



Inhibition of GTPase Rac1 expression by vitamin D mitigates pressure overload-induced cardiac hypertrophy

Ali Moradi^{a,b}, Abdulbaset Maroofi^c, Mahdie Hemati^{b,d}, Tahmine Hashemzade^e,
Nasrin Alborzi^e, Fatemeh Safari^{a,e,*}

^a Yazd Neuroendocrine Research Center, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

^b Department of Clinical Biochemistry, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

^c Department of Exercise Physiology, Faculty of Physical Education & Sport Sciences, University of Guilan, Rasht, Iran

^d Medical Nanotechnology and Tissue Engineering Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

^e Department of Physiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

ARTICLE INFO

Keywords:

Hypertension
Left ventricular hypertrophy
GTPase Rac1
Vitamin D

ABSTRACT

Background: 1, 25-dihydroxy Vitamin D3 (VitD) attenuates left ventricular hypertrophy (LVH), but the mechanisms remain to be portrayed in-depth. The small GTPase Rac1 plays a pivotal role in cardiovascular pathology, especially LVH. Here, we tested whether VitD exerts its anti-LVH effects by counteracting the small GTPase Rac1 expression.

Methods: In Wistar rats, pressure overload-induced LVH was created by abdominal aortic banding. The animals were intraperitoneally administered VitD (0.1 µg/kg/d). Blood pressure was measured via carotid artery cannulation. Real-time RT-PCR and Western blotting were performed to assess the mRNA and protein expression, respectively. Myocardium was stained for determination of cardiomyocytes area and fibrosis. Lipid peroxidation levels and the activities of antioxidant enzymes were measured in left ventricular tissue.

Results: VitD significantly reduced hypertrophy markers (blood pressure, heart-to-body weight ratio, cardiomyocytes area, fibrosis as well as atrial and brain natriuretic peptides mRNA levels) compared to untreated groups ($P < 0.05$). VitD also improved the activity of antioxidant enzymes and reduced lipid peroxidation levels in the myocardium ($P < 0.05$). LVH hearts of untreated animals displayed a significant increase in Rac1 expression compared with the control group ($P < 0.05$). In contrast, cardiac Rac1, at either mRNAs or protein levels, was markedly lower in LVH animals receiving VitD compared with their untreated counterparts ($P < 0.05$).

Conclusion: Our findings attest that VitD mitigates hallmarks of LVH imparted by pressure overload. Notably, VitD appears to perform its anti-LVH function partly through small GTPase Rac1 suppression. This, in turn, provides a robust incentive to consider VitD before LVH culminates in HF devastating disease.

1. Introduction

Cardiovascular disease (CVD) and related complications are the leading causes of mortality worldwide, responsible for nearly 17 million annual deaths [1]. The pathological form of left ventricular hypertrophy (LVH), which is caused by chronic pressure or volume overload [2], contributes to the development of CVD [3,4]. If left untreated, LVH can lead to heart failure (HF) and malignant arrhythmias, thereby sudden cardiac death [5]. In contrast, successful prevention or amelioration of LVH may result in the reduction of mortality risk [6,7]. Several

medications, such as diuretics, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, and β -adrenergic blocking agents, have been used to cope with LVH and early manifestations of HF [8,9]. Although studies have confirmed the efficacy of these therapies, HF-related mortality still remains on the rise [10]. Beyond that, some pharmacological approaches potentially are accompanied by suboptimal clinical outcomes. For instance, available renin-angiotensin (RAS) inhibitors may lead to compensatory increased renin expression due to disruption of a negative feedback loop [11,12]. These issues have led researchers to develop alternative or supplementary agents to limit the

* Corresponding author at: Department of Physiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Postal Code: 8915173143, Yazd, Iran.
E-mail addresses: f.safari@ssu.ac.ir, sa.physiol@gmail.com (F. Safari).

<https://doi.org/10.1016/j.ijcha.2021.100922>

Received 6 July 2021; Received in revised form 5 November 2021; Accepted 19 November 2021

2352-9067/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

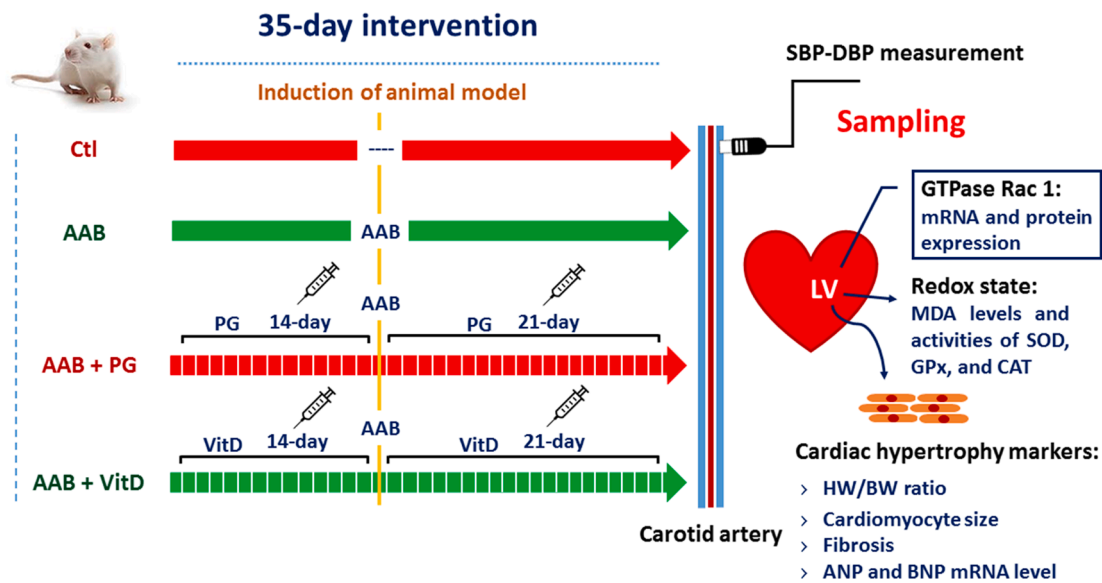


Fig. 1. Experimental design. Wistar rats were divided into the following groups: control or intact rats; Ctl ($n = 10$), abdominal aortic banding (AAB) as an experimental model of left ventricular (LV) hypertrophy; AAB ($n = 10$), rats with AAB who received propylene glycol (PG); AAB + PG ($n = 10$), rats with AAB who received vitamin D (VitD); AAB + VitD ($n = 10$). After the 5-week intervention, hemodynamic parameters, cardiac redox status, expression of Rac1, and markers of hypertrophy were assessed. SBP, systolic blood pressure; DBP, diastolic blood pressure; MDA, malondialdehyde; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; HW/BW, heart weight-to-body weight ratio; ANP and BNP, atrial and brain natriuretic peptide, respectively.

progression of LVH before it culminates in HF.

In recent decades, 1, 25-dihydroxyvitamin D (VitD) has also been considered a promising candidate to counter LVH and related complications [13]. VitD deficiency is highly prevalent in patients suffering from LVH and HF [14,15]. Indeed, research conducted on animals suggests that VitD-deficient or its receptor knockout is intimately associated with the high blood pressure and the manifestation of LVH, including increased cardiomyocyte size and proliferation [16,17]. In contrast, the administration of VitD has been demonstrated to limit unfavorable cardiac remodeling across a wide range of LVH models [18–21]. Likewise, clinical trials have also validated the ability of VitD to ameliorate LVH in HF patients [22]. Anti-LVH effects of VitD occur, at least in part, via reducing the activity of RAS, both in the circulatory and cardiac system [23]. Of note, since the VitD receptor is expressed in cardiomyocytes, the heart is regarded as a VitD target system. Indeed, VitD can directly exert some of its actions on the heart. This concept is supported by *in vitro* studies, where treatment of rat neonatal cardiomyocytes with VitD protects against the isoproterenol-induced increase in the mRNA, protein, and promoter activity of modulatory calcineurin inhibitory protein 1 (MCIP1). Calcineurin/NFAT signaling plays a central role in pathological LVH, which can be activated by MCIP1 expression [24,25]. Moreover, in primary cultures of neonatal rat cardiomyocytes, VitD has been demonstrated to inhibit cell proliferation as well as hypertrophy and its-associated genes, such as *c-myc* and atrial natriuretic peptide (ANP) [26,27]. In a study, Al-Rasheed et al. (2015) indicate that treatment of rats with VitD can reduce LVH through inhibiting the nuclear factor kappa B (NF- κ B) signaling pathway in the heart tissue and, by doing so, tumor necrosis factor- α (TNF- α) [18]. Clearly, direct and indirect effects of VitD can cover each other to cope with hypertrophy. However, the anti-LVH cellular and molecular mechanisms of VitD remain to be fully clarified.

Cardiomyocytes regularly convert the pressure overload to biochemical cascades [28]. Indeed, sustained pressure overload paves the way to the development of LVH by activating various intracellular signaling in the myocardium [28]. Among others, Rac1, a central small GTP-binding protein, has been suggested to play a pivotal role in the pathogenesis of LVH [28,29]. In the heart, over-expression, thus activation of Rac1 causes LVH [29,30]. Rac1 not only regulates several cellular processes, such as proliferation, survival, migration, and

trafficking [31] but also triggers NADPH oxidase, which in turn contributes to the generation of oxidative stress [29,32,33]. Oxidative stress *per se* can instigate a wide variety of molecular signaling involved in the development of LVH and cell dysfunction [34]. According to this view, reduction of oxidative stress is capable of coping with LVH and remodeling caused by pressure overload [34,35]. Rac1 is an essential component of the signaling pathway in the myocardium initiating LVH [29]. Inhibition of Rac1 has been highly suggested as a promising strategy for the prevention and treatment of LVH [28,29]. In this regard, experimental research has shown that cardiac-specific Rac1 deletion limits oxidative stress and LVH in mice [29]. Despite its decisive role in cardioprotection [13], the impact of VitD on the expression of Rac1 in pressure overload hearts has, to our knowledge, not been investigated so far.

Therefore, this study aimed to describe the influences of VitD supplementation on LVH-related markers as well as small GTPase Rac1 mRNA and protein expression in rats subjected to pressure overload-induced LVH. Further, given the role of Rac1 in the regulation of oxidative stress and redox state in hypertrophied myocardium [29,36], the cardiac levels of lipid peroxidation and the activities of antioxidant enzymes were also evaluated.

2. Materials and methods

2.1. Research design and left ventricular hypertrophy model

This study was performed in conformity with the guiding principles for animal research provided by the American Physiological Society and with approval the Ethics Committee of School of Medicine-Shahid Sadoughi University of Medical Sciences, Yazd, Iran (ID: IR: SSU/9321). Female Wistar rats (170–210 g) were kept in an animal house under controlled conditions (12-h circadian light/dark cycle) with free access to a standard pellet diet and tap water throughout the research. Animals were randomly divided into the following experimental groups:

1. Control (Ctl) group includes intact animals
2. Abdominal aortic banding (AAB) group in which the animals underwent aortic banding without receiving any treatment

- The AAB animals with intraperitoneal injection (IP) of propylene glycol (PG) as the solvent of vitamin D (AAB + PG).
- The AAB animals which received IP injection of VitD (0.1 $\mu\text{g}/\text{kg}$ of body weight/days; DarouPakhsh-Iran) for 14 days before AAB operation and for 21 days after banding of aorta (AAB + VitD). Our pilot study, has shown that injection of 0.1 $\mu\text{g}/\text{kg}/\text{day}$ of 1, 25-dihydroxyvitamin D does not alter the serum levels of calcium and resting blood pressure.

AAB was performed in conformity with earlier protocols [19,37]. In short, after anesthetizing animals with IP injection of a mixture of ketamine (70 mg/g) and Xylazine (10 mg/g), an incision was made in the left flank to rapidly obtain access to the suprarenal abdominal aorta. AAB was completed by ligation of the artery (7–0 silk sutures) and removing a 21-gauge needle from the loop. Once the surgery site was closed, the animals were placed in separate cages equipped with a heat lamp and monitored until complete recovery.

At the end of the 35-day intervention, the animals were weighed, then anesthetized. The systolic and diastolic blood pressure (SBP and DBP, respectively) were measured by cannulation of the carotid artery connected to a power lab system (ADInstruments, Australia). After scarifying, the animal hearts were removed, cleaned in ice-cold saline, and weighted to calculate heart weight-to-body weight ratio (HW/BW), an important marker of cardiac hypertrophy. Then the left ventricles were separated and fixed in 10% buffered formalin solution for histological assessment or stored at $-80\text{ }^{\circ}\text{C}$ for molecular studies. In our

present study, two rats died in the AAB and AAB + PG groups within the first week after aortic banding, who were excluded from the experiment. Thus, the study was performed on 10 rats/groups. In each group, three hearts were fixed in formaldehyde for histological assessments and the rest (seven hearts) were kept for molecular studies. Of note, the blood pressure and HW/BW analyses were recorded in all rats ($n = 10/\text{group}$). The experimental scheme framework is shown in Fig. 1.

2.2. Western blotting

Left ventricular (LV) samples were homogenized in a standard lysis buffer solution (lysis buffer solution containing: 150 mM NaCl, 1% Triton X-10, 0.25% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, 1 mM EDTA, 2 mM EGTA, 20 mM HEPES and 2 mM PMSF) and homogenization, the protein concentration was determined according to Bradford method as detailed in the previous report [38]. Equal amounts of protein (100 μg) were loaded on SDS-PAGE gels. Then the protein bands were transferred to PVDF membranes (Amersham Bioscience, UK). After 60 min of blocking with a buffer containing 1% bovine serum albumin, the membranes were incubated by mouse monoclonal anti-GTPase Rac1 primary antibody (Cat. No. ab33186, Abcam, USA) and secondary antibody of goat anti-mouse IgG-HRP (Cell signaling, Munich, Germany), respectively. The gel bands were quantified by ImageJ software (1.43). β -actin was considered as a reference protein (primary antibody Cat. No. ab8227, Abcam, USA).

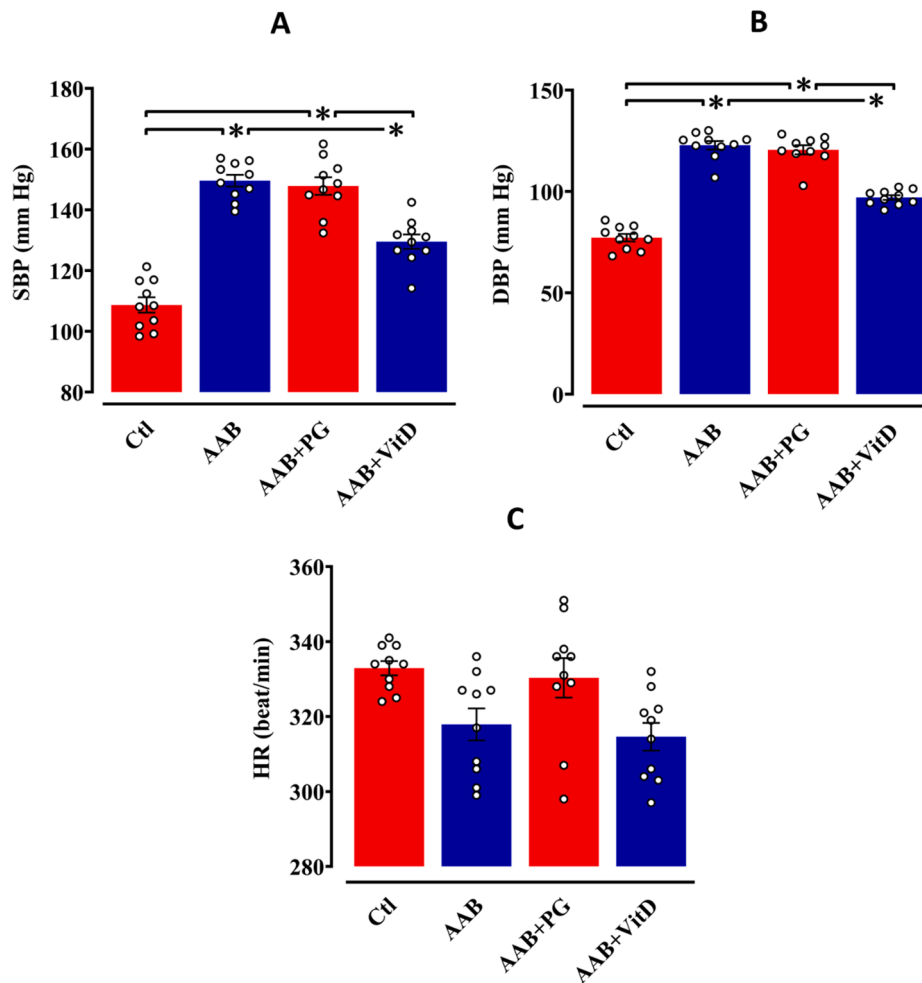


Fig. 2. Hemodynamic parameters. Effect of vitamin D (VitD) on (A) systolic and (B) diastolic blood pressure (SBP and DBP, respectively) and (C) heart rate (HR) in animals subjected to abdominal aortic banding (AAB). Values represent as mean \pm SEM ($n = 10$). * $p < 0.05$ difference between groups. Ctl, control; PG, propylene glycol.

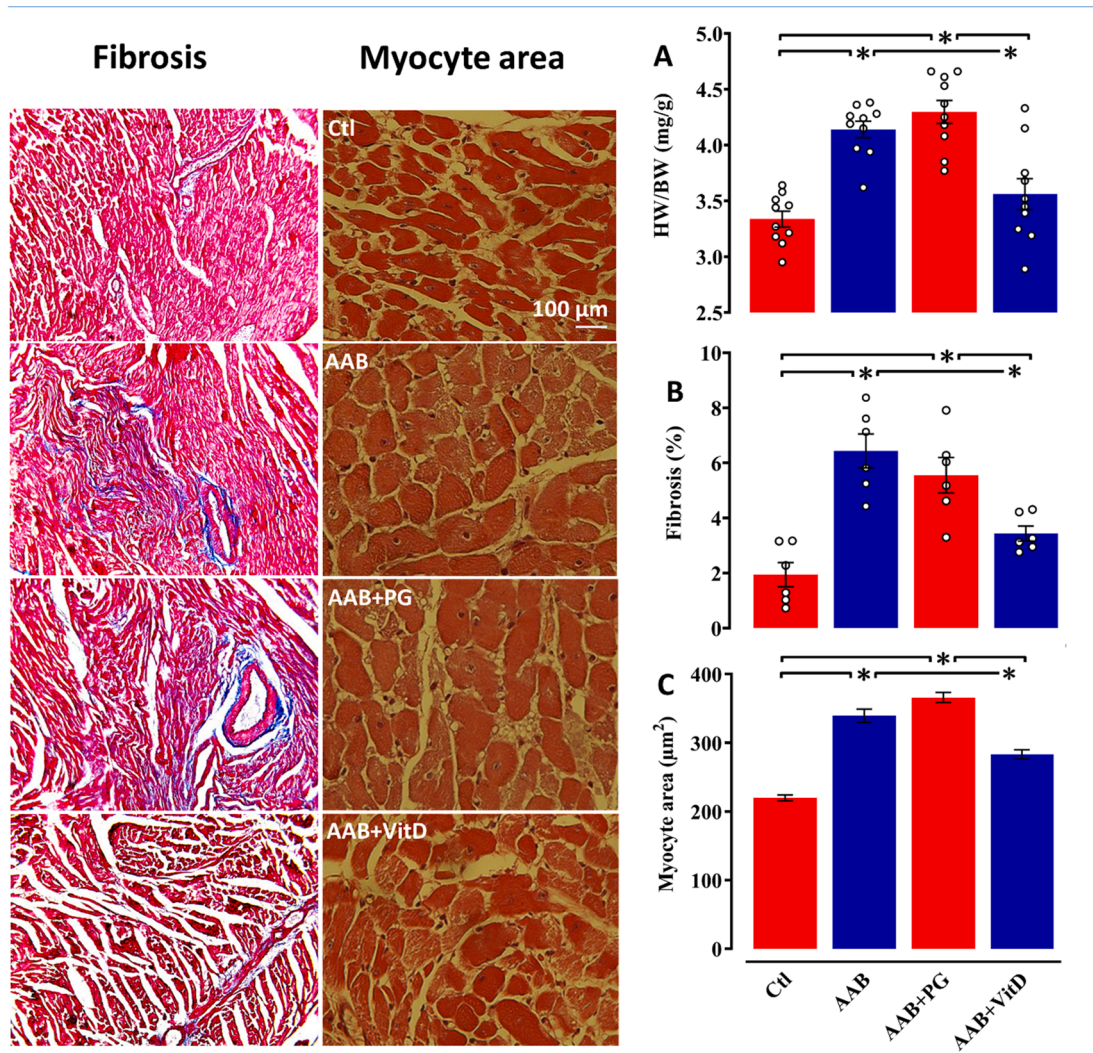


Fig. 3. Histological results and heart-to-body weight ratio. Effect of vitamin D (VitD) on (A) heart-to-body weight ratio (HW/BW, $n = 10$), (B) fibrosis ($n = 6$ sections from three hearts per group), and (C) cardiomyocyte area (150 cells were measured in each group) in animals subjected to abdominal aortic banding (AAB). Values represent as mean \pm SEM. * $p < 0.05$ difference between groups. Ctl, control; PG, propylene glycol. *Note:* the single data points were not considered in the myocyte area graph (C) due to the higher number of samples in this group.

2.3. Real time RT-PCR

Total RNA of the LV tissue was extracted using RNX-Plus (CinnaGen, Iran) in accordance with the instructions supplied by the manufacturer. The isolated RNA was converted to cDNA using the RevertAid TMM MuLV Reverse Transcriptase (Fermentas, USA). Then the PCR amplification was performed using Master Mix containing SYBR Green (Takara Bio, Japan).

The relative transcription levels of Rac1 (primer sequence: sense, 5'-GTAAAACCTGCTGCTCATC-3' and antisense, 5'-GCTTCATCAAACACTGTCTTG-3'), ANP (primer sequence: sense, 5'-GAGGAGAAGATGCCGGTAG-3' and antisense, 5'-CTAGAGAGGGAGCTAAGTG-3'), and BNP (primer sequence: sense, 5'-TGATTCTGCTCTGCTTTTC-3' and antisense, 5'-GTGGATTGTTCTGGAGACTG-3') were evaluated against the housekeeping β -actin gene (primer sequence: sense, 5'-GAACCCTAAGGCCAACGTGAA-3' and antisense, 5'-ATAGCAGCCACAAAAGGGA AA-3') by the $2^{-\Delta\Delta Ct}$ method.

2.4. Histological examination

Masson's trichrome and hematoxylin-eosin (H&E) staining methods were performed to evaluate LV fibrosis and cardiomyocytes area,

respectively. In brief, LV samples ($n = 3$ for each group) were fixed in 10% formalin. After processing steps and paraffinization, the tissue samples were sectioned at a thickness of 5- μ m. Following the completion of staining, the prepared slices were analyzed by an optical microscope.

2.5. Lipid peroxidation assay

Studies have shown that the progression of myocardial hypertrophy is accompanied by oxidative stress-induced lipid peroxidation. Therefore, the level of malondialdehyde (MDA) as the main marker of lipid peroxidation was measured in the LV tissue of experimental groups according to the protocol of the MDA Assay kit (ZellBio, GmbH Company, Germany).

2.6. Enzymatic antioxidant assay

2.6.1. Catalase activity

The activity of the antioxidant factor catalase (CAT) was measured spectrophotometrically according to Aebi's method [39]. Briefly, the LV tissue was lysed and homogenized in a lysis buffer containing NaCl, Tris, Triton X-100, EDTA, SDS, EGTA, HEPES, and PMSF. After centrifugation (13000 rpm for 45 min at 4 °C), the supernatant was collected and

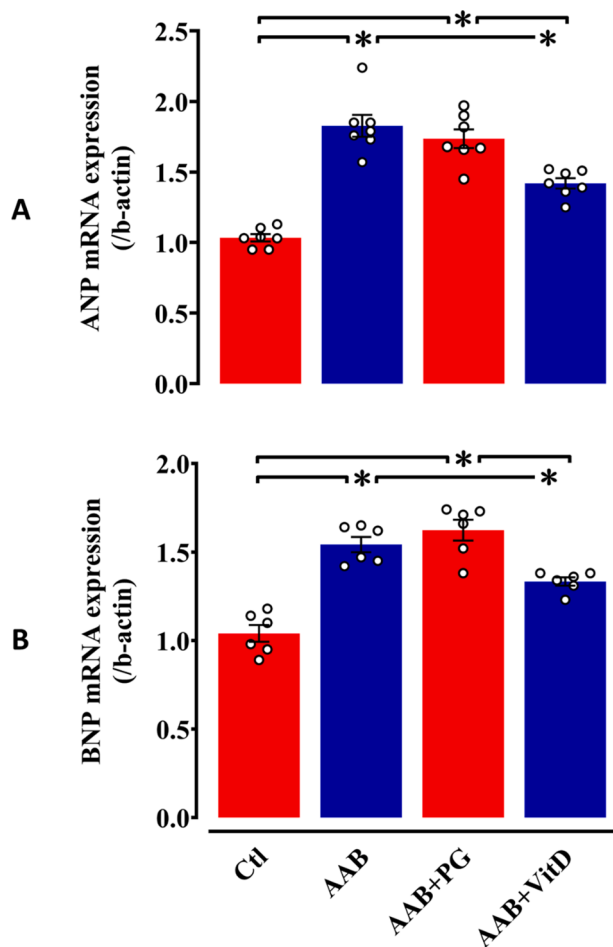


Fig. 4. Molecular markers of hypertrophy. Effect of vitamin D (VitD) on cardiac mRNA expression of (A) atrial and (B) brain natriuretic peptides (ANP and BNP, respectively) in animals subjected to abdominal aortic banding (AAB). Values represent as mean \pm SEM (n = 7). * p < 0.05 difference between groups. Ctl, control; PG, propylene glycol.

diluted with PBS. After adding H_2O_2 , the absorbance was read at 240 nm for 3 min.

2.6.2. Superoxide dismutase

The enzymatic activity of the antioxidant factor superoxide dismutase (SOD) was determined using the SOD assay kit (ZellBio GmbH Company, Germany). SOD activity unit was defined as the amount of SOD required to catalyse the conversion of 1 μ mole of superoxide radical anions into oxygen molecules and hydrogen peroxide in one minute.

2.6.3. Glutathione peroxidase assay

The antioxidant factor glutathione peroxidase (GPx) plays a crucial role in cardiomyocyte defense by detoxifying hydrogen peroxide (H_2O_2). In this study, the activity of GPx was measured using the GPx assay kit (ZellBio GmbH Company). GPx activity unit was defined as the amount of enzyme that required to catalyse the oxidation of 1 μ mole NADPH in one minute.

2.6.4. Statistical analysis

Data were analyzed by GraphPad Prism software (version 5.0) and expressed as mean \pm SEM, with a P -value of <0.05. Kruskal–Wallis and Dunn's Post-Hoc test were used to compare blood pressure among groups. Changes in HW/BW, cell area, ANP and BNP mRNA levels, as well as Rac1 mRNA and protein expression, were assessed by one-way

ANOVA followed by Tukey Post-Hoc test.

3. Results

3.1. VitD counteracts AAB-induced SBP and DPB increase

As shown in Fig. 2 (A and B, respectively), in the AAB group, animals exhibited a significant increase in SBP and DBP compared with Ctl (P < 0.05). In contrast, those enrolled in AAB + VitD had significantly lower levels of SBP and DBP in relation to the AAB group (P < 0.05). Heart rate (Fig. 2C) was not affected by either AAB or VitD. No significant differences were found in blood pressure between AAB and AAB + PG.

3.2. VitD ameliorates AAB-induced LVH manifestations

Figs. 3 and 4 shows that the LVH-related markers, both histological and molecular features, including HW/W ratio (Fig. 3A), fibrosis (Fig. 3B), cardiomyocyte area (Fig. 3C), and mRNA levels of ANP (Fig. 4A) or BNP (Fig. 4B), were increased markedly in the AAB animals compared with Ctl (P < 0.05). However, the AAB + VitD group exhibited significantly reduced all hypertrophy-related markers (P < 0.05). No significant differences were found in the hallmarks of LVH between AAB and AAB + PG.

3.3. VitD alleviates AAB-induced oxidative stress in the heart

As shown in Fig. 5 (A, B, C, D, respectively), AAB induction caused a significant increase in MDA formation (an end product of lipid peroxidation), while reducing the activities of antioxidant enzymes, including SOD, GPx, and CAT in the heart tissue compared with Ctl (P < 0.05). These alterations, except for SOD, were significantly ameliorated in the VitD-treated group (P < 0.05).

3.4. VitD abrogates AAB-induced cardiac GTPase Rac1 mRNA and protein over-expression

To explore the molecular mechanisms underpinning the anti-LVH effects of VitD, the cardiac expression of GTPase Rac1 was assessed. Fig. 6 shows that AAB significantly increased GTPase Rac1 expression, both at protein ($116.9 \pm 16\%$) (Fig. 6A) and mRNA ($92.2 \pm 17\%$) (Fig. 6B) levels compared with Ctl (P < 0.05). Instead, VitD administration significantly ameliorated the over-expression of GTPase Rac1, whether mRNA or protein, in AAB + VitD compared with AAB animals (P < 0.05). No significant differences were found in GTPase Rac1 expression between AAB and AAB + PG.

4. Discussion

Our main finding is that VitD counteracted LVH owing to AAB, at least in part, by abrogating GTPase Rac1 over-expression, whether gene or protein, in myocardial tissue. To our knowledge, no studies so far have investigated the influence of VitD on GTPase Rac1, an important molecule involved in LVH [28,29], in animals with AAB-induced pressure overload. Therefore, this study fosters the concept that reducing the expression, thus activation of cardiac GTPase Rac1 can play a leading role in coping with LVH [28,29].

AAB, with its pressure overload, acts as a trigger for LVH [2], helping future HF [5]. In accordance with previous studies [37,40], our present data reveal that AAB induced LVH successfully, as validated by the increased cardiomyocyte size, HW/BW ratio, ANP and BNP upregulation as well as fibrosis. We also observed that AAB is associated with SBP and DBP elevation, which in turn contributes to the development of LVH, probably by increased cardiac workload and consequent cardiomyocyte stretch [41]. The present study found that AAB can lead to a reduction in the activities of antioxidant enzymes, including SOD, GPx, and CAT in the heart. This, in turn, was accompanied by augmentation of MDA

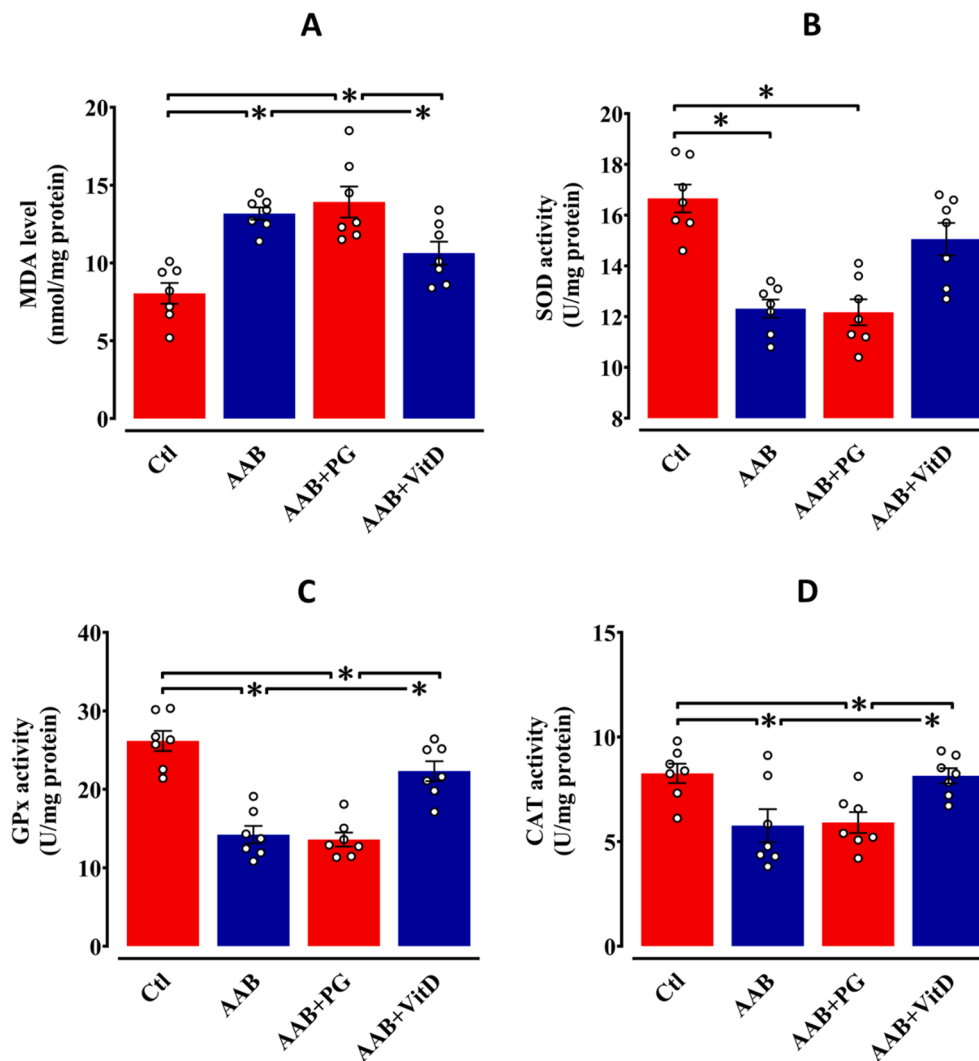


Fig. 5. Oxidative stress and antioxidant status in the heart. Effect of vitamin D (VitD) on (A) malondialdehyde (MDA) content (a marker for oxidative lipid injury) and the activities of antioxidant enzymes, including (B) superoxide dismutase (SOD), (C) glutathione peroxidase (GPx), and (D) catalase (CAT) in animals subjected to abdominal aortic banding (AAB). Values represent as mean \pm SEM ($n = 7$). * $p < 0.05$ difference between groups. Ctl, control; PG, propylene glycol.

levels, a significant indicator of oxidative stress in both cells and tissues [42]. Oxidative stress has been attested to act as a trigger for the induction of various mitogen-activated protein kinases and thereby contributes to cardiac hypertrophy [43]. In-vivo, treatment with antioxidants is able to counteract pressure overload-induced LVH by suppressing oxidative stress and redox-sensitive pathways [35].

Lack of or hypovitaminosis VitD may lead to overt CVD [44]. Numerous research findings confirm the close association between circulating VitD deficiency and the development of LVH [16,17], cardiac dysfunction [45], and HF [14,44]. In turn, supplementation of VitD has been shown to ameliorate experimental LVH [20,21] and reduce the mortality risk in HF patients [44]. Likewise, the data from the current study shows that IP administration of VitD for 35-day can significantly limit the manifestations of LVH in rats subjected to AAB, which was characterized by reduced cardiomyocyte size, HW/BW ratio, and fibrosis. This observation is in accordance with the animal study by Zhang et al. (2018), who demonstrated that treatment with VitD is capable of diminishing pressure overload-induced LVH [46] and, with those of Safari et al. (2015), where VitD protected rat hearts from LVH linked to AAB [37]. In this study, VitD also prevented increases in SBP and DBP in AAB animals. In a good agreement, experimental studies attested that VitD and its analogs, such as paricalcitol and doxercalciferol, can reduce blood pressure, at least in part, through

suppressing renin production [20,47]. It is important to note that a systematic review conducted by Beveridge et al. (2015) has concluded that VitD supplementation is not powerful for controlling blood pressure as much as antihypertensive medications [48]. The present study failed to show a significant effect of VitD on resting heart rate in rats, which is consistent with the findings of Tomson et al. (2018), who revealed that VitD supplementation for 12 months *per se* did not change the heart rate in older individuals [49]. Thus, it is speculated that the cardiac benefits of VitD may occur independently of heart rate modulation.

From a mechanistic point of view, studies have attested that VitD inhibits or activates several molecular agents to counter adverse cardiac remodeling and HF. In this regard, VitD has been shown to reduce the expression or secretion of fetal molecules such as atrial natriuretic peptide in cultured neonatal rat cardiomyocytes [50]. In a study, Wei et al. (2017) showed that VitD supplementation in rats ameliorates diabetic cardiomyopathy by suppressing the β -catenin/TCF4/GSK-3 β /mTOR pathway and thereby activation of autophagy [51]. Lack of VitD or its receptor knockout increases RAS activity [44] and sympathetic nerve activation [41]. This directly stimulates cardiomyocytes and initiates hypertrophic signaling pathways [41]. However, there is evidence indicating VitD supplementation can destroy such deteriorate scenario by reducing the RAS activity [44,52]. VitD also attenuates inflammation, oxidative stress, and apoptosis that is crucial for LVH [18,46]. It has

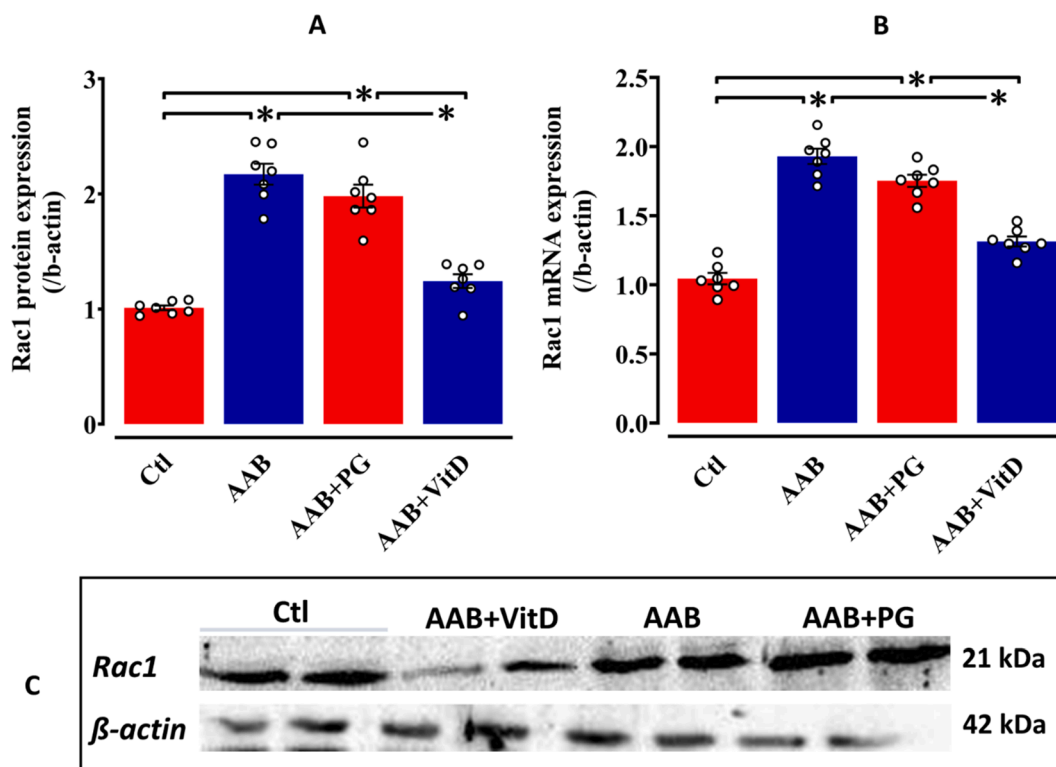


Fig. 6. GTPase Rac1 protein and mRNA expression. Effect of vitamin D (VitD) on cardiac GTPase Rac1 (A) protein and (B) mRNA expression in animals subjected to abdominal aortic banding (AAB). (C) Representative Western blot of Rac1 protein expression in rat myocardium. Values represent as mean \pm SEM (n = 7). * p < 0.05 difference between groups. Ctl, control; PG, propylene glycol.

been shown that injection of VitD in LVH rats reduces the risk of pro-inflammatory mediator TNF- α over-expression by abrogating NF- κ B/p65 signaling [18]. Likewise, Zhang et al. (2018) found that VitD can lower the cardiac NADPH oxidase expression in a rat model of pressure overload [46]. It is important to note that activation of NADPH oxidase leads to reactive oxygen species (ROS) generation, which is a critical driver of redox signaling, thus LVH and HF [53-55]. In this study, besides restoring the activities of antioxidant enzymes (except for SOD), VitD attenuated the MDA formation in the myocardium of AAB rats. This observation is in accordance with the previous animal studies, where VitD leads to an increase in activities of antioxidant enzymes (including SOD) in the heart while reducing MDA levels [56,57]. The inconsistency in the SOD-related finding can be attributed, at least in part, to the difference in the doses of VitD applied by various studies. Importantly, the dose of VitD in our study was lower than in other investigations [56,57]. VitD may reduce the MDA levels in different ways, including both beneficial effects on the antioxidant system and suppressing NADPH oxidase [46], a key producer of ROS, thus MDA [42].

To further uncover the molecular mechanisms by which VitD unleashes protection against AAB or pressure overload-induced LVH, we measured the expression of Rac1 GTPase in rat myocardium. This molecule has been undisputedly shown to promote the proliferation of vascular smooth muscle cells, endothelial dysfunction, hypertension, atherosclerosis, adverse cardiac remodeling, and atrial fibrillation [58-60]. As supported by evidence, Rac1 GTPase plays a large part in the origin of LVH. In fact, persistent activation of the Rac1 GTPase renders the heart more susceptible to LVH, chambers dilation, fibrosis, contractile dysfunction, and ultimately HF [29,30,61-63]. Disruption or inhibition of Rac1 GTPase signaling ameliorates LVH, myocardial remodeling and fibrosis, cardiac ischemic-reperfusion injury, and ventricular arrhythmia [62,64-66]. It is believed that the most majority, if not all, of the Rac1 GTPase-imparted LVH and diverse cardiovascular problems, occurs via NADPH oxidase and ROS [29,32,53,54].

Specifically, in pressure-overload hearts, Ayuzawa et al. (2015) demonstrated that Rac1 GTPase mediates the production of NADPH oxidase by recruiting the cardiac mineralocorticoid receptor [53]. Moreover, findings from a study attested that Rac1 GTPase deficiency is associated with abolished NADPH oxidase, inhibition of endoplasmic reticulum stress, and reduced myocardial remodeling in diabetic mice [66]. On these grounds, the inactivation of Rac1 GTPase can be considered an indispensable target for coping with LVH.

Similar to previous research, this study attested that AAB increases the expression of cardiac Rac1 GTPase, at either gene or protein levels [28,53]. In turn, our present data reveal, for the first time, that VitD injection suppresses the Rac1 GTPase expression in the heart. Importantly, this effect was coupled with reduced the robust histological and molecular LVH-related markers. Thus, our findings suggest that cardiac Rac1 GTPase may be a pivotal mediator for anti-LVH effects of VitD. At present, it is not clear how VitD serves to disrupt the relationship between Rac1 GTPase and LVH. Still, in light of the evidence that activation of Rac1 GTPase increases NADPH oxidase [53], ROS [67], and TNF- α expression [68], it is very plausible that VitD prevents LVH, at least in part, by repressing Rac1 GTPase and, by doing so, inflammation and redox signaling. To alter the expression of Rac1 GTPase, VitD acts potentially through different scenarios. For example, VitD may attenuate LVH via counteracting hypertension, thereby suppressing the upregulation of Rac1 GTPase that occurs secondary to the hypertrophy. Moreover, the ability of VitD to directly target the cardiac myocytes [19,25,26] allows us to speculate that VitD alters the protein level of Rac1 GTPase independent of influence upon hemodynamic parameters, in fact, by a direct effect on cardiomyocytes and suppression of LVH-associated (pro-hypertrophic) factors. Some likelihood also is that VitD reduces the protein level of Rac1 GTPase in hypertrophied cardiomyocytes exclusively via impact on signaling pathways involved in the antioxidant system or those related to the control of survival, i.e., independent of anti-hypertrophy or hypertensive effects.

5. Conclusion

Data from the current study demonstrate that VitD has remarkable anti-LVH properties. This effect appears to be mediated through suppression of the cardiac Rac1 GTPase. It is therefore very reasonable to consider VitD before LVH culminates in HF devastating disease.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

References

- [1] H. Thomas, J. Diamond, A. Vieco, S. Chaudhuri, E. Shinnar, S. Cromer, et al., Global atlas of cardiovascular disease 2000–2016: the path to prevention and control, *Glob Heart*. 14 (2018) 97.
- [2] A. Oláh, B.T. Németh, C. Mátyás, L. Hidi, Á. Lux, M. Ruppert, D. Kellermayer, A. A. Sayour, L. Szabó, M. Török, A. Meltzer, L. Gellér, B. Merkely, T. Radovits, Physiological and pathological left ventricular hypertrophy of comparable degree is associated with characteristic differences of in vivo hemodynamics, *Am. J. Physiol. Heart Circ. Physiol.* 310 (5) (2016) H587–H597.
- [3] A. González, S. Ravassa, B. López, M.U. Moreno, J. Beaumont, G. San José, et al., Myocardial remodeling in hypertension: toward a new view of hypertensive heart disease, *Hypertension* 72 (2018) 549–558.
- [4] N. Latic, R.G. Erben, Vitamin D and cardiovascular disease, with emphasis on hypertension, atherosclerosis, and heart failure, *Int. J. Mol. Sci.* 21 (2020) 6483.
- [5] C.-H. Tsai, C.-T. Pan, Y.-Y. Chang, Z.-W. Chen, V.-C. Wu, C.-S. Hung, Y.-H. Lin, Left ventricular remodeling and dysfunction in primary aldosteronism, *J. Hum. Hypertens.* 35 (2) (2021) 131–147.
- [6] B.H. Lorell, B.A. Carabello, Left ventricular hypertrophy: pathogenesis, detection, and prognosis, *Circulation* 102 (2000) 470–479.
- [7] E. Bisping, P. Wakula, M. Poteser, F.R. Heinzel, Targeting cardiac hypertrophy: toward a causal heart failure therapy, *J. Cardiovasc. Pharmacol.* 64 (2014) 293–305.
- [8] P. Van Zwieten, The influence of antihypertensive drug treatment on the prevention and regression of left ventricular hypertrophy, *Cardiovasc. Res.* 45 (2000) 82–91.
- [9] M. Itoh, Y. Takeishi, S. Nakada, T. Miyamoto, Y. Tsunoda, H. Takahashi, I. Kubota, H. Tomoike, Long-term treatment with angiotensin II type 1 receptor antagonist, CV-11974, restores β -catenin mRNA expression in volume-overloaded rabbit hearts, *Heart Vessels*. 17 (1) (2002) 36–41.
- [10] G. Savarese, L.H. Lund, Global public health burden of heart failure, *Card Fail Rev.* 3 (2017) 7.
- [11] D.N. Müller, F.C. Luft, Direct renin inhibition with aliskiren in hypertension and target organ damage, *Clin J Am Soc Nephrol.* 1 (2) (2006) 221–228.
- [12] Y.C. Li, Renoprotective effects of vitamin D analogs, *Kidney Int.* 78 (2) (2010) 134–139.
- [13] R.K. Al-Ishaq, P. Kubatka, M. Brozmanova, K. Gazdikova, M. Caprnda, D. Büsselberg, Health implication of vitamin D on the cardiovascular and the renal system, *Arch Physiol. Biochem.* 127 (3) (2021) 195–209.
- [14] L.C.Y. Liu, A.A. Voors, D.J. van Veldhuisen, E. van der Veer, A.M. Belonje, M. K. Szymanski, H.H.W. Silljé, W.H. van Gilst, T. Jaarsma, R.A. de Boer, Vitamin D status and outcomes in heart failure patients, *Eur. J. Heart Fail.* 13 (6) (2011) 619–625.
- [15] A.L. Marsland, P.J. Gianaros, D.-C.-H. Kuan, L.K. Sheu, K. Krajina, S.B. Manuck, Brain morphology links systemic inflammation to cognitive function in midlife adults, *Brain. Behav. Immun.* 48 (2015) 195–204.
- [16] S. Panizo, S. Barrio-Vazquez, M. Naves-Diaz, N. Carrillo-Lopez, I. Rodriguez, A. Fernandez-Vazquez, J.M. Valdivielso, R. Thadhani, J.B. Cannata-Andia, Vitamin D receptor activation, left ventricular hypertrophy and myocardial fibrosis, *Nephrol. Dial Transplant.* 28 (11) (2013) 2735–2744.
- [17] O. Gezmish, M. Tare, H.C. Parkington, R. Morley, E.R. Porrello, K.J. Bubb, M. J. Black, Maternal vitamin D deficiency leads to cardiac hypertrophy in rat offspring, *Reprod. Sci.* 17 (2) (2010) 168–176.
- [18] N. Al-Rasheed, Y. BASSIOUNI, Vitamin D attenuates pro-inflammatory TNF- α cytokine expression by inhibiting NF- κ B/p65 signaling in hypertrophied rat hearts, *J Physiol Biochem.* 71 (2015) 1–9.
- [19] Fatemeh Jahanifar, Akram Astani, Shahnaz Shekarforoosh, Mohabbat Jamhiri, Fereshteh Safari, Farideh Zarei, Fatemeh Safari, 1.25 Dihydroxyvitamin D3 attenuates hypertrophy markers in cardiomyoblast H9c2 cells: evaluation of sirtuin3 mRNA and protein level, *Int. J. Vitam. Nutr. Res.* 89 (3–4) (2019) 144–151.
- [20] Juan Kong, Gene H. Kim, Minjie Wei, Tao Sun, George Li, Shu Q. Liu, Xinmin Li, Ishir Bhan, Qun Zhao, Ravi Thadhani, Yan Chun Li, Therapeutic effects of vitamin D analogs on cardiac hypertrophy in spontaneously hypertensive rats, *Am. J. Pathol.* 177 (2) (2010) 622–631.
- [21] Jun H. Choi, Qingen Ke, Soochan Bae, Ji Yoo Lee, Yu Jin Kim, Ui Kyoung Kim, Cynthia Arbeen, Ravi Thadhani, Peter M. Kang, Doxercaliferol, a pro-hormone of vitamin D, prevents the development of cardiac hypertrophy in rats, *J. Card Fail.* 17 (12) (2011) 1051–1058.
- [22] K.K. Witte, R. Byrom, J. Gierula, M.F. Paton, H.A. Jamil, J.E. Lowry, et al., Effects of vitamin D on cardiac function in patients with chronic HF: the VINDICATE study, *J. Am. Coll. Cardiol.* 67 (2016) 2593–2603.
- [23] W. Xiang, J. Kong, S. Chen, L.-P. Cao, G. Qiao, W. Zheng, et al., Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems, *Am. J. Physiol. Endocrinol. Metab.* 288 (2005) E125–E132.
- [24] B. Sanna, E.B. Brandt, R.A. Kaiser, P. Pfluger, S.A. Witt, T.R. Kimball, et al., Modulatory calcineurin-interacting proteins 1 and 2 function as calcineurin facilitators in vivo, *Proceedings of the National Academy of Sciences.* 103 (2006) 7327–7332.
- [25] Songcang Chen, Christopher S. Law, Christopher L. Grigsby, Keith Olsen, Ting-Ting Hong, Yan Zhang, Yerem Yeghiazarians, David G. Gardner, Cardiomyocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy, *Circulation* 124 (17) (2011) 1838–1847.
- [26] T. D. O'Connell, J. E. Berry, A. K. Jarvis, M. J. Somerman, R. U. Simpson, 1, 25-Dihydroxyvitamin D3 regulation of cardiac myocyte proliferation and hypertrophy, *American Journal of Physiology-Heart and Circulatory Physiology.* 272 (1997) H1751–H1758.
- [27] J Wu, M Garami, T Cheng, D G Gardner, 1, 25 (OH) 2 vitamin D3, and retinoic acid antagonize endothelin-stimulated hypertrophy of neonatal rat cardiac myocytes, *J. Clin. Investig.* 97 (7) (1996) 1577–1588.
- [28] H. Nakagami, K. Jensen, J. Liao, A novel pleiotropic effect of statins: prevention of cardiac hypertrophy by cholesterol-independent mechanisms, *Ann. Med.* 35 (2003) 398–403.
- [29] M. Satoh, H. Ogita, K. Takeshita, Y. Mukai, D. J. Kwiatkowski, J. K. Liao, Requirement of Rac1 in the development of cardiac hypertrophy, *Proc Natl Acad Sci U S A.* 103 (2006) 7432–7437.
- [30] Yoshiharu Higuchi, Kinya Otsu, Kazuhiko Nishida, Shinichi Hirotsani, Hiroyuki Nakayama, Osamu Yamaguchi, Shungo Hikoso, Kazunori Kashiwase, Toshihiro Takeda, Tetsuya Watanabe, Toshiaki Mano, Yasushi Matsumura, Hikaru Ueno, Masatsugu Hori, The small GTP-binding protein Rac1 induces cardiac myocyte hypertrophy through the activation of apoptosis signal-regulating kinase 1 and nuclear factor- κ B, *J. Biol. Chem.* 278 (23) (2003) 20770–20777.
- [31] E. Bosco, J. Mulloy, Y. Zheng, Rac1 GTPase: a “Rac” of all trades, *Cell Mol Life Sci.* 66 (2009) 370–374.
- [32] F Custodis, M Eberl, H Kilter, M Bohm, U Laufs, Association of RhoGDI α with Rac1 GTPase mediates free radical production during myocardial hypertrophy, *Cardiovasc Res.* 71 (2) (2006) 342–351.
- [33] Masao Takemoto, Koichi Node, Hironori Nakagami, Yulin Liao, Michael Grimm, Yaeko Takemoto, Masafumi Kitakaze, James K. Liao, Statins as antioxidant therapy for preventing cardiac myocyte hypertrophy, *J. Clin. Invest.* 108 (10) (2001) 1429–1437.
- [34] Eiki Takimoto, David A. Kass, Role of oxidative stress in cardiac hypertrophy and remodeling, *Hypertension* 49 (2) (2007) 241–248.
- [35] Ikuko Tsujimoto, Shungo Hikoso, Osamu Yamaguchi, Kazunori Kashiwase, Atsuko Nakai, Toshihiro Takeda, Tetsuya Watanabe, Masayuki Taniike, Yasushi Matsumura, Kazuhiko Nishida, Masatsugu Hori, Mikihiko Kogo, Kinya Otsu, The antioxidant edaravone attenuates pressure overload-induced left ventricular hypertrophy, *Hypertension* 45 (5) (2005) 921–926.
- [36] Can M. Sag, Celio X.C. Santos, Ajay M. Shah, Redox regulation of cardiac hypertrophy, *J. Mol. Cell. Cardiol.* 73 (2014) 103–111.
- [37] M. Jamhiri, F. Safi Dahaj, A. Astani, S.H. Hejazian, Z. HafiziBarjin, M. Ghobadi, et al., Carvacrol Ameliorates Pathological Cardiac Hypertrophy in Both *In-vivo* and *In-vitro* Models, *Iran J Pharm Res* 18 (3) (2019) 1380–1394, <https://doi.org/10.22037/ijpr.2019.1100766>.
- [38] F Safari, G Bayat, S Shekarforoush, S Hekmatimagoddam, Z Anvar, M Forouzandeh Moghaddam, S Hajizadeh, Expressional profile of cardiac uncoupling protein-2 following myocardial ischemia reperfusion in losartan- and ramiprilat-treated rats, *J Renin Angiotensin Aldosterone Syst.* 15 (3) (2014) 209–217, <https://doi.org/10.1177/1470320312474050>.
- [39] H. Aebi, Catalase in vitro, *Methods Enzymol.* 105 (1984) 121–126.
- [40] Balázs Tamás Németh, Csaba Mátyás, Attila Oláh, Árpád Lux, László Hidi, Mihály Ruppert, Dalma Kellermayer, Gábor Kökény, Gábor Szabó, Béla Merkely, Tamás Radovits, Cinaciguat prevents the development of pathologic hypertrophy in a rat model of left ventricular pressure overload, *Sci Rep.* 6 (1) (2016), <https://doi.org/10.1038/srep37166>.
- [41] Mordecai P. Blaustein, How does pressure overload cause cardiac hypertrophy and dysfunction? High-ouabain affinity cardiac Na⁺ pumps are crucial, *Am. J. Physiol. Heart Circ. Physiol.* 313 (5) (2017) H919–H930.
- [42] E. Ho, K.K. Galougahi, C.-C. Liu, R. Bhandi, G.A. Figtree, Biological markers of oxidative stress: applications to cardiovascular research and practice, *Redox Biol.* 1 (2013) 483–491.
- [43] M. Seddon, Y. H Looi, A. M Shah, Oxidative stress and redox signalling in cardiac hypertrophy and heart failure, *Heart* 93 (8) (2007) 903–907.
- [44] J.-D. Zhao, J.-J. Jia, P.-S. Dong, D. Zhao, D.-L. Li, H.-F. Zhang, Effect of vitamin D on ventricular remodelling in heart failure: a meta-analysis of both in vivo and controlled trials, *BMJ open.* 8 (2018), e20545.

- [45] H.L. Nizami, P. Katare, P. Prabhakar, Y. Kumar, S.K. Arava, P. Chakraborty, et al., Vitamin D deficiency in rats causes cardiac dysfunction by inducing myocardial insulin resistance, *Mol. Nutr. Food Res.* 63 (2019) 1900109.
- [46] Liang Zhang, Xiao Yan, Yun-Long Zhang, Jie Bai, Tesfaldet Habtemariam Hidru, Qing-Shan Wang, Hui-Hua Li, Vitamin D attenuates pressure overload-induced cardiac remodeling and dysfunction in mice, *J. Steroid Biochem. Mol. Biol.* 178 (2018) 293–302.
- [47] C. Legartha, D. Grimm, M. Wehland, J. Bauer, M. Krüger, The impact of vitamin D in the treatment of essential hypertension, *Int. J. Mol. Sci.* 19 (2018) 455.
- [48] L.A. Beveridge, A.D. Struthers, F. Khan, R. Jorde, R. Scragg, H.M. Macdonald, et al., Effect of vitamin D supplementation on blood pressure: a systematic review and meta-analysis incorporating individual patient data, *JAMA Int. Med.* 175 (2015) 745–754.
- [49] J. Tomson, H. Hin, J. Emberson, R. Kurien, M. Lay, J. Cox, et al., Effects of vitamin D on blood pressure, arterial stiffness, and cardiac function in older people after 1 year: BEST-D (Biochemical Efficacy and Safety Trial of Vitamin D), *J. Am. Heart Assoc.* 6 (2017), e005707.
- [50] J. Wu, M. Garami, L. Cao, Q. Li, D. Gardner, 1, 25 (OH) 2D3 suppresses expression and secretion of atrial natriuretic peptide from cardiac myocytes, *Am J Physiol Endocrinol Metab.* 268 (1995) E1108-E1113.
- [51] Huili Wei, Hua Qu, Hang Wang, Baolan Ji, Yao Ding, Dan Liu, Yang Duan, Huimin Liang, Chuan Peng, Xiaoqiu Xiao, Huacong Deng, 1, 25-Dihydroxyvitamin-D3 prevents the development of diabetic cardiomyopathy in type 1 diabetic rats by enhancing autophagy via inhibiting the β -catenin/TCF4/GSK-3 β /mTOR pathway, *J. Steroid. Biochem. Mol. Biol.* 168 (2017) 71–90.
- [52] Yan Chun Li, Juan Kong, Minjie Wei, Zhou-Feng Chen, Shu Q. Liu, Li-Ping Cao, 1, 25-Dihydroxyvitamin D 3 is a negative endocrine regulator of the renin-angiotensin system, *J. Clin. Invest.* 110 (2) (2002) 229–238.
- [53] Nobuhiro Ayuzawa, Miki Nagase, Kohei Ueda, Mitsuhiko Nishimoto, Wakako Kawarazaki, Takeshi Marumo, Atsu Aiba, Takayuki Sakurai, Takayuki Shindo, Toshiro Fujita, Rac1-mediated activation of mineralocorticoid receptor in pressure overload-induced cardiac injury, *Hypertension* 67 (1) (2016) 99–106.
- [54] Jian-Mei Li, Nick P. Gall, David J. Grieve, Mingyou Chen, Ajay M. Shah, Activation of NADPH oxidase during progression of cardiac hypertrophy to failure, *Hypertension* 40 (4) (2002) 477–484.
- [55] C Murdoch, M Zhang, A Cave, A Shah, NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure, *Cardiovasc. Res.* 71 (2) (2006) 208–215.
- [56] J. Yang, Y. Zhang, Y. Pan, C. Sun, Z. Liu, N. Liu, et al., The protective effect of 1, 25 (OH) 2D3 on myocardial function is mediated via sirtuin 3-regulated fatty acid metabolism, *Front. Cell Dev. Biol.* 9 (2021) 966.
- [57] M.A. Farhangi, G. Nameni, G. Hajilulian, M. Mesgari-Abbasi, Cardiac tissue oxidative stress and inflammation after vitamin D administrations in high fat-diet induced obese rats, *BMC Cardiovascular Disorders* 17 (2017) 1–7.
- [58] Hamdy H. Hassanain, David Gregg, Maria Luisa Marcelo, Jay L. Zweier, Heraldo P. Souza, Balakrishnan Selvakumar, Qi Ma, Moustafa Moustafa-Bayoumi, Phillip F. Binkley, Nicholas A. Flavahan, Mariana Morris, Chunming Dong, Pascal J. Goldschmidt-Clermont, Hypertension caused by transgenic overexpression of Rac1, *Antioxid. Redox Signal.* 9 (1) (2007) 91–100.
- [59] Oliver Adam, Ulrich Laufs, Rac1-mediated effects of HMG-CoA reductase inhibitors (statins) in cardiovascular disease, *Antioxid. Redox Signal.* 20 (8) (2014) 1238–1250.
- [60] A. Carrizzo, M. Forte, M. Lembo, L. Formisano, A.A. Puca, C. Vecchione, Rac-1 as a new therapeutic target in cerebro-and cardio-vascular diseases, *Curr. Drug Targets.* 15 (2014) 1231–1246.
- [61] M. A. Hassan Talukder, Mohammad T. Elnakish, Fuchun Yang, Yoshinori Nishijima, Mazin A. Alhaj, Murugesan Velayutham, Hamdy H. Hassanain, Jay L. Zweier, Cardiomyocyte-specific overexpression of an active form of Rac predisposes the heart to increased myocardial stunning and ischemia-reperfusion injury, *Am. J. Physiol. Heart Circ. Physiol.* 304 (2) (2013) H294–H302.
- [62] Daniel Lavall, Pia Schuster, Nadine Jacobs, Andrey Kazakov, Michael Böhm, Ulrich Laufs, Rac1 GTPase regulates 11 β hydroxysteroid dehydrogenase type 2 and fibrotic remodeling, *J. Biol. Chem.* 292 (18) (2017) 7542–7553.
- [63] J.-C. Reil, M. Hohl, M. Oberhofer, A. Kazakov, L. Kaestner, P. Mueller, et al., Cardiac Rac1 overexpression in mice creates a substrate for atrial arrhythmias characterized by structural remodeling, *Cardiovasc Res.* 87 (2010) 485–493.
- [64] Lili Zhang, Xiangru Lu, Le Gui, Yan Wu, Stephen M. Sims, Guoping Wang, Qingping Feng, Inhibition of Rac1 reduces store overload-induced calcium release and protects against ventricular arrhythmia, *J. Cell Mol. Med.* 20 (8) (2016) 1513–1522.
- [65] Limei Shan, Jianmin Li, Meng Wei, Jian Ma, Li Wan, Wei Zhu, Ying Li, Huaqing Zhu, J. Malcolm O. Arnold, Tianqing Peng, Disruption of Rac1 signaling reduces ischemia-reperfusion injury in the diabetic heart by inhibiting calpain, *Free Radic. Biol. Med.* 49 (11) (2010) 1804–1814.
- [66] Jianmin Li, Huaqing Zhu, E Shen, Li Wan, J. Malcolm O. Arnold, Tianqing Peng, Deficiency of rac1 blocks NADPH oxidase activation, inhibits endoplasmic reticulum stress, and reduces myocardial remodeling in a mouse model of type 1 diabetes, *Diabetes* 59 (8) (2010) 2033–2042.
- [67] Sandip Patil, Melisa Bunderson, Jason Wilham, Stephen M. Black, Important role for Rac1 in regulating reactive oxygen species generation and pulmonary arterial smooth muscle cell growth, *Am. J. Physiol. Lung Cell Mol. Physiol.* 287 (6) (2004) L1314–L1322.
- [68] T. Zhang, X. Lu, F. Beier, Q. Feng, Rac1 activation induces tumour necrosis factor- α expression and cardiac dysfunction in endotoxemia, *J. Cell Mol. Med.* 15 (2011) 1109–1121.