

Involvement of 1,25-dihydroxyvitamin D₃ in regulating myocardial calcium metabolism: Physiological and pathological actions

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ABSTRACT — The role of the prohormone vitamin D₃ in regulating calcium and phosphate metabolism in the intestine, kidney, and bone has been known for several decades [1]. Recent studies have provided evidence that vitamin D₃ may also play an important role in regulating metabolism in other organs, including heart [2]. This role has been suggested by the identification of a specific receptor for 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], the active metabolite of vitamin D₃, in these tissues [3-5], as well as the presence of a 1,25(OH)₂D₃-dependent calcium binding protein [6, 7].

Although administration of excessive quantities of vitamin D₃ has been shown in many studies to produce myocardial calcinosis and heart failure [8, 9], the importance of vitamin D₃ in regulating myocardial metabolism under normal conditions has only recently been demonstrated [10, 11]. The purpose of the present review is to assess the current status of research regarding the pathological and physiological actions of vitamin D₃ on the heart. The initial section of this report will focus on the pathological effects of excessive vitamin D₃ on cardiovascular function, while the latter sections will describe recent studies related to the involvement of 1,25(OH)₂D₃ in regulating calcium homeostasis in ventricular cells and the relationship between vitamin D₃ and myocardial contractility.

Historical role of vitamin D₃ and the myocardium

Nearly fifty years ago Seyle [12] demonstrated that administration of very high doses of vitamin D₃ induced calcification in both cardiac and vascular muscle, resulting in a condition similar to Mönckeberg's sclerosis. Wrzolkowa and Zydowo [13] later showed that in rats hypervitaminosis D₃ produced a complex pattern of myocardial damage, including proliferation of endoplasmic reticulum, a

decrease in myofibril number, and appearance of calcium deposits and myelin-like structures. In vascular smooth muscle, hypervitaminosis D₃ was associated with calcification of elastic tissue in the medial layer [9]. Frey and coworkers [14] have shown that in rats a single toxic dose of vitamin D₃ administered intramuscularly can increase the rate of calcium uptake into the wall of the mesenteric artery by 35 to 40 times within three days. The authors also showed that toxic doses of vitamin D₃ lead to an increase in the rate of calcium uptake by the

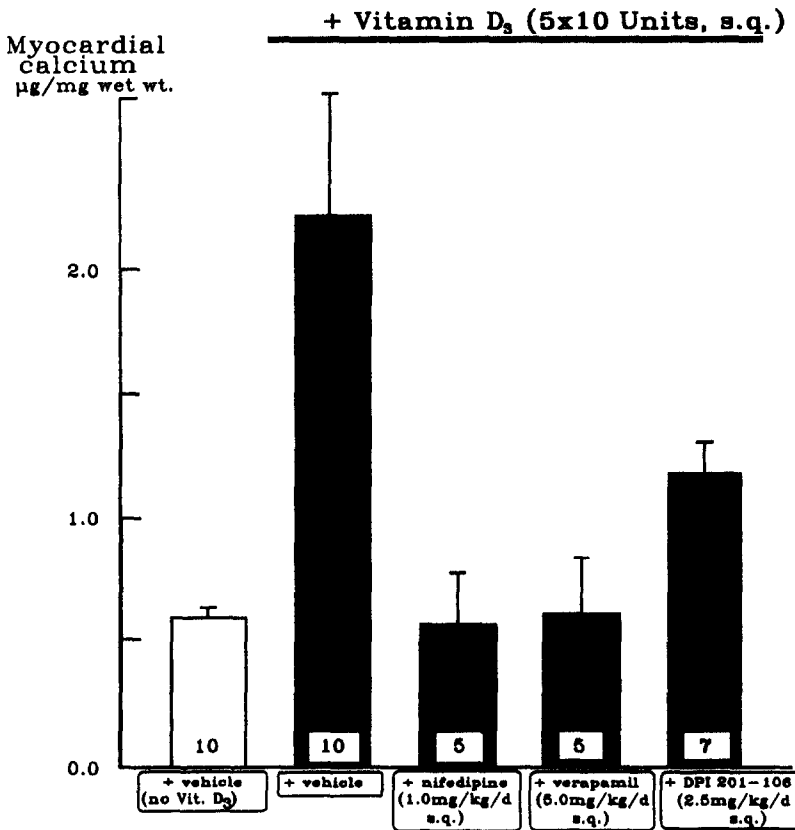


Fig. 1 Inhibition of vitamin D₃-induced myocardial calcium accumulation in rats by the calcium channel blockers nifedipine, verapamil, and DPI 201-106. For these studies rats received a single intramuscular injection of vitamin D₃ or vehicle (corn oil), and were sacrificed five days later. The calcium channel blockers were administered twice daily throughout the study. Myocardial calcium was measured using atomic absorption spectrometry as described previously by Weishaar et al. [17]

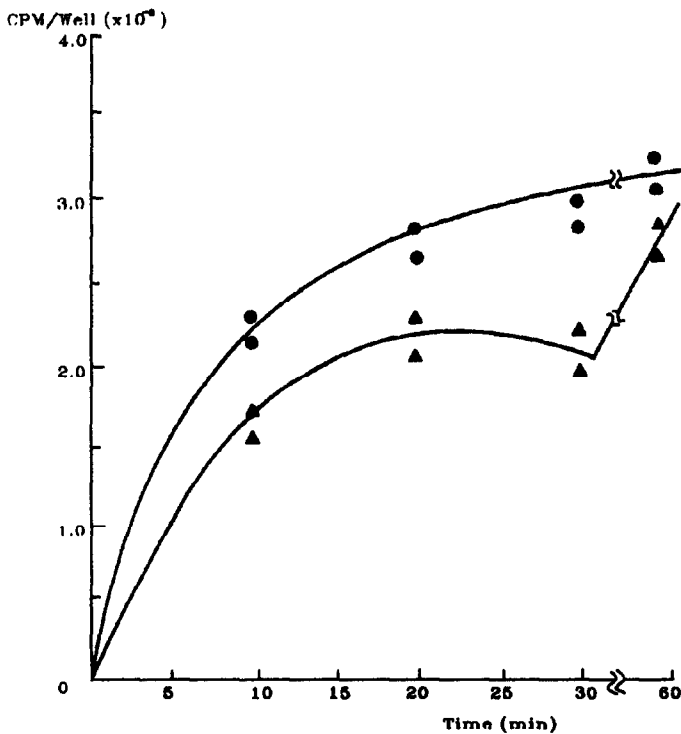
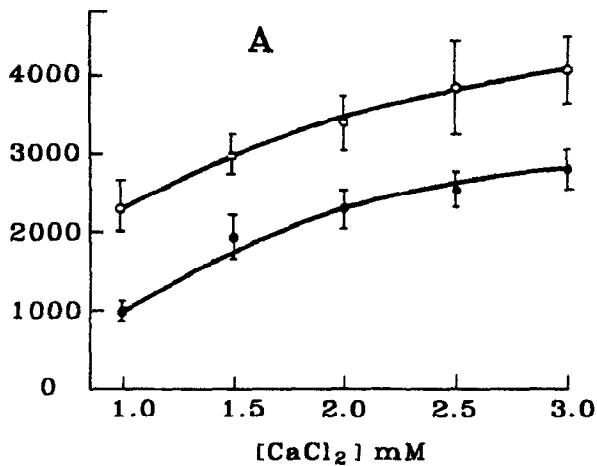
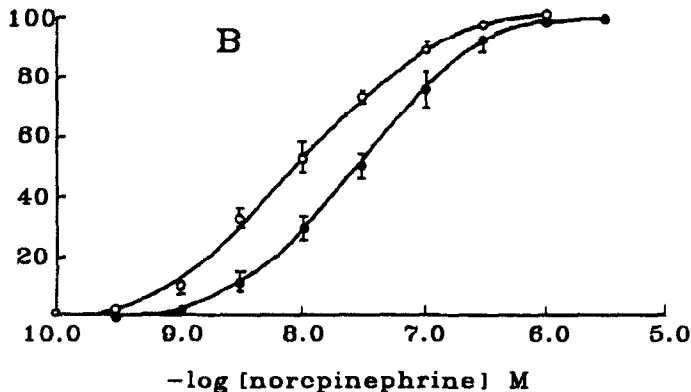


Fig. 2 Time course for ⁴⁵Ca uptake in primary culture of spontaneously beating heart cells. Cells were maintained in Dulbecco's modified Eagles media supplemented with 10% fetal calf serum and antibiotics in Costar multi-well (16 mm diameter) flasks. Cells were treated with 5 nM 1,25(OH)₂D₃ (●) or 0.1% ethanol (▲) for 24 hours. Calcium uptake was initiated by addition of 0.2 Ci/ml ⁴⁵CaCl₂ in Krebs-Ringer buffer. ⁴⁵Ca associated with heart cells was assessed by stopping the reaction with three successive rinses at noted times using Krebs-Ringer buffer. All wells contained similar cell numbers. Data are representative of four separate experiments

$+dP/dt_{\max} (\text{mmHg} \cdot \text{s}^{-1})$


% of max. response


Fig. 3

Panel A. Changes in $+dP/dt$ in response to changes in extra-cellular calcium in isolated, Langendorff perfused hearts from vitamin D₃-deficient rats maintained for nine weeks 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 five to seven hearts from each group. Statistically significant differences ($P < 0.05$) were observed between the two groups

Panel B. Changes in the sensitivity of isolated aortic rings to exogenous norepinephrine in vitamin D₃-deficient rats maintained for nine weeks 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 four to six rings from each group. Statistically significant differences ($P < 0.05$) were observed between the two groups

aorta and the ventricle, although the increases were somewhat smaller than in the mesenteric artery [14]. Fleckenstein et al. [15] have also shown similar increases in arterial calcium in the elderly, and have suggested that the vitamin D₃-intoxicated rat represents a useful animal model of human pathology.

The mechanism responsible for the pathological changes which accompany hypervitaminosis D₃ is thought to be the substantial increase in circulating calcium which accompanies administration of excessive vitamin D₃ [13, 16], rather than a direct effect of vitamin D₃ on cardiac or vascular muscle metabolism. In support of this hypothesis, Figure 1 shows that in rats the large increase in myocardial calcium which occurs following administration of excessive amounts of vitamin D₃ can be markedly attenuated by treatment with the calcium channel

blockers nifedipine, verapamil, or DPI 201-106. Fleckenstein has also shown that the increase in ⁴⁵Ca uptake into vascular smooth muscle from vitamin D₃-intoxicated rats can be prevented by verapamil or the calcium channel blocker diltiazem [15].

Although these studies suggest that vitamin D₃ can influence cardiac and vascular muscle metabolism at pharmacological doses, they provide little insight into the involvement of vitamin D₃ under physiological conditions. Recent studies in rats, however, have shown that vitamin D₃-deficiency results in profound changes in the contractility of cardiac, vascular, and skeletal muscle, suggesting that vitamin D₃ or its metabolite 1,25(OH)₂D₃ plays an important role in maintaining normal contractile function [10, 11, 18].

In the paragraphs to follow, the basis for an

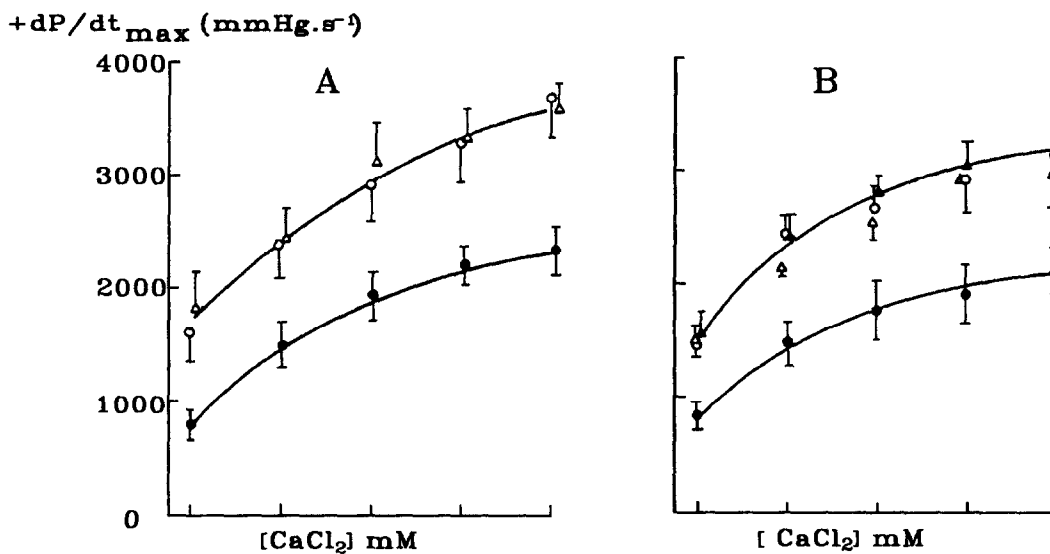


Fig. 4 Panel A. Changes in $+dP/dt$ in response to changes in extracellular calcium in isolated, Langendorff-perfused hearts from rats maintained for nine weeks on: (1) a vitamin D_3 -deficient diet containing 0.4% calcium and 0.4% phosphate (\circ); (2) a vitamin D_3 -deficient diet containing 2.5% calcium and 1.5% phosphate (Δ); or (3) a vitamin D_3 -sufficient diet (\bullet). Each symbol represents the mean \pm SEM of five to seven hearts from each group. A statistically significant difference ($P < 0.05$) was observed between the two vitamin D_3 -deficient groups and the vitamin D_3 -sufficient group

involvement of vitamin D_3 in regulating cardiovascular function under physiological conditions will be presented.

Effects of 1,25-dihydroxyvitamin D_3 on cultured cardiac cells

The observation that $1,25(OH)_2D_3$ receptors exist in heart muscle suggests that myocardial cells may represent a target for $1,25(OH)_2D_3$ regulation. It is now recognized that $1,25(OH)_2D_3$ modulates calcium uptake and handling in various cell types such as intestine, bone, blood, and pancreas [2, 19]. Thus, we wished to determine if $1,25(OH)_2D_3$ alters calcium uptake in cultured, beating rat cardiac myocytes.

Rat heart myocytes were isolated and placed in culture by methods previously described [20]. The resulting preparation reproducibly yielded static

Panel B. Changes in $+dP/dt$ in response to changes in extracellular calcium in isolated, Langendorff-perfused hearts from: (1) rats maintained for 18 weeks on a vitamin D_3 -sufficient diet (\bullet); (2) rats maintained for 18 weeks on a vitamin D_3 -deficient diet containing 0.4% calcium and 0.4% phosphate (\circ); and (3) rats initially maintained for nine weeks on a vitamin D_3 -deficient diet containing 0.4% calcium and 0.4% phosphate and subsequently maintained for an additional nine weeks on either a vitamin D_3 -deficient diet containing 2.5% calcium and 1.5% phosphate (Δ) or on a vitamin D_3 -sufficient diet (\blacktriangle). Each symbol represents the mean \pm SEM of four to seven hearts from each group. A statistically significant difference ($P < 0.05$) was observed between the three groups originally maintained on vitamin D_3 -deficient diet and vitamin D_3 -sufficient group

cultures of spontaneously beating myocytes four days post isolation. Figure 2 shows the effects of inclusion of 5 nM $1,25(OH)_2D_3$ in the culture medium for 24 hours on ^{45}Ca uptake by cultured heart cells. We have observed small but reproducible increases in calcium uptake in several similar experiments. We have also noted that this increase was not observed after four hours treatment, suggesting that the action may require *de novo* synthesis of proteins as expected of steroid hormones. These data are consistent with those recently reported by Walters et al. [21]. Importantly, these data are consistent with our observation of activities and receptors for $1,25(OH)_2D_3$ being present in heart cells. Much work remains to be done to determine if the

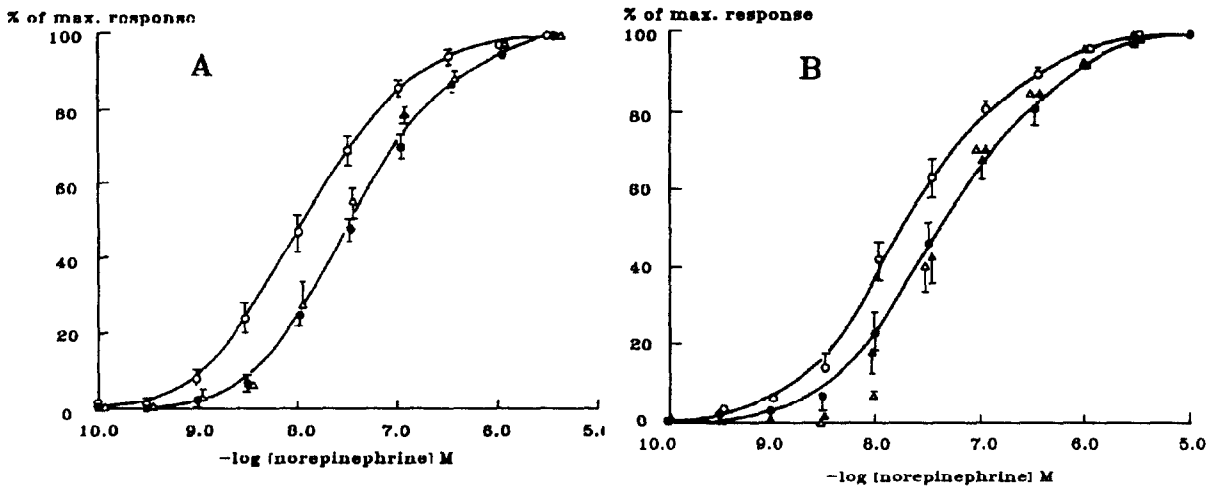


Fig. 5

Panel A. Changes in sensitivity to exogenous norepinephrine of isolated aortic rings from rats maintained for nine weeks on: (1) a vitamin D₃-deficient diet containing 0.4% calcium and 0.4% phosphate (○); (2) a vitamin D₃-deficient diet containing 2.5% calcium and 1.5% phosphate (Δ); or (3) a vitamin D₃-sufficient diet (●). Each symbol represents the mean \pm SEM of nine or more different aortic rings. A statistically significant difference ($P < 0.05$) was observed between the response of rings from rats maintained for nine weeks on vitamin D₃-deficient diet containing 0.4% phosphate and the other two groups

observed alterations of heart cell calcium handling induced by 1,25(OH)₂D₃ is related to the alteration of myocardial function in animals depleted of endogenous 1,25(OH)₂D₃ [10, 11] that will be described in the following sections.

Effects of chronic vitamin D₃-deficiency on cardiac and vascular smooth contractility

Two approaches were taken to evaluate the involvement of vitamin D₃ with regulating cardiac and vascular muscle contractile function. In the first approach, isolated tissues were removed from vitamin D₃-replete rats and exposed to increasing concentrations of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃]. In the second approach, rats were depleted of endogenous vitamin D₃ after which tissues were removed and contractile function evaluated. Although 1,25(OH)₂D₃ has no direct effect on the contractility of isolated cardiac and vascular muscle from vitamin D₃-sufficient rats at

Panel B. Changes in sensitivity to exogenous norepinephrine of isolated aortic rings from: (1) rats maintained for 18 weeks on a vitamin D₃-sufficient diet (●); (2) rats maintained for 18 weeks on a vitamin D₃-deficient diet containing 0.4% calcium and 0.4% phosphate (○); and (3) rats initially maintained for nine weeks on a vitamin D₃-deficient diet containing 0.4% calcium and 0.4% phosphate and subsequently maintained for an additional nine weeks on either a vitamin D₃-deficient diet containing 2.5% calcium and 1.5% phosphate (Δ) or on a vitamin D₃-sufficient diet (▲). Each symbol represents the mean \pm SEM of five to seven different aortic rings. A statistically significant difference ($P < 0.05$) was observed between the response of rings from rats maintained for 18 weeks on vitamin D₃-deficient diet containing 0.4% calcium and 0.4% phosphate and other three groups

concentrations as high as 1.0 μ M (data not shown), maintenance of rats on a vitamin D₃-deficient diet for nine weeks resulted in significant increases in cardiac and vascular muscle contractile function (Figs 3A, 3B). These increases suggest that endogenous vitamin D₃ plays an important role in maintaining normal contractile function. Vitamin D₃-deficiency was also associated with transient increases in systolic blood pressure and circulating creatine phosphokinase [10]. These latter changes appeared to coincide with the decrease in circulating calcium observed in the vitamin D₃-deficient rats.

To determine whether the changes in cardiac and vascular muscle contraction function which accompany vitamin D₃-deficiency represent a direct response to vitamin D₃ or to the hypocalcemia which accompanies depletion of endogenous vitamin D₃, rats were placed on a vitamin D₃-deficient diet containing high calcium to

Table I Effect of vitamin D₃-deficiency on the heart weight/body weight ratio and on myocardial collagen content

<i>Group</i>	<i>Heart Wt/Body Wt</i> (x1000)	<i>Myocardial collagen</i> (μ g hydroxyproline/g wet wt)
Vitamin D ₃ -sufficient Normocalcemic	3.06 \pm 0.09 [14]	244.3 \pm 13.5 [18]
Vitamin D ₃ -deficient Hypocalcemic	3.50 \pm 0.24 [10]*	310.3 \pm 12.4 [17]*
Vitamin D ₃ -deficient Normocalcemic	3.71 \pm 0.21 [6]*	306.0 \pm 41.3 [10]*

Values represent the mean \pm S.E.M. of the number of separate samples shown in parentheses
* $P < 0.05$, compared to the vitamin D₃-sufficient group

maintain circulating levels of calcium in the normal range [11]. As Figure 4A shows, preventing hypocalcemia in vitamin D₃-deficient rats did not prevent the increase in cardiac contractile function in the vitamin D₃-deficient rats. In addition, transfer of vitamin D₃-deficient, hypocalcemic rats to the vitamin D₃-deficient, high calcium diet did not reverse the changes in cardiac contractility (Fig. 4B). In contrast, the changes in vascular muscle contractile function in the vitamin D₃-deficient rats could be prevented by preventing hypocalcemia and reversed by restoring circulating calcium to normal levels (Figs 5A, 5B).

The possibility that vitamin D₃-deficiency altered cardiac contractile function by influencing the physical or morphological properties of the heart was evaluated by examining changes in the heart weight/body weight ratio, and changes in myocardial collagen in the vitamin D₃-deficient rats. Vitamin D₃-deficiency was associated with a significant increase in the heart weight/body weight ratio, which could not be blocked by preventing hypocalcemia [11]. This increase was also accompanied by an increase in myocardial collagen. These changes are illustrated in Table I. As with the increase in cardiac contractility, the increases in heart weight/body weight ratio and in myocardial collagen in the vitamin D₃-deficient rats could not be blocked by preventing hypocalcemia, indicating

that the increase represented a direct response to vitamin D₃-deficiency.

The relationship between the vitamin D₃-dependent increases in the heart weight/body weight ratio and in myocardial collagen, and the increases in cardiac contractile function which accompany vitamin D₃-deficiency is unclear at the present time. This relationship is complicated by the fact that at least five genetically distinct subclasses of collagen has been characterized, which can respond independently of each other [22]. Interestingly, Raisz and coworkers [23] have shown that in cultured bone cells from vitamin D₃-replete rat fetuses low concentrations of vitamin D₃ metabolites inhibit collagen synthesis. The rate of collagen synthesis is also enhanced in cartilage from vitamin D₃-deficient chicks [24].

Summary and Conclusions

The purpose of this review has been to summarize current concepts regarding the involvement of vitamin D₃ in the regulation of myocardial metabolism, and to distinguish the pathological and physiological actions of vitamin D₃ on the heart. Although early investigations in this area demonstrated that administration of excessive amounts of vitamin D₃ produced calcification of

cardiac and vascular smooth muscle, these studies employing supranormal quantities of vitamin D₃, making it difficult to assess the potential involvement of vitamin D₃ in modulating myocardial metabolism under physiological conditions.

Recently, Simpson et al. [3, 4] identified a specific receptor for 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], the biologically active form of vitamin D₃, in cardiac cells. Thomasset and coworkers [7] have also shown that myocardial tissue contains a vitamin D₃-dependent calcium binding protein. In addition, low concentrations of 1,25(OH)₂D₃ have been shown here and by others to stimulate the uptake of ⁴⁵Ca into cultured cardiac cells [21]. The relevance of these findings is supported by the observation that depletion of endogenous vitamin D₃ is associated with a number of changes in cardiovascular metabolism, including increases in cardiac and vascular muscle contractile function, changes in the physical and morphological properties of the heart, and transient increases in systolic blood pressure. Although some of these changes clearly represent a response to the hypocalcemia which accompanies vitamin D₃-deficiency, others such as the increase in cardiac contractility and the increase in myocardial collagen content, represent a direct response to vitamin D₃-deficiency. Studies are currently underway to assess the possible involvement of parathyroid hormone in these latter changes.

These new findings add additional support for the involvement of the endocrine system in modulating cardiovascular metabolism. In addition, the possibility also exists that vitamin D₃ may play an important role in the changes in cardiovascular function which accompany diabetes [25], lengthy bed rest or immobilization [26], or prolonged periods of weightlessness [27], since circulating levels of 1,25(OH)₂D₃ are also reduced in these conditions.

Acknowledgements

This research was supported by a grant-in-aid from the American Heart Association, with funds contributed in part by the Michigan Heart Association (Grant 85-846), by the National Dairy Board, administered in cooperation with the National Dairy Council, and by the National Institutes of Health (Grant CA-36507).

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Received : 8 August 1988

Accepted : 9 September 1988