




Resveratrol Suppresses Cardiac Renin Angiotensin System in the Late Phase of Left Ventricular Hypertrophy

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Abstract

Background and objectives: Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a natural polyphenole phytoalexin which exerts potential cardioprotective effects, but the cellular and molecular mechanisms responsible for these effects are still unknown. Cardiac renin angiotensin system (RAS) over-activation plays an important role in pathogenesis of left ventricular hypertrophy (LVH) progression. The aim of the study was to investigate the effects of resveratrol on the main components of RAS during early and late phase of myocardial hypertrophy. **Methods:** To consider the early and late phase of LVH, the rats were studied two and sixteen weeks after abdominal aorta banding without treatment (H2w and H16w groups, respectively) or with resveratrol (R) treatment. Intact animals served as control (Ctl). Arterial blood pressure was recorded by carotid cannulation. Angiotensin II (Ang II) level was measured using ELISA kit. Gene expression was evaluated by Real time RT-PCR technique. Cardiomyocyte size and fibrosis were assessed using haematoxylin/eosin and Masson trichrome staining, respectively. **Results:** Results of this study showed that in H2w group AT1a mRNA level was increased significantly ($p < 0.05$ vs. Ctl). In H16w group cardiac level of Ang II and also renin, angiotensinogen, and AT2R mRNA levels were increased significantly ($p < 0.001$ vs. Ctl). In H16w+R group, tissue concentration of Ang II as well as renin and angiotensinogen mRNA level decreased significantly compared with H16w group ($p < 0.001$, $p < 0.5$ and $p < 0.05$, respectively). **Conclusion:** Progression of LVH is accompanied by dynamic changes in RAS components expression in myocardial tissue. Resveratrol protects the heart against pressure overload-induced hypertrophy in part via RAS suppression.

Keywords: left ventricular hypertrophy; renin-angiotensin system; resveratrol

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Introduction

Nowadays, hypertension (HTN) is considered as a widespread disease which can cause complications such as left ventricular hypertrophy (LVH) and heart failure [1,2]. LVH represents the adaptive compensatory response of

the heart to chronic pressure and volume overload, myocardial ischemia and valvular diseases. LVH is characterized by increased heart mass which is associated with cardiomyocytes growth and extracellular matrix remodeling. If

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the causes of adaptive hypertrophy (early phase) persist, it can eventually enter the mal-adaptive phase (late phase) and cause complications such as cardiomyopathy, systolic and diastolic dysfunction, heart failure and sudden cardiac death [3,4]. Despite increasing number of studies, cellular and molecular mechanisms responsible for LVH progression are not known precisely.

Strong evidence suggests the important role of renin angiotensin system (RAS) activity in pathogenesis of cardiovascular diseases [5-7]. Recent studies have revealed the presence of a "local cardiac RAS"; angiotensin II (Ang II) is produced locally in the myocardial tissue, independent of the components of systemic RAS. Overproduction of intracardiac Ang II leads to hypertension, LVH and heart failure [8,9]. Therefore, pharmacological blockade of the RAS at the level of Ang II production or function ameliorates cardiac remodeling in clinical [10,11] and experimental [12,13] models of heart disease. The main cardiovascular effects of Ang II are mediated by type 1 (AT1aR, AT1bR) and type 2 receptors (AT2R), which are expressed in cardiomyocytes and vascular cells [14]. The mechanisms by which RAS participates in hypertrophy progression and thereby heart failure are enhanced production of reactive oxygen species (ROS), pro-fibrotic proteins, proliferative and proapoptotic factors leading to increased cardiac mass without any positive effect on pumping function of the heart [15,16].

There are many therapeutic strategies available for treating hypertrophy such as β -blockers, angiotensin receptor blockers or angiotensin converting enzyme inhibitors (ACEIs), but mortality associated with heart failure is still rising. Thus, it is necessary to find new pharmacological approaches for the treatment of mal-adaptive hypertrophy.

Recent studies have revealed that the polyphenol resveratrol (3,5,4'-trihydroxy-trans-stilbene), which is found in grapes, peanuts and berries, exhibits powerful antioxidant, anti-inflammation and anti-aging properties [17]. Although there are few findings about the cardiovascular effects of resveratrol and the underlying mechanisms, several lines of studies demonstrated that resveratrol can prevent hypertension and cardiac hypertrophy and remodeling [18-20]. It also protects the heart against coronary heart disease and ischemia-reperfusion injury and inhibits platelet aggregation [21,22]. Our recent study has

shown that, three weeks after aortic banding, the cardiac level of Ang II and AT1a mRNA expression increases and resveratrol prevents hypertrophy-induced AT1a receptor upregulation in adaptive hypertrophy [23], although the progression of hypertrophy to mal-adaptive phase was not investigated.

Despite the well-known role of RAS in the pathophysiology of cardiac hypertrophy and the novel cardioprotective effects of resveratrol, there has been no report on the possible effects of resveratrol on RAS components level during progression of cardiac hypertrophy. Therefore, in the current study, we aimed to investigate the effect of resveratrol on the expression of key components of RAS in both early and late phases of pressure overload-induced LVH in rats.

Materials and Methods

Ethical considerations

All procedures involving animals were approved by the Animal Ethics Care and Use Committee of Shahid Sadoughi University of Medical Science (Code: 3894, 18-02-2016).

Animals and treatments

Male Wistar rats (150-190 g) were kept under standard conditions (12 h light/dark cycle; 25 °C temperature). The rats were randomly divided into five groups. Two groups of rats underwent abdominal aortic banding to induce cardiac hypertrophy without any treatment. Samples were taken 2 and 16 weeks after surgery (H2w and H16w groups, respectively). Two separate groups of rats were treated with resveratrol and subjected to aortic banding (H2w+R and H16w+R groups). In our study, 2 and 16 weeks after aortic banding were considered as the early and progressive phases of hypertrophy, respectively [24,25]. Intact rats served as control (Ctl).

Treatment groups received resveratrol (Cat No. R5010, Sigma-Aldrich, USA.) (2.5 mg/kg/day, dissolved in DMSO 4% and diluted in distilled water, intraperitoneal injection) from the day of surgery until the end of experiment. The previous studies have shown that resveratrol at the mentioned dose exerts cardioprotective effects in experimental models of HTN and LVH without lowering blood pressure [26,27].

Induction of LVH by aortic banding

Suprarenal abdominal aorta was constricted to

induce pressure overload-induced LVH. Briefly, the rats were anesthetized by IP injection of ketamine (70-90 mg/kg) and xylazine (10 mg/kg). Incision was made in the left flank. After exposing the suprarenal aorta, a 21 gauge needle was placed beside the artery and a suture was tied around it. The needle was removed after partial banding of the artery. Abdominal wall muscles and skin were sutured. In hypertrophied groups, tetracycline was administered intramuscularly for six days. Two and sixteen weeks after the surgery, the rats were weighted, anesthetized again, and arterial blood pressure was measured directly by carotid artery cannulation connected to a power lab system. The heart was excised quickly, washed with cold saline and weighted to evaluate heart weight to body weight ratio (HW/BW). Then, left ventricular tissue was separated and kept in -80 °C for further molecular studies [23,28]. Three samples from each group were fixed in formaldehyde 10% to investigate the effect of the interventions on cardiomyocytes size, as the main marker of cardiac hypertrophy. Following tissue processing, the cardiac sections (5 µm) were stained with hematoxylin and eosin. Then, the pictures were captured by a Nikon microscope equipped with a Sony, Syber-shot, DSCWX200 camera. The cell area was measured using image J software and reported relative to control. Areas of at least 180 cells in each group were measured.

Real-time quantitative RT-PCR

Left ventricular tissue was lysed by RNX-plus solution (Sinagen, Iran) and homogenized (T10Bhomogenizer, Germany). RNA was extracted according to the manufacturer's instructions. The RNA concentration was measured using the nanodrop set (Biotech Instrument Model: Box998, USA) at 260 nanometer wavelength. For cDNA synthesis, reverse transcription reaction was done using Revert AidTMM-MuLV Reverse Transcriptase (Fermentas, USA). cDNA from experimental

groups underwent real time RT-PCR reaction in presence of specific primers using MasterMix containing SYBR green (Takara, Japan). The primer sequences used for the reaction have been presented in table 1. The GAPDH gene was selected as the reference. Gene expression was compared according to $2^{-\Delta\Delta ct}$ method [29].

Cardiac level of Angiotensin II assay by ELISA test

To evaluate Ang II level, left ventricular tissue was lysed and homogenized using 1 mL lysis solution (containing 20 mM HEPES, 2 mM EGTA, 1 mM EDTA, 50 mM Tris-HCl, SDS 0.1%, sodium deoxycholate: 0.25%, Triton 1%, 150 Mm NaCl, supplemented with protease inhibitor PMSF). The homogenized tissue was centrifuged (45 min/4 °C at 13000 RPM) and then the supernatant was used for measurement of Ang II using ELISA kit (Phoenix, USA) following the kit instructions.

Statistical analysis

Blood pressure and heart weight to body weight ratio were analyzed using Kruskal-Wallis test with Dunn's post-test for multiple comparison. Ang II and transcriptional level of target genes were analyzed using one-way ANOVA followed by Tukey post-test. $P < 0.05$ was considered to be statically significant. Data have been presented as mean \pm SEM. Statistical analysis was done using Prism software (version 5.0).

Results and Discussion

Certain plants such as peanuts, soy, grapes, cocoa and berries produce resveratrol in response to stress, fungal invasion and injury. Therefore, this phytoalexin phenolic compound acts as the defensive response in a number of plant species [30]. Studies have shown that regular consumption of peanut (three to five times per week) decreases the risk of coronary heart disease.

Table 1. Primer sequences used in the study

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
AT1a	CCATTCACCTGCCTCAG	ACGGCTTTGCTTGGTTACTC
AT1b	ATGTCTCCAGTCCCCTCTCA	TGACCTCCCATCTCCTTTTG
AT2	CAATCTGGCTGTGGCTGACTT	TGCACATCACAGGTCCAAAGA
Angiotensinogen	CAGCACGACTTCCTGACTTGGAT	GGATGCTGTTGAGAACCTCTCCCA
Renin	AGGATCAGTGTGAATGGGGTGA	GGTTGTGAATCTCACAGGCAGTGT
GAPDH	AACGACCCCTTCATTGAC	TCCACGACATACTCAGCAC

Since peanut contains high concentration of stilbenoids such as resveratrol, especially at the early stage of growth, it is suggested that the cardioprotective effect of this nut is related to the presence of resveratrol [31]. Itadori tea which is used as the traditional herbal remedy for cardiovascular diseases contains high concentration of resveratrol too [30].

In the first part of current study we investigated the effect of resveratrol on hemodynamic parameters in experimental groups. As presented in table 2, systolic and diastolic blood pressure increased significantly in H2w group ($p < 0.001$ and $p < 0.01$ vs. Ctl, respectively). In H2w+R group, systolic and diastolic pressure was significantly different from those in the H2w group ($p < 0.05$ and $p < 0.01$, respectively). In H16w and H16w+R groups, blood pressure was not significantly different compared to that in the Ctl group, suggesting progression of compensated hypertrophy.

Furthermore, a significant increase in HW/BW ratio was observed in both hypertrophied groups (H2w and H16w: $p < 0.001$; vs. Ctl). However, in H2w+R and H16w+R groups, which were treated with resveratrol, HW/BW ratio decreased significantly in comparison to non-treated groups ($p < 0.001$ and $p < 0.01$, respectively) (figure 1). Cardiomyocyte size also increased in hypertrophied groups ($p < 0.001$) and resveratrol pretreatment prevented this increase (figure 2).

In this study, we tried to evaluate the effects of resveratrol on LVH progression and we could examine its effects on some LVH parameters. The important point was that resveratrol inhibited abdominal aorta banding-induced hypertension. The increase in left ventricular mass was also completely prevented in animals receiving resveratrol. The effect of resveratrol on aortic banding-induced hypertension has been indicated in previous studies including our recent study. Therefore, it is suggested that resveratrol does not exert hypotensive effect at baseline, but it prevents aortic banding-induced hypertension [23,26,27]. In our study, resveratrol decreased

HW/BW ratio and this effect was accompanied by the decrease of cell area which reveals the antihypertrophic effect of resveratrol. It is possible that the antihypertrophic effect of resveratrol is through regulation of blood pressure. In other words, resveratrol exerted its antihypertrophic effect by preventing aortic banding-induced hypertension. However, it should be noted that resveratrol can suppress pro-hypertrophic signaling pathways independent of its effect on blood pressure. For example, resveratrol inhibits calcineurin-nuclear factor of activated T cells (NFAT) [32] and Akt pathways and activates AMP-activated protein kinases, thus suppressing protein synthesis in cardiomyocytes [33]. Resveratrol also activates AMPK via LKB1 and inhibits Akt, thus suppressing protein synthesis and gene transcription.

Many lines of evidence have confirmed that resveratrol applies most of its protective properties by activation of sirtuin-1 deacetylase, which is shown to protect cardiomyocytes against oxidative injury [34,35]. Resveratrol abolished aorta banding induced-hypertrophy in rats through upregulation of nitric oxide synthase [36]. It has prevented hypertension through activation of LKB1-AMP-eNOS signaling pathway and suppressed cardiac hypertrophy in spontaneously hypertensive rats as well as mice under Ang infusion [18].

Cardioprotective effects of resveratrol were also pointed out in ischemic injury. Resveratrol decreases infarct area size in ischemic myocardium, suppresses cardiac tissue ANP level and enhances cardiac function [37]. Our earlier study also showed that low doses of resveratrol in combination with vitamin D decreased infarct size and ischemic reperfusion arrhythmias prevalence and reinforced antioxidant factors expression [38].

Results from the next part of our study showed that progression of pressure overload-induced LVH was accompanied by dynamic changes in cardiac levels of Ang II.

Table 2. Heart to body weight ratio and hemodynamic parameters

	Ctl	H2w	H2w+R	H16w	H16w+R
Systolic pressure (mm Hg)	110.3±7.5	149.6±8.3 ^{***}	127.8±7.1 [#]	125.2±6.8	131.2±5.9
Diastolic pressure (mm Hg)	71.6±3.8	113.3±5.1 ^{**}	96.5±3.6 ^{##}	86.3±4.7	79.4±5.0
Heart Rate	301±14	276±16	285±16	251±14	288±12

Hemodynamic parameters in control (Ctl) and hypertrophied groups two and sixteen weeks after aortic banding (H2w and H16w, respectively) in the presence or absence of resveratrol (R);

^{**} $p < 0.01$ and ^{***} $p < 0.001$ vs. Ctl. [#] $p < 0.05$ and ^{##} $p < 0.01$ vs. H2w

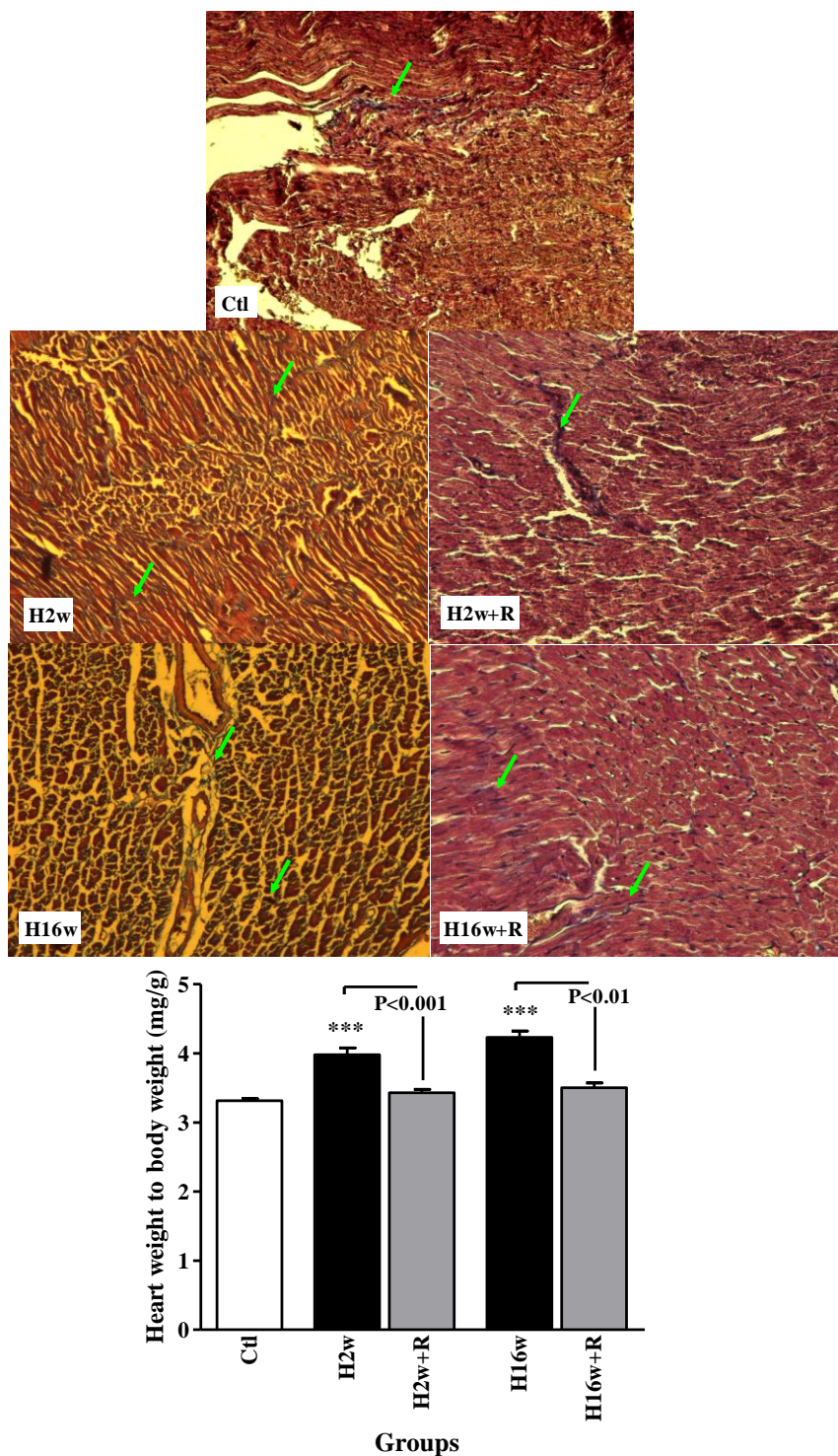


Figure 1. Heart to body weight ratio; two or sixteen weeks after aortic banding the heart weight and body weight of untreated rats (H2w and H16w groups, respectively) and resveratrol (R)-treated rats were measured. Intact animals served as control (Ctl). Heart sections were stained with Masson's Trichrome to confirm cardiac fibrosis. Images of four sections per heart (three hearts per group) were taken at a magnification of $\times 20$. *** $P < 0.001$ vs. Ctl.

As illustrated in figure 3, Ang II level increased significantly in left ventricular tissue of H16w group compared with the control group ($p < 0.001$). However, in H16w+R group, in which the

animals were treated with resveratrol, Ang II level was significantly different from that in the H16w group ($P < 0.001$).

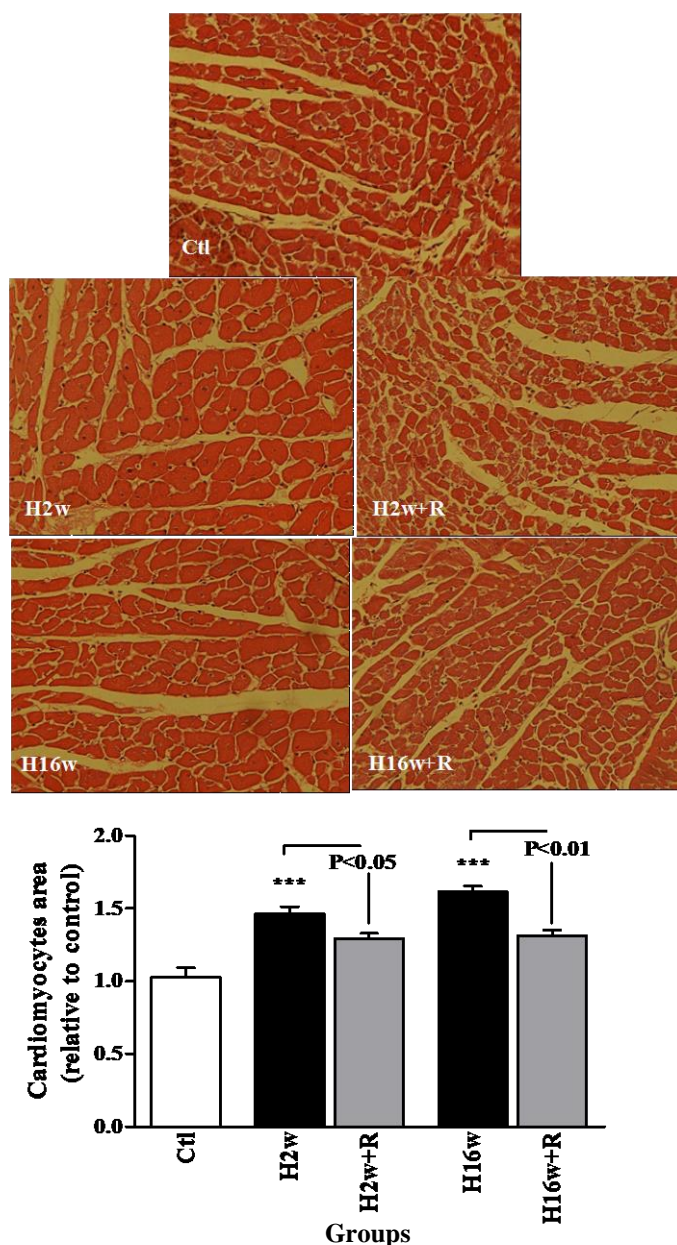


Figure 2. Cardiomyocytes area; two or sixteen weeks after aortic banding, heart sections of untreated rats (H2w and H16w groups, respectively) and resveratrol(R)-treated rats were stained with Hematoxyline/eosin and images were taken at a magnification of $\times 20$. Areas of 180 cells per group (60 cells per heart) were measured using Image J software. Intact animals served as control (Ctl). ***P<0.001 vs. Ctl.

In H2w groups, the change in the tissue level of Ang II was not statistically significant. In our study, tissue level of Ang did not change two weeks after abdominal aorta banding (early phase of hypertrophy), but our recent study showed that Ang II level increased in left ventricular tissue three weeks after banding of artery [23]; therefore, it is possible that progression of LVH from two weeks to three weeks is associated with gradual increase of Ang II production in the

heart. The important role of RAS in pathogenesis of cardiovascular diseases such as hypertrophy has been evaluated in several studies. RAS can be divided into 2 parts: 1. Classical RAS: also known as circulatory RAS, it is identified as the important component in blood pressure and body water volume regulatory system. 2. Local cardiac RAS: studies have shown that all of the needed ingredients to produce Ang II are available in the heart tissue and expression of renin,

angiotensinogen, ACE and Ang receptors in myocardial tissue is proved. Therefore, researchers believe that a notable amount of Ang II in heart is produced in situ [39]. Cardiac RAS overactivation plays an important role in pathogenesis of hypertension and LVH.

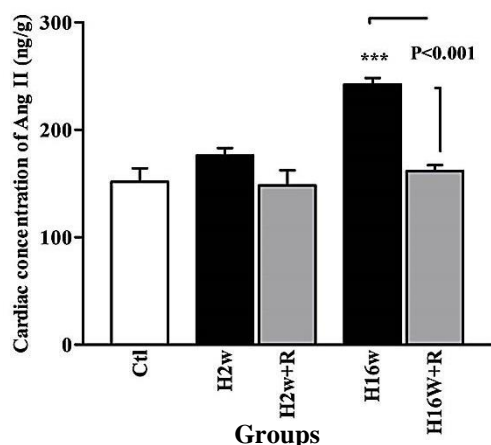


Figure 3. Cardiac level of Angiotensin II (Ang II); in the left ventricular tissue of non-treated and resveratrol-treated (R) rats two and sixteen weeks after aortic banding Ang II concentration was measured (H2w and H16w, respectively). Intact animal served as control (Ctl); *** $p < 0.001$ vs. Ctl

Ang II triggers the molecular pathways of hypertrophy by activation of phospholipase C and D, mitogen-activated protein kinase (MAPK) and JAK-STAT signaling pathways [40]. Chronic Ang II prescription causes a rise in expression of fibrotic marker proteins such as collagen through activation of transforming growth factor beta (TGF- β) [41]. When the heart is exposed to volume or pressure overload, stretched myocytes release Ang II, indicating that Ang II can be a mediator for stretch-induced hypertrophy [42]. As Ang II level increases dramatically in the late phase of hypertrophy, the key role of this system is confirmed in pathogenesis of hypertrophy progression.

Previous studies have demonstrated that volume or pressure overload of the heart is accompanied by dynamic changes in transcription level of Ang II receptors [7,23]. Therefore, this issue was assessed in the current study. Evaluating transcriptional level of the main receptors of Ang II in the left ventricular tissue indicated that AT1aR mRNA level increased in H2w group by 1.5 fold, which shows a significant difference in comparison to the Ctl ($p < 0.05$) (figure 4).

There was no significant difference among the experimental groups regarding AT1bR mRNA level.

In other words, AT1aR mRNA level increased in the early phase of hypertrophy, but it returned to the normal level in the late phase. It is supposed that upregulation of AT1aR in the early stage of LVH increases the sensitivity of the heart to Ang II.

Several studies have demonstrated that AT1R, especially AT1a receptor, was responsible for pathological effects of Ang in the heart. Animals with AT1R overexpression showed severe cardiac hypertrophy and fibrosis [43]. AT1R is expressed in cardiac cardiomyocytes and fibroblasts while AT2R is expressed in fibroblasts and endothelial cells. In rats, AT1R consists of 2 sub-types named AT1aR and AT1bR which are respectively located on chromosomes No. 17 and 2.

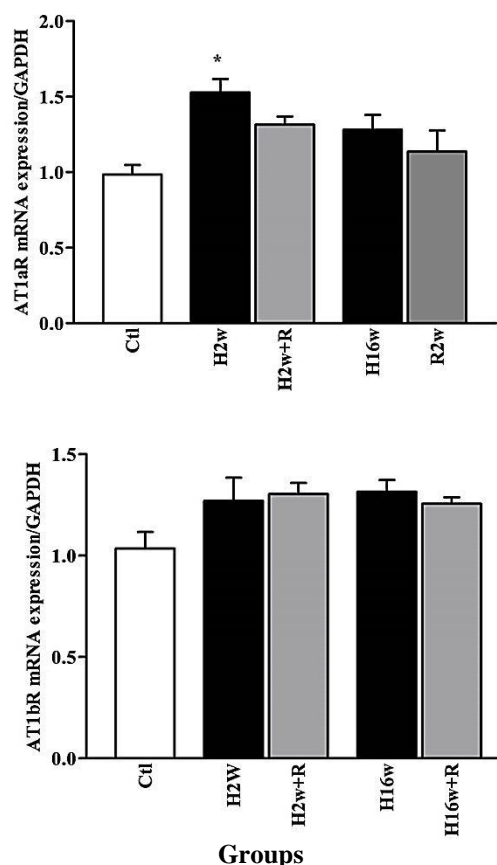


Figure 4. Transcriptional levels of Ang II receptors; the mRNA levels of Ang II type 1 (AT1aR, AT1bR) and type 2 (AT2R) receptors was evaluated in the left ventricular tissue of non-treated and resveratrol-treated (R) hypertrophied rats two and sixteen weeks after aortic banding (H2w and H16w). Intact animal served as control (Ctl). * $p < 0.05$, *** $p < 0.001$ vs Ctl

Increase of AT1R density in cardiac tissue following hypertension and cardiomyopathy has also been reported [14,44]. Ang II applies vasoconstriction and negative inotropic effects on heart using AT1aR rather than AT1bR [45]. There is little information available about AT2R mediated cardiovascular effects. Being only 3% similar to AT1R, AT2R is highly expressed in the heart and aorta tissue during fetal period and is expressed in liver, lung and kidney at lower volumes. However, its expression reduces intensely after birth. In the adulthood period, expression of this receptor increases again in the cardiovascular system under pathological conditions. Some studies demonstrated that AT2R antagonizes AT1R mediated effects, but there is still a big controversy on this subject [14]. For example, in a study by D D'Amore et al., AT2R activation caused hypertrophy in isolated cardiomyocytes and did not oppose AT1R induced hypertrophy [46]. However, in another study, deletion of AT2R exacerbated myocardial ischemia-induced heart failure in mice [47].

In our study, as indicated in figure 4, AT2R mRNA level increased by 1.63 fold in H16w group, which is statistically different from the Ctl group ($p < 0.001$). This indicated re-induction of fetal gene expression during hypertrophy progression.

Our data is in agreement with the results of the study by Kurabayashi et al. which showed that AT1R expression decreased in human failed heart despite AT2R density while AT2R mRNA level increased. It is possible that AT1R depletion in human failed heart is the result of Ang II elevation. This is while AT1R depletion was not observed in non-failed hypertrophied ventricle [48].

Changes in the Ang II receptor expression level in heart tissue were examined in some animal models of hypertrophy and heart failure. Suzuki et al. showed that AT1R and AT2R density as well as AT1a mRNA level increased in SHR rats [46]. Elevation of AT1aR and AT2R mRNA level in infarcted and non-infarcted area of the left ventricle followed by coronary ligation in rats was also explained in [50]. However, AT1aR and AT2R mRNA level did not change in rats with left ventricular failure followed by aorto-caval shunt (an instance for volume overload induced heart failure) [51]. Different models of heart failure may be the main reason of the difference

between the result of the mentioned study and that of ours.

Our findings are also consistent with those obtained by Schultz et al. which showed that ascending aorta banding in dog for 9 months increased Ang concentration in left ventricular tissue, but decreased AT1R expression [52]. Based on these data, it can be concluded that when the heart is exposed to pressure overload cardiac AT1R density increases, but when the hemodynamic overload persists and cardiac hypertrophy progresses to failure stage it will decrease. In the late phase of hypertrophy cardiac level of Ang II increased dramatically therefore, it could be possible that there was a negative feedback loop between Ang II and At1R receptor. Given the importance of renin and angiotensin in local RAS in myocardial tissue, transcription level of these genes was also evaluated. The results showed that angiotensinogen mRNA level increased by 1.56 fold in H16W ($p < 0.001$ vs. Ctl), but angiotensinogen mRNA level increased by 1.2 fold in H16w+R group which was treated with resveratrol. This shows a significant difference with non-treated rats in the H16w group ($p < 0.05$).

As shown in figure 5, the transcription level of renin augmented by 1.7 fold in the H16w group which is significantly different from the Ctl ($p < 0.001$). In the H16w+R group, the renin mRNA reached 1.27 fold, which shows a significant difference in comparison with the H16w group ($p < 0.05$).

There are lots of findings about renin expression and its important role in myocardial tissue. Some studies claim that renin expression is low in myocardial tissue and that its main portion is produced in kidneys provided by renin uptake from the circulation [39]. In heart failure patients under ACEI treatment, the renin level in circulation and heart tissue increased, indicating elevated renin uptake in these patients. At the same time, angiotensinogen concentration decreased in the heart tissue, suggesting significant increase in Ang production in failed heart [53]. Renin mRNA level was also increased in infarcted left ventricle of rats [54], so it is possible that pathological conditions may stimulate renin secretion in heart. Angiotensinogen expression is also very low in normal heart, but it may increase under pathological conditions. Our data have shown that angiotensinogen mRNA increased in the late

phase of hypertrophy while the increase was not significant in the early phase of LVH, suggesting that the progression of LVH was accompanied by overexpression of key components of local RAS in the cardiac tissue.

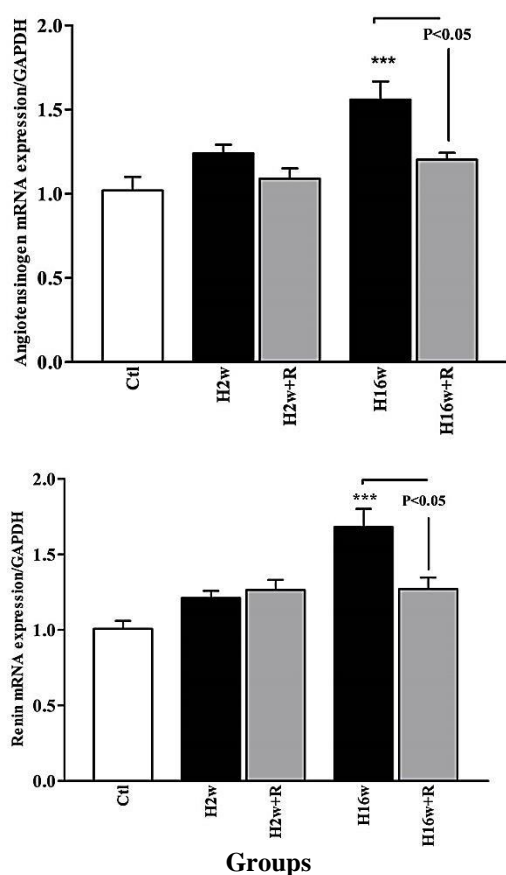


Figure 5. Angiotensinogen and renin mRNA levels; cardiac transcriptional levels of angiotensinogen and renin were assessed in the left ventricular tissue of non-treated and resveratrol-treated (R) hypertrophied rats two and sixteen weeks after aortic banding (H2w and H16w). Intact animal served as control (Ctl). *** $p < 0.001$ vs. Ctl

In this study, we aimed to investigate the effects of resveratrol polyphenol on RAS component changes during myocardial hypertrophy progression. The findings showed that resveratrol inhibited abdominal aorta banding induced hypertension in rats and moderated the rise of HW/BW ratio. It was interesting that this anti-hypertrophic effect was associated with RAS suppression in myocardial tissue as resveratrol could prevent tissue Ang II level elevation as well as renin and angiotensinogen mRNA upregulation in hypertrophied tissue.

The results of our previous study showed that resveratrol suppressed AT1R upregulation in the early phase of hypertrophy [23]. However, this

effect was not observed in the current study. It seems that the main cause of this inconsistency is the duration of resveratrol treatment: in our previous study, animals received drug from two weeks before hypertension induction until three weeks after surgery (five weeks), which shows a longer treatment duration than that of the present work.

In the present study, longer periods of hypertrophy were also investigated and renin and angiotensinogen expression were further evaluated so that it can provide us with a more comprehensive view on the effects of resveratrol on RAS components in progression of hypertrophy. In a study by Ichiki et al., resveratrol suppressed Ang II-induced senescence of vascular smooth muscle cells through inhibition of AT1R [55]. It could be concluded that the impact of resveratrol on RAS component is not only mediated through its direct effect, but also, resveratrol might suppress RAS over-activation through inhibition of hypertension and hypertrophy. Even though resveratrol has no direct effect on RAS, considering the importance of RAS in pathogenesis of myocardial hypertrophy, its indirect effects can also be valuable. To find a more exact answer to the question that “how do RAS changes occur in response to resveratrol?”, schematizing more accurate in vitro and in vivo studies can help gathering more comprehensive results.

A limitation of our study was that renin and ACE activities were not measured. It is, therefore, possible that Ang level have risen as a result of these enzymes over-activation. Although it is proved that RAS components are upregulated anyway, measuring the enzymes activity in further studies can present more accurate information.

Based on the findings of the present study, it could be concluded that progression from compensated hypertrophy to decompensated phase is accompanied by dynamic changes in RAS component. As in the early phase of LVH, AT1aR transcription level was increased, while in the late phase Ang II and the elements involved in Ang II production, such as renin and angiotensinogen, were upregulated. Resveratrol decreased hypertrophy markers in both early and late phase and these protective effects are associated with suppression of RAS expression.

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Author contributions

Tahmineh Hashemizadeh, Fahimeh Dorri Mashhadi and Fereshteh Safari performed the experiments; Ali Pedarzadeh contributed to the study design, data analysis and interpretation; Aryan Naghedi prepared materials and a draft of paper; Javad Zavvar Reza participated in the interpretation of the data; Fatemeh Safari generated the idea, designed the experiments and revised the article.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

BP: blood pressure; HR: heart rate; RAS: renin angiotensin system; LVH: left ventricular hypertrophy; Ang II: angiotensin II; AT1a: angiotensin II type 1 receptor; AT2R: angiotensin II type 2 receptor; ARB: angiotensin receptor blockers; ACE: angiotensin converting enzyme ; LKB1-AMP: liver kinase B1, one of the many upstream kinases of AMPK; eNOS: endothelial nitric oxide synthase; SERCA2: sarco/endoplasmic reticulum Ca²⁺-ATPase; MAPK: mitogen-activated protein kinase; HTN: hypertension; VSMC: vascular smooth muscle cell; SHR: spontaneously hypertensive rat