorff perfused hearts from vitamin D₃-deficient rats maintained for 9 wk on a low (0.4%) calcium, low (0.4%) phosphate diet (○), from vitamin D₃-deficient rats maintained for 9 wk on a high (2.5%) calcium, high (1.5%) phosphate diet (Δ), and from vitamin D₃-sufficient rats maintained for 9 wk on the low calcium, low phosphate diet (●). Each symbol represents the mean ± SEM of 5–7 hearts from each group.

Statistically significant differences ($P < 0.05$) were observed between the vitamin D₃-sufficient rats and both groups of vitamin D₃-deficient rats. No statistically significant differences were observed between the two groups of vitamin D₃-deficient groups.

tractility, these results indicate that vitamin D₃ may play a direct role in regulating cardiovascular function.

Little information is presently available regarding the direct influence of vitamin D₃ on cardiovascular function under normal conditions or conditions such as ischemia, heart failure, or hypertension, where changes in cellular Ca homeostasis are apparent. In skeletal muscle, vitamin D₃ depletion is associated with a prolongation of muscle contraction kinetics (24). This change is apparently not the result of hypocalcemia or alterations in parathyroid hormone metabolism. Wassner et al. have also found that vitamin D₃ depletion increases skeletal muscle degradation (25). Several investigators have described a specific cardiomyopathy associated with end-stage renal disease (26, 27). Administration of either 25(OH)D₃ (28) or 1- α -hydroxyvitamin D₃ (27) has been shown to improve left ventricular function in these patients. Whether this improvement was due to restoration of a 1,25(OH)₂D₃-dependent metabolic process in cardiac muscle or the ability of 1,25(OH)₂D₃ to suppress the hyperparathyroidism observed in these patients is not known.

In the present study, vitamin D₃ deficiency was associated with significant changes in cardiovascular function, including increases in cardiac and vascular muscle contractile responses and systolic BP. The changes in cardiac contractile function are of interest in that whereas only modest differences in cardiac contractile function were apparent after 6 wk of vitamin D₃ depletion, after 9 wk a profound increase in cardiac contractility was observed in the vitamin D₃-deficient rats. This change in contractile function appears to represent the direct response to vitamin D₃ depletion and not a response to hypocalcemia that accompanies depletion, because increasing serum Ca from 5.6 to 9.0 mg/dl during the 9-wk period of vitamin D₃ depletion does not prevent the change in cardiac contractile function. The possibility exists, however, that hypocalcemia could still contribute to the changes in ventricular contractile function observed in the vitamin D₃-deficient rats, because rats placed on the 2.5% Ca vitamin D₃-deficient diet remained slightly hypocalcemic compared to rats on the vitamin D₃-sufficient diet (9.0 vs. 10.3 mg/dl).

In addition to an increase in the rate of myocardial pressure development, hearts from the vitamin D₃-deficient rats also displayed an increase in the rate of myocardial relaxation. Katz and others have suggested that the rate of myocardial relaxation is closely associated with the rate of Ca sequestration by the sarcoplasmic reticulum (29–31), possibly indicating that vitamin D₃ deficiency can increase the rate at which such sequestration occurs. Likewise, an increase in the rate of Ca release by the sarcoplasmic reticulum may represent the basis for the increase in contractility observed in the hearts of the vitamin D₃-deficient rats, because Ca release by the sarcoplasmic reticulum represents the principle initiating event in myocardial contraction (30, 31). Fabiato and Fabiato have shown that Ca release by the sarcoplasmic reticulum is triggered by an increase in cytosolic free Ca (30), which occurs during each depolarization when Ca enters the cell through the Ca slow channel.

An alternative explanation for the increase in myocardial contractility in the vitamin D₃-deficient rats is the absence of 1,25(OH)₂D₃-dependent Ca-binding proteins, which under normal conditions might serve as a Ca buffer in cardiac muscle. Thomasset et al. has previously demonstrated that cardiac muscle from rats maintained on a vitamin D₃-sufficient diet contains a 1,25(OH)₂D₃-dependent Ca-binding protein immunochemically similar to that found in intestinal cells (10), and it has been

suggested that this protein may play an important role in calcium handling in a number of organs and cells (32, 33). The loss of such a protein in cardiac muscle following vitamin D₃ depletion might allow the cytosolic free Ca level to rise to a higher than normal level after each depolarization, thereby triggering the release of a greater quantity of Ca from the sarcoplasmic reticulum, resulting in a greater contraction. Both alternative explanations are currently under investigation in our laboratory.

Vitamin D₃ depletion also resulted in profound changes in vascular muscle contractile function. These changes temporally paralleled the changes in cardiac muscle contractility, in that whereas no significant differences in the contractile function of isolated aortic rings were observed after 6 wk of vitamin D₃ depletion, after 9 wk large differences in vascular contractility were observed between the vitamin D₃-deficient and vitamin D₃-sufficient groups. The direct/indirect role of vitamin D₃ in modulating these changes in vascular contractility are not currently known, but preliminary evidence suggests the hypocalcemia may play a more important role in modulating these changes than vitamin D₃ (manuscript in preparation).

Whereas the changes in vascular and cardiac contractility may represent direct alterations in calcium homeostasis in cardiac and vascular muscle, the basis for the change in BP that accompanies vitamin D₃ depletion is not clear. It is noteworthy that the increase in blood pressure in the vitamin D₃-deficient rats coincided with the development of hypocalcemia in these animals. McCarron has previously demonstrated that hypocalcemia is associated with increases in BP, both in patients and experimental animals (34). Administration of Ca to young spontaneously hypertensive rats has also been shown to attenuate the development of hypertension in these animals (35), and will reverse "fixed" hypertension in adult animals (36). However, while serum Ca remained reduced throughout the present study, the increase in BP in the vitamin D₃-deficient rats was transient. This observation suggests that a compensatory mechanism exists which is capable of overcoming the initial hypertensive response. One such compensatory mechanism might be an increase in serum PO₄ which can accompany vitamin D₃ depletion (24, 25). Lau et al. have previously reported a strong correlation between increases in serum PO₄ and hypotension (37). However, in the present study only slight increases in serum PO₄ were observed in the vitamin D₃-deficient rats, which were never more than 15% above levels in the vitamin D₃-sufficient rats.

A significant increase in serum CPK was also observed in the vitamin D₃-deficient rats, which is indicative of muscle damage in these animals. Because only total CPK was measured in the present study, it cannot be said for certain whether the increase in serum CPK in the vitamin D₃-deficient rats is due to damage to myocardial or skeletal muscle or both. As with the increase in BP, the increase in serum CPK appeared to correlate with the onset of hypocalcemia, suggesting that both events may represent a metabolic consequence of the reduction in circulating Ca. The subsequent decline in serum CPK, like the return of BP to normotensive levels, suggests the presence of adaptive mechanisms to compensate for the initial insult.

The present study demonstrates that vitamin D₃ deficiency produces profound changes in cardiovascular function, including heightened contractile responses of isolated cardiac and vascular smooth muscle, hypertension, and elevation of serum CPK. These observations indicate that vitamin D₃ plays an important role in maintaining normal cardiovascular function. This involvement appears to be a direct one in cardiac muscle. However,

in vascular muscle the change in function may be mediated by hypocalcemia secondary to vitamin D₃ deficiency (manuscript in preparation). Whether changes in vitamin D₃ also play an important role in cardiovascular disease states is not known. It is important to note that there is a decline in circulating levels of both 1,25(OH)₂D₃ and Ca with increasing age (38), and a correlation between increased BP and hypocalcemia has been demonstrated in patients and animal models of hypertension (34). In addition, the possibility exists that vitamin D₃ may play a role in the changes in cardiovascular function that can accompany diabetes (39), lengthy bedrest or immobilization (40, 41), or prolonged periods of weightlessness (41), because circulating levels of 1,25(OH)₂D₃ are reduced by these conditions (42-45). In conclusion, the results of this study strongly suggest that the vitamin D₃-endocrine system contributes to the regulation of cardiovascular function, either directly as is the case with cardiac muscle or indirectly through the effects of 1,25(OH)₂D₃ on Ca or other circulating factors.

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