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High-intensity interval training improves blood pressure and adropin plasma levels in elderly with hypertensive treatment

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Abstract

Background: Adropin is a newly identified bioactive protein that is important in energy hemostasis and vascular endothelial function.

Aim: The purpose of the present study was to investigate plasma levels of adropin and nitrite/nitrate (NO), in elderly treated hypertensive subjects at baseline and follow-up after 6 weeks of high-intensity interval training (HIIT).

Material and Methods: Forty-four elderly participants with treated hypertensives (age 61.09±5.82 years, 25 male and 19 female, BMI= 25.7±1.31 kg/m^2) were randomly assigned to either the high-intensity interval training (HIIT) or control group. The HIIT group received an intervention consisting of 10 intervals of 1.5 min at 85% to 90% of their heart rate reserve (HRR), separated by 2 min of rest at 50% to 55% of their HRR, in three sessions per week for a duration of six weeks. Plasma levels of adropin and NO were measured using enzyme-linked immunosorbent assay. The statistical analysis was performed by two-way repeated ANOVA to determine differences between groups and Pearson's correlation coefficient to determine correlation.

Results: The results showed that, following the six-week HIIT intervention, the plasma levels of adropin and NO significantly increased when compared to both control group (P= 0.0016) and the baseline (P= 0.0003) measurements. Peak oxygen consumption was increased after exercise training compared to the control group (P=0.005). Δ adropin in the HIIT group showed a positive correlation with increased Δ NO (r= 0.707, P= 0i002) and Δ VO_2peak (r= 0.836, P= 0.001), and a negative correlation with Δ DBP (r= 0.643, P= 0.025) and Δ SBP (r= 0.691, P= 0.013).

Conclusions: The study findings suggests that HIIT can enhance both blood pressure and cardiorespiratory fitness. The observed increase in plasma level of adropin may have contributed to reduction of blood pressure by promoting nitric oxide production.

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1. Introduction

Hypertension (HTN) is the major risk factor for cardiovascular and cerebrovascular diseases characterized by increased systemic blood pressure and is one of the primary causes of morbidity and mortality [1]. More than 1 billion people suffer from it in worldwide and raised up incidence as a result of aging [2]. It sounds that a middle-age individual with normal blood pressure is highly likely to become a hypertensive patient, during the next two decades [2].

High blood pressure in elderly subject is an important predictor of the risk of cardiovascular, which is doubled when diastolic and systolic blood pressure increase for 10/20 mmHg in elderly individual [3]. Furthermore, cardiorespiratory fitness (CRF), also is similarly a key predictor of future cardiovascular events [4]. It has been suggested that an inverse correlation between CRF levels and HTN development, even in elderly individuals [5].

Previous studies suggested that regular exercise training improves CRF in older individuals with chronic disease [6, 7, 8] especially in HTN and associated with decrease mortality risk in elderly hypertensive individual [9]. Exercise training is a nonpharmacological strategy recommended for the prevention, treatment and control of blood pressure and cardiovascular risk factors in chronic disease [8, 9, 10, 11].

Adropin is a peptide hormone encoded by the *ENHO* (energy homeostasis associated) gene, which is expressed in multiple tissues including liver, skeletal muscle, heart, and nervous system [12]. Additionally, it was shown that vascular endothelial cells are another source of adropin and trigger an intracellular signaling transduction pathway, which may in turn be involved in endothelial function. Adropin binds to vascular endothelial growth factor receptor 2 (VEGFR2) and activation of the PI3K-Akt and ERK1/2 pathways which results in increases endothelial nitric oxide synthesis (eNOS) [13]. Clinical studies reported that lower levels of adropin in patients with T2DM and HTN compared to healthy people were associated with cardiovascular disease [11, 14].

The balance between vasodilation and vasoconstriction is impaired in hypertension and aging by increasing oxidative stress, reducing eNOS expression, decreased nitric oxide [10] and alterations in vascular phenotype [15].

Aerobic exercise training improves endothelial dysfunction concomitant with increases in plasma levels of nitric oxide (NO) in HTN individuals [8]. Previous research has demonstrated aerobic exercise training elevated plasma levels of adropin and NO along with decreased arterial stiffness and blood pressure in persons with hypertension [11, 15]. Evidence to date suggests that the intensity of exercise training is the most important factor in the reported improvements in cardiorespiratory fitness, glycemic index and blood pressure [7, 8]. Previous studies found that high-intensity interval training (HIIT) has superior effects on cardiovascular risk factors and improvements in vascular function in persons with chronic diseases [7, 8, 16]. It appears that different types of exercise training may have different effects on cardiovascular risk factors, cardiorespiratory fitness, vascular function and blood pressure.

The mechanisms responsible for the effect of HIIT on vascular function have not been fully explained. Evidence suggests that aerobic exercise training increases plasma levels of adropin and NOx in middle-aged and older healthy persons [11]. However, no study we are aware of having

investigated whether HIIT is capable of improving blood pressure via adropin and NO in elderly hypertensive subjects. In this study we hypothesized that HIIT may improve blood pressure via increasing adropin and NOx levels. To test our hypothesis, we measured adropin, NOx, blood pressure, and cardiorespiratory fitness in an elderly hypertensive subject.

2. Materials and Methods

2.1. Human experiments

Forty-four patients from the HTN clinic of Ashrafi Esfahani hospital in Tehran voluntarily participated in this study. The inclusion criteria were: age 50-70 years, treated hypertensive subjects (systolic blood pressure< 140 mmHg and diastolic blood pressure< 90 mmHg by used antihypertensive drugs) who had been given medication therapy for at least 12 months (with at least 3 months of the medication being performed without complications), and no exercise training in the previous one year. The exclusion criteria were body mass index over 28 kg/m^2, diabetes and obesity, cardiovascular disease, liver disease, mental illness, functional limitations (such as osteoarthritis), drug discontinuation during the study and smoking.

After the patients were evaluated for eligibility, the participants were randomized (by gender and age) to the HIIT and control (CTR) group with an online randomization tool (https://www.randomizer.org/). Block randomization was used with a block size of two. The present study protocol was approved by the Ethics Committee of Tehran University and was conducted according to the Helsinki declaration and is registered at http://www.irct.ir/ (IRCT2016092823002N3). Before participation in the study, written informed consent was received from all patients.

2.2. Study design

This study investigated the effect of HIIT on adropin, NO, blood pressure and cardiorespiratory fitness in elderly treated hypertensive subjects. Resting diastolic and systolic blood pressure were measured at baseline and at the end of 6 weeks (after a 1-h rest in supine position) with a sphygmomanometer (Beurer GmbH, Ulm, Germany). Fasting blood samples were taken from the antecubital vein at the beginning and end of the exercise training period to measure plasma levels of adropin, NO. Anthropometric characteristics (height, weight and body mass index [BMI]), peak oxygen consumption (VO_{2peak}) and maximal heart rate (HR_{max}) were measured at baseline and at the end of the 6-week intervention. An incremental exercise test (1 week before and after exercise training) was performed on a bicycle ergometer. Respiratory gases were collected using a facemask and breath-by-breath analysis was performed using a commercial system (ZAN 600CEPT, Ergorespiratory, ZAN Mesgerate GMbH, Oberthulba, Germany) to determine VO_{2peak} and also measure HR_{max}. The workload in the incremental cycle exercise test was initially set at 30 W for men and 20 W for women, for 2 min, and power output was increased every 1 min by 10 W until the participant could not continue and maintained a fixed pedaling frequency of 40 rpm. All participants wore a heart monitor (Polar Beat, Polar Electro, Kempele, Finland) during the exercise test to verify the assigned exercise intensity (HR_{max}) [11, 17].

2.3. Exercise training

All participants received 6 weeks of HIIT, 3 days per week, while those in the CTR group continued their usual activities without any regular training. Exercise training was provided with a bicycle

ergometer (894E Monark Ergomedic Peak Bike, Varberg, Sweden). Participants in the HIIT group performed 10 intervals (1.5 min) at 85% to 90% heart rate reserve (HRR) separated by 2 min at 50% to 55% HRR in each session of exercise training (Figure 1).

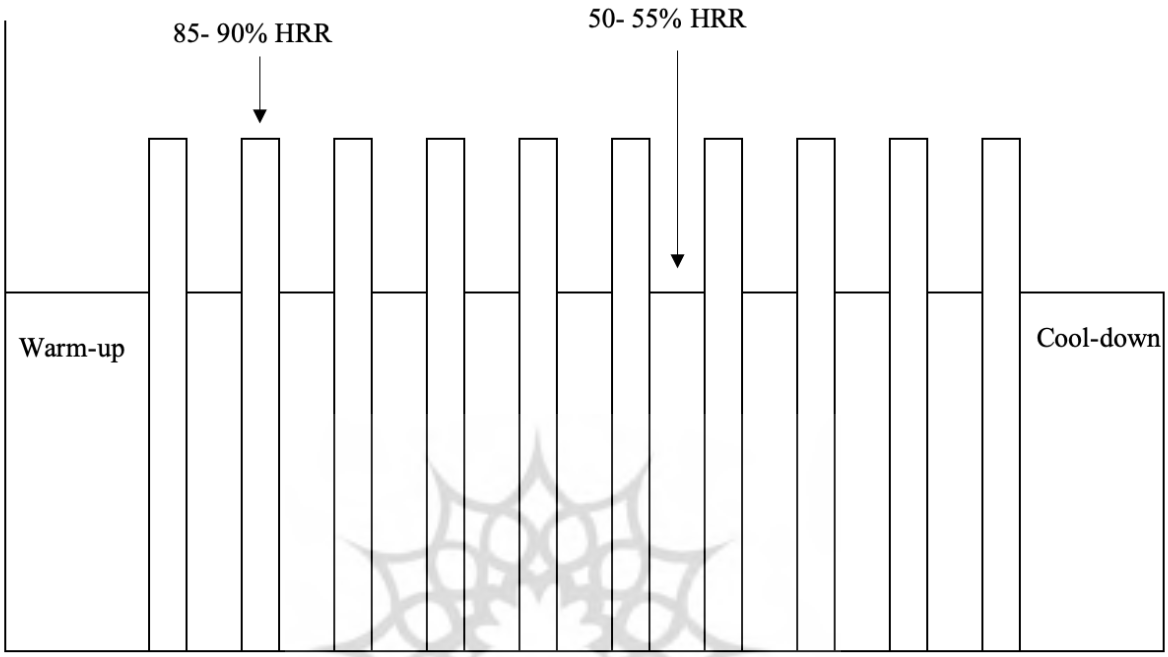


Figure 1. HIIT protocol flow-chart

HRR was calculated as the difference between peak and resting heart rate, multiplied by the intensity of exercise and added to resting heart rate, according to the Karvonen method. Peak and resting heart rate were obtained during the exercise test. The treadmill velocity was continually adjusted along as training adaptations occurred, to ensure that every training session was carried out at the desired HRR throughout the 6-week training period. To ensure proper intensity, all subjects wore heart rate monitors (Polar Beat, Polar Electro, Kempele, Finland) in the exercise session.

The control group subjects were encouraged to maintain their daily activities without exercise training during the 6-week period. Additionally, subjects in both groups were advised to maintain the food intake and follow their usual daily schedule during the 6 weeks of experimental period [11, 17].

2.4. Blood sample analyses and anthropometric assessment

Blood samples were collected from the antecubital vein after an overnight fast at baseline and 48 h after the last exercise training session. All blood samples were immediately centrifuged at 1800 g for 15 min at 4 C. The plasma samples were placed in liquid nitrogen, transferred to the laboratory, and stored at −80 C. Plasma levels of adropin were measured by ELISA (LifeSpan BioSciences, Inc. LS-F7643, Seattle, WA, USA), and NO was measured with the Griess method (R&D System Abingdon Science Park, Bio-tech, Abingdon, UK) according to the manufacturer's instructions.

Height was measured with a

stadiometer (SECA, Hamburg, Germany) and weight was recorded with a calibrated electric digital scale (AMZ 12; Tokyo, Japan) while participants wore light clothing but no shoes. BMI was calculated as weight (kg) divided by height (m^2).

2.5. Statistical analysis

The data are expressed as the mean±standard deviation. All statistical analyses were performed with SPSS statistical software (version 16.0; SPSS Inc., Chicago, IL, USA) with a significance level (for two-tailed tests) of $P<0.05$. The Shapiro–Wilk test was used to verify the normality of data distribution. Two-way repeated ANOVA was used in order to determine differences between groups. The relationships between adropin and NO with blood pressure was assessed with Pearson's correlation coefficient.

3. Results

The clinical and demographic characteristics of participants and types of medication therapy are shown in Table 1. It was shown that no significant differences between groups in demographic or clinical characteristics or medication at baseline (Table 1).

The results shown that no significant for height, weight, BMI, maximal heart rate, VO_{2peak}, DBP, SBP and medical therapy at baseline (Table 1). Compared to the baseline value, weight and BMI decreased in HIIT group ($P<0.05$), and showed that weight and BMI decreased compared to the CTR group ($P\leq0.032$). The value of DBP and SBP have no significant after the 6-week exercise intervention between groups, however, interaction time × group significantly decreased in DBP ($P=0.007$) and SBP ($P=0.035$) in the follow-up (Table 2).

The value of HR_{max} ($P=0.019$) and VO_{2peak} ($P=0.032$) significantly increased compared to baseline and CTR group. Additionally, it was demonstrated that 6-week of HIIT reduced resting heart rate and heart rate recovery at 1 in HIIT group.

Table 1. Clinical characteristics of elderly hypertensive subjects at baseline

	CTR (n= 15)	HIIT (n= 15)	P value
Gender (male/female)	8 / 7	9 / 7	0.960
Age (years)	60.40 ± 6.35	61.86 ± 5.23	0.231
Weight (kg)	70.26 ± 3.86	72.43 ± 3.64	0.253
Height (cm)	167.53 ± 4.40	169.06 ± 4.75	0.198
BMI (kg/m^2)	25.92 ± 1.67	25.43 ± 1.07	0.648
Maximal heart rate (bpm)	168.13 ± 11.17	166.66 ± 12.16	0.419
VO_{2peak} ($mL\ kg^{-1}\ min^{-1}$)	28.20 ± 5.41	27.20 ± 5.15	0.521
Exercise time	10.64 ± 1.64	10.23 ± 1.45	0.953
DBP (mm Hg)	81.13 ± 4.67	82.33 ± 3.71	0.506
SBP (mm Hg)	129.86 ± 6.94	130.46 ± 6.71	0.871
Diuretic	9 (60%)	8 (53%)	0.713
ACE inhibitors	6 (40%)	5 (33%)	0.705
β-blockers	5 (33%)	4 (26%)	0.690
CCI	3 (20%)	3 (20%)	0.990

Data are presented as means ± SD. P values calculated with independent-t for demographic variables, and with the chi-squared test for medication use.

Abbreviations: HIIT, high-intensity interval training; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; ACE-inhibitors, angiotensin-converting enzyme inhibitors; CCI, calcium channel inhibitor.

Table 2. Baseline and follow-up results for exercise tests and biochemical measurements

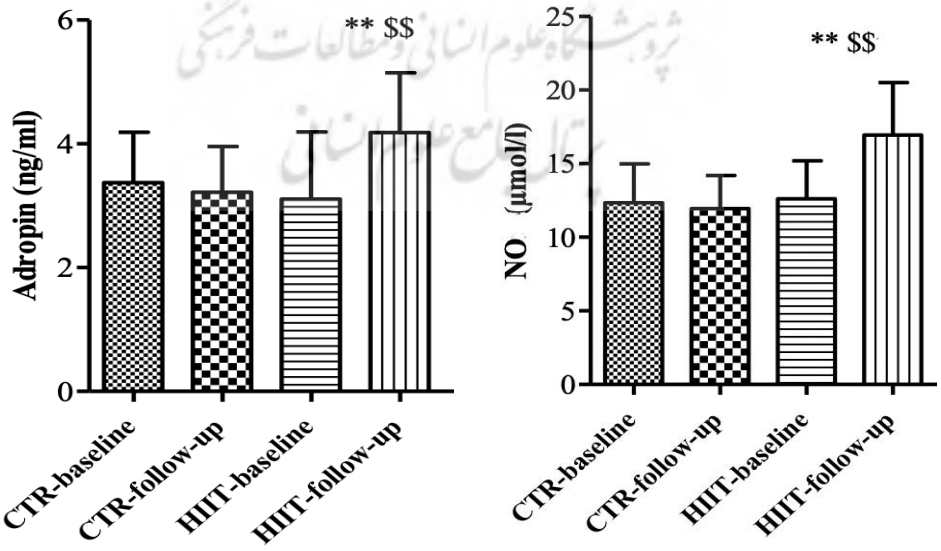
	CTR		HIIT		*P*
	Baseline	**Follow-up**	**Baseline**	**Follow-up**	
Weight (kg)	70.26 ± 3.86	71.06 ± 4.06	72.43 ± 3.64	69.31 ± 3.75*	0.043
BMI (kg/m^2)	25.92 ± 1.67	26.01 ± 1.51	25.43 ± 1.07	24.93 ± 1.05*	0.032
DBP (mm Hg)	81.13 ± 4.67	82.06 ± 3.43	82.33 ± 3.71	80.20 ± 3.60 *	0.007
SBP (mm Hg)	129.86 ± 6.94	130.53 ± 5.39	130.46 ± 6.71	127.03 ± 6.35 *	0.035
VO_{2peak} (mL kg^{-1} min^{-1})	28.20 ± 5.41	27.53 ± 5.39	27.20 ± 5.15	30.73 ± 4.99 *$	0.005
Exercise time (min)	10.64 ± 1.64	10.61 ± 1.60	10.24 ± 1.45	11.43 ± 1.43 *$	0.009
HR_{max} (bpm)	168.13 ± 11.17	164.86 ± 11.46	166.66 ± 12.16	170.13 ± 9.62	0.062
HRR at 1 min (bpm)	141.93 ± 9.66	141.60 ± 9.77	140.46 ± 12.81	129.00 ± 12.68 *$	0.001
HRR at 2 min (bpm)	122.20 ± 7.65	123.40 ± 7.80	121.20 ± 14.18	119.26 ± 13.72 *	0.019
HRR at 3 min (bpm)	103.66 ± 9.46	103.60 ± 9.36	102.40 ± 11.40	98.53 ± 11.69 *	0.040

Data are presented as means ± SD. *P value< 0.05 vs. CTR; $ P value< 0.05 vs. Baseline.

Abbreviations: CTR, control; HIIT, high-intensity interval training; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR_{max}, maximal heart rate; VO_{2peak}, peak oxygen uptake; HRR, heart rate recovery.

Baseline and follow-up adropin and NO plasma levels are shown in Figure 2. The results showed no significant differences in plasma levels of adropin and NO at baseline between groups. There was a significant increase in adropin after 6- weeks HIIT (*P*=0.003). Compared to the baseline value, plasma adropin levels increased 38.68% in HIIT (time × group interaction, *P*=0.001). After the 6-week exercise intervention, NO concentrations increased in HIIT group (time × group interaction, *P*= 0.003). Plasma levels of NO in the HIIT group compared to baseline were increased by 31.09% (*P*<0.001).

The results showed that Δ adropin in the HIIT group was associated with increased Δ NO (r= 0.707, P= 0.002) and Δ VO_{2peak} (r= 0.836, P= 0.001). Additionally, there is a negative correlation between Δ adropin with Δ DBP and Δ SBP. It was shown that increase in Δ adropin was associated with decrease Δ DBP (r= 0.643, *P*= 0.025) and Δ SBP (r= 0.691, *P*= 0.013).



** *P* < 0.01 vs. CTR group, $$ *P*<0.01 vs. baseline.

Figure 2. Plasma levels of adropin and NO at baseline in control (CTR) and high-intensity interval training (HIIT) groups

Table 3. Adjusted Pearson's correlation [r (P-value)] for %FMD and cardiovascular risk factors

	Δ NO	Δ VO_{2peak}	Δ DBP	Δ SBP
Δ Adropin				
CTR	-0.376 (0.298)	-0.281 (0.546)	0.121 (0.668)	0.139 (0.622)
HIIT	0.707 (0.002)	0.836 (0.001)	- 0.543 (0.056)	- 0.613 (0.043)

Data are presented as r (P-value).

Abbreviations: Δ, Change in value baseline and follow-up; HIIT, high-intensity interval training; VO_{2peak}, peak oxygen uptake; NOx, nitrite/nitrate; DBP, diastolic blood pressure; SBP, systolic blood pressure.

4. Discussion

The main finding of this study is that 6 weeks of HIIT led to decreased blood pressure associated with increases in plasma levels of adropin and NO in elderly treated hypertensive patients. Plasma levels of the adropin and NO increased in the HIIT group compared to CTR. Additionally, changes in plasma levels of adropin showed a positive correlation with Δ NO and Δ VO_{2peak} following exercise training. However, there is a negative correlation between value change of plasma levels of adropin with Δ DBP and Δ SBP in the HIIT group.

Previous studies have been shown that HIIT had a greater benefit for cardiorespiratory fitness than traditional endurance training in chronic disease [6, 7, 8, 16]. Our results indicated that VO_{2peak} ($ml.kg^{-1}.min^{-1}$) was increased after 6- weeks of HIIT training. Prevalence of HTN increased in subjects who have a lower level of cardiorespiratory fitness. Increase of cardiorespiratory fitness levels via exercise training could prevent the proportion of HTN. Earlier research found that the intensity of exercise training had the clearest effect on cardiorespiratory improvements [6, 7, 8, 16]. Increased blood flow by HIIT, through fluctuations between low and high intensities, is a great challenge to the heart, improves cardiorespiratory fitness indicators such as stroke volume and oxygen uptake [18].

Diastolic and systolic blood pressure were significantly decreased compared to baseline and to the control group. Improved blood pressure is associated with increased cardiorespiratory fitness in chronic patients [7]. The improved blood pressure induced by HIIT appears to be related to increased VO_{2peak}, shear stress, and metabolic production in blood vessels along with local factors associated with exercise training [8, 9].

Adropin is a newly identified secretory protein encoded by the protein energy homeostasis-related gene *ENHO* [19]. A clinical study demonstrated that low serum adropin was associated with coronary atherosclerosis in healthy and chronic subjects [14]. Plasma levels of adropin were increased after aerobic exercise training in adolescents with obesity [20] and middle-aged and older people [14]. A previous study indicated that 12 weeks of aerobic exercise at 60–80% HR_{max} increased adropin plasma levels from 2.62±087 to 3.45±0.65 ng/mL in adolescents with obesity [14]. The present study shows that a 6- week HIIT intervention significantly increased plasma levels of adropin in elderly treated hypertensive.

Adropin is highly expressed in brain, heart, skeletal muscle, liver, kidney and endothelial cells [12]. However, Fujie et al. (2021) showed that only mRNA adropin expression in the aorta followed the same pattern as circulating adropin plasma levels in aging and exercise training, suggesting reduced arterial adropin synthesis as an explanation for the lower circulating adropin in aging and chronic disease [21]. Given that the arterial tree is one of the most important sources of circulatory adropin, its

adaptation to the mechanical stresses associated with exercise training, may explain our observed increases in plasma levels of adropin following HIIT.

Endothelial dysfunction is a main mechanism suggested to explain the effects of adropin in patients with cardiovascular disease [22]. The endothelium plays a crucial role in maintaining vascular hemostasis and (in part) nitric oxide production and bioavailability [23]. Low levels of nitric oxide are associated with endothelial function. Lovren et al. (2010) reported that endothelial cells are an important source of adropin, which upregulates eNOS expression– a factor responsible for nitric oxide production in the endothelium [13]. In addition, changes in arterial adropin concentrations, associated with aerobic training, are reported to correlate positively with changes in arterial eNOS phosphorylation and NOx levels [21]. Our results support this, in that change of plasma levels of adropin were correlated with plasma levels of NOx following HIIT.

The mechanisms by which HIIT increases nitric oxide production and bioavailability is not fully understood. Nevertheless, a previous study showed that increased anterograde shear and decreased retrograde shear led to increased nitric oxide production [24]. Oxidative stress is another possible mechanism associated with decreased nitric oxide bioavailability, given that in combination with nitric oxide, it favors peroxynitrite production [25].

One previous study showed that greater shear stress and increased antioxidant capacity were seen after aerobic interval training [16]. In addition, adropin led to increased nitric oxide production through the activation of VEGFR2 and the downstream PI3K/Akt or ERK1/2 signaling pathway [13]. Aerobic training increased VEGF-2 protein expression, Akt and eNOS phosphorylation which was associated with NO arterial concentration [21].

Systolic and diastolic blood pressure were decreased after 6- weeks of exercise training in our participants. In this connection, Zhang et al. (2017) reported that increased plasma levels of adropin were associated with decreased blood pressure and increased reactive hyperemia index [15]. Moreover, increased plasma levels of adropin after aerobic exercise training were accompanied by a concomitant decrease in arterial stiffness in middle-aged and older adults [20]. HIIT may decrease blood pressure through an increase in plasma levels of adropin, concomitant with evaluation of plasma NOx levels. The present study shows that elevated adropin levels were negatively associated with diastolic ($r=-0.530$, $P=0.035$) and systolic blood pressurec($r=-0.606$,. $P=0.013$) after the HIIT intervention. There was a positive correlation between plasma adropin levels and NOx and VO_{2peak} in both exercise training groups studied here.

This study has several limitations. The percent of adherence to exercise training was 68% for HIIT group, which is a low level of adherence, a greater proportion of participants was lost to follow-up. Losses of follow-up could have decreased our ability to see a significant intervention effect. Despite the relatively small sample, we used numerous selection criteria, and the whole sample was adequately homogenous. Participants were allowed to be taking several medications which could affect vascular health, and this could have decreased the effect of exercise training.

5. Conclusion

We investigated the effect of 6- weeks of HIIT on plasma adropin and NO concentration and blood pressure in elderly hypertensive subjects. This finding suggests that HIIT increased plasma levels of

adropin along with NO. Additionally, there was a negative correlation between adropin and blood pressure in the HIIT group. Thus, increased plasma levels of adropin may help to lower blood pressure by increasing nitric oxide production.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to the original idea, study design.

Ethical considerations

The author has completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc.

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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