



# Comparing the Apelin Level in Hypertensive Patients Who Received Hypertension Drugs

Mina Hemmati <sup>1,2,\*</sup>, Fatemeh Abharzanjani <sup>3</sup>, Tooba Kazemi <sup>2</sup> and Fateme Estantesi <sup>4</sup>

<sup>1</sup>Biochemistry Department, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

<sup>2</sup>Cardio Vascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran

<sup>3</sup>Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran

<sup>4</sup>Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran

\*Corresponding author: Biochemistry Department, Zanjan University of Medical Sciences, Zanjan, Iran. Tel: +98-2433140301, Fax: +98-2433449553, Email: mhemmati@zums.ac.ir

Received 2020 June 07; Revised 2020 December 16; Accepted 2021 January 26.

## Abstract

**Background:** Apelin, an adipokine secreted from adipose tissue, plays an important role in regulating blood pressure and hypertension.

**Objectives:** The current study aimed to compare the plasma Apelin level in hypertensive patients under treatment with amlodipine, losartan, and amlodipine + losartan.

**Methods:** In this case-control study, the serum level of Apelin was compared in four groups of (A) Healthy subjects (n = 31); (B) Hypertensive patients, received amlodipine (n = 31); Hypertensive patients, received losartan (n = 45); and patients (n = 33) that received amlodipine and losartan. Apelin level in serum samples was measured using Human Apelin ELISA Kit according to the manufacturers' instructions. Data were analyzed using SPSS version 19 (Chicago: SPSS Inc.), at the significant level of  $\alpha = 0.05$ .

**Results:** The mean blood level of Apelin in the control group and groups receiving amlodipine, losartan, and amlodipine + losartan was  $366.16 \pm 36.04$ ,  $247.19 \pm 27.77$ ,  $282.93 \pm 47.08$ , and  $289.84 \pm 32.20$  g/dl, respectively. Losartan + amlodipine group had a higher level of Apelin compared with amlodipine alone ( $P < 0.05$ ).

**Conclusions:** This study demonstrated that Apelin has a definite protective effect in preventing hypertension. Also, according to the results, the renin-angiotensin-aldosterone system inhibitors, such as losartan, caused a higher increase in the Apelin, resulting in better blood pressure control.

**Keywords:** Apelin, Hypertension, Amlodipine, Losartan, Calcium Channel Blocker Receptor, Angiotensin II Receptor Antagonists

## 1. Background

Hypertension is a major health problem that significantly increases the risk of several health problems, such as cardiovascular disease (CVDs), coronary heart disease (CHD), congestive heart failure (CHF), ischemic and hemorrhagic stroke, kidney failure, and peripheral vascular disease. Although antihypertensive therapy significantly reduces the risk of CVDs and renal problems, most people with hypertension do not receive treatment at all, or their treatment is inadequate (1). There are different levels of hypertension and age-related increase in blood pressure. In industrial societies, blood pressure increases steadily during the first two decades of life (2).

Recent studies have shown that adipose tissue hormones or adipokines are involved in the pathogenesis of obesity complications such as hyperlipidemia, diabetes mellitus, hypertension, atherosclerosis, and heart failure.

Apelin, as an Adipokine, exists in the body in at least three forms of 13, 17, and 36 amino acids, all of which originate from a 77 amino acid precursor. Apelin is a multifunctional peptide involved in the regulation of the cardiovascular system (including regulation of blood pressure and cardiovascular function control). So obviously, Apelin has a role in the pathophysiology of hypertension and heart diseases associated with high blood pressure (3, 4).

It also plays a role in angiogenesis and apoptosis of endothelial and smooth muscle cells. Besides, it has an anti-vascular endothelial growth factor. In blood flow, Apelin modifies the expression of the endothelial nitric oxide synthase enzyme (eNOS) and causes vasodilation related to eNOS, and acts against the vasoconstriction associated with angiotensin II. Therefore, it has positive inotropic and protective effects on the heart. Apelin is a protein that affects the vascular endothelium-dependent vasodilation

and with positive inotrope. Apelin not only can reduce the ventricular afterload and preload, but also can increase the strength of cardiac contraction. Apelin plasma level is a practical guide for evaluating the severity of heart failure (3, 4).

This peptide acts through Apelin's receptor (APJ receptor) and is much similar to angiotensin II. Apelin is widely spread in many tissues, mostly vascular endothelium (5). Apelin/APJ System has several physiological effects on water/electrolyte balance, blood sugar control, and safety and nutritional status of the body, but its main purpose is the cardiovascular system (6). Some studies showed that the apelin/APJ system has an essential role in body normal function as well as CVDs such as atherosclerosis, CHD, heart failure, and systemic/pulmonary hypertension (7).

Apelin generally creates autocrine and paracrine effects, but its plasma level also has physiological effects. Apelin plasma concentration is around 10 g/mL, and its half-life is less than 5 minutes. The slow, stable, and inotropic effects of apelin have also been proved in very low concentrations up to the point that Apelin has been known as the strongest inotrope endogenous molecule, even more, effective than adrenomedullin and endothelin (6, 8, 9).

On the other hand, the mutual effect of Apelin-APJ receptor on vein surface causes vascular vasodilation induction through NO release from endothelial cells and declines vascular load in the left ventricle and its filling pattern improvement through decreasing the systemic vascular resistance. APJ receptor exists in the smooth veins and causes its plasma level maintenance (10, 11).

## 2. Objectives

Since Apelin plays an important role in blood pressure regulation, the plasma Apelin level in hypertensive patients receiving hypertensive drugs has been measured in this study in order to inform the hypertensive patients about the importance of this peptide as well as preventing the usage of its devastating materials.

## 3. Methods

The study population of the present research was all hypertension patients admitted to the cardiology clinic of Vali-e-Asr Hospital of Birjand in 2014 with the arrival conditions. Participants were selected using a non-random sampling method. The inclusion criteria were being aged 25 to 85 years, satisfaction to participate, the absence of comorbidities such as diabetes, dyslipidemia, CAD, hypertension diagnosis for patients in the recent two years, complete treatment period, regular referring to the cardiology

clinics, and regular consumption of medications, lack of uncontrolled hypertension, absence of any secondary hypertension, chronic renal and hepatic diseases, diabetes, CAD, and history of, or current risk of cancer.

In total, 140 eligible subjects were divided into four groups: (A) 45 hypertensive patients who were treated with an Angiotensin II receptor blockers (ARB) drug such as losartan; (B) 31 hypertensive patients treated with calcium channel blockers (CCB) drugs such as amlodipine; (C) 33 hypertensive patients, simultaneously treated with an ARB drug such as losartan and a CCB drug such as amlodipine; and (D) 31 healthy subjects as controls. Demographic characteristics of participants were collected using questionnaires, and their blood samples were also collected.

All sampling procedures were performed by nurses under sterile conditions. Patients were ensured that their blood samples only will be used for research purposes, and their information would remain confidential. To take a blood sample, 5 cc of blood was taken from the patients referring to the cardiology clinics. After centrifugation, the blood serum samples were divided into 5 mL tubes and then frozen and stored at -20°C.

Collected samples were then analyzed within 2 to 4 weeks, and Apelin levels were evaluated using an ELISA kit (East Biopharm Company of Thailand). Blood pressure measurement was performed using a mercury manometer in a quiet environment on the cases' right hands in a sitting position. The patients were asked not to use energetic substances, narcotics, tea, soft drinks, and cigarettes an hour before assessing their blood pressure, and they were also asked to rest on a chair for 5 minutes before the assessment. The study was approved by the research ethics committee of the Birjand University of Medical Sciences (ethic code: Ir.bums.1394.287). Besides, the principles of the Declaration of Helsinki and its amendment were followed.

Data were analyzed using SPSS (V.16) in order to determine the distribution of the main variable (Apelin). According to the results of the Kolmogorov-Smirnov test ( $P = 0.144$ ), the non-normality assumption was rejected, and therefore the distribution was assumed to be normal. In addition to descriptive statistics (i.e., mean and standard deviation), data were analyzed using the statistical tests (t-independent, ANOVA, and Tukey tests). Statistical significance was considered when  $P$ -value  $< 0.05$ .

## 4. Results

In the present study, 140 eligible subjects were evaluated, as follows: 31 subjects in the healthy group, 31 in the amlodipine group, 45 in the losartan group, and 33 in the combination therapy group (amlodipine + losartan). There was no significant difference between the

groups concerning gender and mean age ( $P = 0.467$  and  $P = 0.276$ , respectively) (Table 1). However, significant differences were observed among the different groups of patients compared to healthy controls concerning mean systolic blood pressure, according to ANOVA test results ( $P = 0.01$ ). According to the results of the Tukey HSD test, the only significant difference was between the mean diastolic blood pressure of healthy people and other therapeutic groups, and in this regard, there was no difference among treatment groups (Table 2). The results of the ANOVA test for comparing the values of Apelin demonstrated a significant difference between the groups; therefore, the post hoc test was used to identify the precise difference.

The results of the Tukey's test indicated that the highest level of Apelin existed in order in the amlodipine + losartan, losartan, and amlodipine treatment groups, respectively. Meanwhile, Apelin level in patients receiving losartan and the combination of losartan and amlodipine was significantly higher compared to the amlodipine-treated group ( $P \leq 0.05$ ). Compared to the healthy individuals, the groups treated with amlodipine, losartan, and combined therapy had lower circulating levels of apelin, respectively (Table 3). In the present study, most of the participants (119 patients, 85%) had a low level of Apelin (less  $\geq 365$  pg/mL). Assessing the quality level of Apelin among different groups showed a significant correlation ( $P = 0.01$ ) (Table 4).

The results depicted a significant association between the mean systolic and diastolic blood pressure levels and the level of Apelin; i.e. those with a low Apelin level had much higher mean systolic and diastolic blood pressures compared to those with normal Apelin level ( $P < 0.01$  and  $P < 0.05$ , respectively) (Table 5).

## 5. Discussion

Losartan is an antihypertensive drug acting as a selective angiotensin II type 1 (AT1) receptor antagonist (12). In addition, Amlodipine is a CCBs drug that exerts its action through inhibition of calcium influx into vascular smooth muscle cells and myocardial cells. Indeed, Amlodipine decreases peripheral vascular resistance (PVR), which is indicated for treating high blood pressure (13).

The current study aimed to compare the serum Apelin levels in high blood pressure patients treated with amlodipine, losartan, and amlodipine + losartan. The highest Apelin level was observed in patients treated with amlodipine + losartan, which was significantly higher than those who received losartan and amlodipine alone. The systolic blood pressure was higher in the amlodipine + losartan group compared to the amlodipine and losartan groups, but this difference was not significant. Furthermore, the

diastolic blood pressure level was higher in amlodipine group compared to the losartan and amlodipine + losartan groups; however, it was not statistically significant. Those in the control group had a lower systolic and diastolic blood pressure and higher levels of Apelin compared to the treatment groups. Regarding the higher blood Apelin level in groups treated with losartan or losartan + amlodipine, compared to the group treated solely with amlodipine, we can mention the results of Hung et al. (14), which attempted to analyze the performance of Ang II receptor during Adipocytes differentiations. They concluded that inhibiting the renin-angiotensin-aldosterone system causes increased secretion of Apelin; a conclusion that is in line with the results of the present study (14). Furthermore, Siddiquee et al. (15), in a study on the protective effects of Apelin against the cardiovascular fibrosis resulting from angiotensin II and PAI-1 production decline, demonstrated that Apelin has protective properties against vascular remodeling and cardiac fibrosis through the direct regulation of PAI-1 gene expression. The mentioned protective effect is induced by the synergistic inhibition of Ang II signaling and NO production rise due to Apelin (15). The results obtained regarding the lower level of diastolic blood pressure in the group treated with losartan, as compared to treatment groups, can be justified in this respect.

Akcilar et al. (16) also showed that apelin decreases blood pressure in DOCA-salt rats and can be used as a therapeutic agent in the treatment of high blood pressure in the future. The result obtained in our study is in line with this study (16).

It is noteworthy that Apelin is not only effective in systemic blood pressure, but it also plays an important role in regulating pulmonary hypertension, as reported by Wannamethee et al. (17) and Azizi et al. (18). According to the literature, there is a predictive effect of Apelin polymorphism in patients with hypertension treated with losartan, in women unlike men, that means the observed decrease in systolic blood pressure after 24 weeks of treatment with losartan was significantly different in dominant and recessive genotype models. Therefore, it has been concluded that there is a relationship between the existent of specific Apelin genotype and a better response to treatment with Angiotensin II inhibitor (losartan) (19).

In line with the results of this study regarding the higher Apelin levels in healthy people with normal blood pressure, Przewlocka-Kosmala et al. (20) demonstrated that the Apelin level decreased in blood circulation in the patients with high blood pressure and the low plasma apelin level in this patients can independently aggravate the ventricular systolic and diastolic functional disorder.

Andersen et al. (21) investigated the association between the Apelin level and pulmonary hypertension and

**Table 1.** Different Groups of Patients Based on Gender and Age

Group	Sexuality, No. (%)		Chi Square Test	Groups	Age		ANOVA
	Male	Female			Range	Mean $\pm$ SD	
Healthy control	13 (40)	18 (60)	Value = 2.607; df = 3; P = 0.467	Healthy control	39 - 76	9.63 $\pm$ 52.56	F = 1.303; df = 3; P = 0.276
Amlodipine	10 (32.3)	21 (67.7)		Amlodipine	39 - 76	9.56 $\pm$ 52.32	
Losartan	22 (48.9)	23 (51.1)		Losartan	34 - 80	9.22 $\pm$ 53.33	
Amlodipine+ losartan	16 (48.5)	17 (51.5)		Amlodipine+ losartan	44 - 73	7.74 $\pm$ 56.27	
Total	60 (43.2)	79 (56.8)		Total	34 - 80	9.09 $\pm$ 53.64	

**Table 2.** Different Groups of Patients Based on Systolic and Diastolic Blood Pressure

	Range	Mean $\pm$ SD	ANOVA
<b>Systolic pressure</b>			F = 39.830; df = 3; P = 0.01
Healthy control	105 - 125	117.60 $\pm$ 5.81	
Amlodipine	120 - 150	136.74 $\pm$ 7.68	
Losartan	110 - 150	135.00 $\pm$ 9.17	
Amlodipine + losartan	110 - 160	137.37 $\pm$ 9.44	
Total	105 - 160	132.17 $\pm$ 12.27	
<b>Diastolic pressure</b>			F = 5.552; df = 3; P = 0.01
Healthy control	75 - 85	79.19 $\pm$ 3.74	
Amlodipine	70 - 100	86.93 $\pm$ 9.36	
Losartan	70 - 100	85.60 $\pm$ 8.54	
Amlodipine + losartan	70 - 100	84.88 $\pm$ 8.94	
Total	70-100	84.26 $\pm$ 5.85	

**Table 3.** Different Groups of Patients Based on the Serum Apelin Level

Group	Range	Mean $\pm$ SD	ANOVA
<b>Apelin, pg/mL</b>			F = 55.308; df = 3; P = 0.01
Healthy control	290 - 415	366.16 $\pm$ 36.04	
Amlodipine	199 - 301	247.19 $\pm$ 27.77	
Losartan	198 - 375	282.93 $\pm$ 47.08	
Amlodipine + losartan	210 - 335	289.84 $\pm$ 32.20	
Total	198 - 415	295.07 $\pm$ 55.49	

showed that patients with primary pulmonary hypertension (PPH) had a lower level of plasma and decreased expression of Apelin in lung endothelial cells. So Apelin has been presented as a potential marker for PPH. In addition, Apelin plays a role in angiogenesis and regulating

**Table 4.** Different Groups of Patients Based on the Quality Level of Serum Apelin

Group	Normal, No. (%)	Low, No. (%)	Exact Fisher Test
<b>Apelin, pg/mL</b>			Value = 47.656; P = 0.01
Healthy control	18 (58.1)	13 (41.9)	
Amlodipine	0 (0)	31 (100)	
Losartan	3 (6.7)	42 (93.3)	
Amlodipine + losartan	0 (0)	33 (100)	
Total	21 (15)	119 (85)	

**Table 5.** Average Level of Systolic and Diastolic Blood Pressure in Subject with Normal and Low Level of Apelin

Apelin	Mean $\pm$ SD	Independent t-test
<b>Systolic blood pressure</b>		t = 8.63; df = 137; P = 0.01
Low level	143.91 $\pm$ 9.56	
Normal	115.90 $\pm$ 5.46	
<b>Diastolic blood pressure</b>		t = 3.14; df = 137; P = 0.02
Low level	85.16 $\pm$ 8.68	
Normal	78.90 $\pm$ 4.75	

the apoptosis of endothelial, smooth muscle cells. Chronic treatment with Apelin could reduce the pulmonary hypertension progression in animal models, and researchers have suggested APLNR as an interesting potential therapeutic target for PPH (22-24).

Some studies have investigated the effect of Apelin on blood pressure. For example, Fan et al. (25) investigated the role of Apelin in the prevention of pulmonary hypertension induced by hypoxia in rats. They reported that Apelin showed an important role in the treatment of hypoxic pulmonary hypertension of rats based on vasodilation of pulmonary artery and inhibition of oxidative stress (25).

This study demonstrated that Apelin has a protective effect in the prevention of hypertension in healthy sub-

jects, and for those who suffer from hypertension, it can decline the level of Apelin. Also, according to the findings, renin-angiotensin-aldosterone system inhibitor was associated with an increased level of Apelin, which translates into better response to treatment.

Considering the effects of Apelin gene polymorphism (additive, dominant, and recessive genotypes) on the response rate of patients suffering from high blood pressure to antihypertensive therapies, the researchers suggest evaluating this association in future studies. More comprehensive studies with larger sample sizes that contain other high blood pressure groups such as malignant hypertension are suggested. Furthermore, considering the effect of Apelin level in more optimal control of blood pressure and improving the response to antihypertensive treatments, clinical trial studies are highly recommended.

### Acknowledgments

This study is a part of a thesis proposal for a medical degree. The authors thank all the patients and laboratory personnel.

### Footnotes

**Authors' Contribution:** MH, FE, and TK make substantial contributions to conception and design, and acquisition of data, and analysis and interpretation of data. FA and MH participate in drafting the article or revising it critically for important intellectual content. All authors give final approval of the version to be submitted and any revised version.

**Conflict of Interests:** The authors declare no conflict of interest.

**Ethical Approval:** The study protocol has been designed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the Birjand University of Medical Sciences. In addition, the study is approved by the Ethical Committee of Birjand University of Medical Sciences (code: Ir.bums.1394.287). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

**Funding/Support:** This study was supported by the Birjand University of Medical Sciences.

**Informed Consent:** Informed consent was obtained from all patients.

### References

- Oh GC, Cho HJ. Blood pressure and heart failure. *Clin Hypertens.* 2020;**26**:1. doi: [10.1186/s40885-019-0132-x](https://doi.org/10.1186/s40885-019-0132-x). [PubMed: [31908841](https://pubmed.ncbi.nlm.nih.gov/31908841/)]. [PubMed Central: [PMC6939331](https://pubmed.ncbi.nlm.nih.gov/PMC6939331/)].
- Oliveira IM, Duarte YAO, Zanetta DMT. Prevalence of Systemic Arterial Hypertension Diagnosed, Undiagnosed, and Uncontrolled in Elderly Population: SABE Study. *J Aging Res.* 2019;**2019**:3671869. doi: [10.1155/2019/3671869](https://doi.org/10.1155/2019/3671869). [PubMed: [31565434](https://pubmed.ncbi.nlm.nih.gov/31565434/)]. [PubMed Central: [PMC6745120](https://pubmed.ncbi.nlm.nih.gov/PMC6745120/)].
- Kleinz MJ, Davenport AP. Emerging roles of apelin in biology and medicine. *Pharmacol Ther.* 2005;**107**(2):198-211. doi: [10.1016/j.pharmthera.2005.04.001](https://doi.org/10.1016/j.pharmthera.2005.04.001). [PubMed: [15907343](https://pubmed.ncbi.nlm.nih.gov/15907343/)].
- Tatemoto K, Takayama K, Zou MX, Kumaki I, Zhang W, Kumano K, et al. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul Pept.* 2001;**99**(2-3):87-92. doi: [10.1016/s0167-0115\(01\)00236-1](https://doi.org/10.1016/s0167-0115(01)00236-1). [PubMed: [11384769](https://pubmed.ncbi.nlm.nih.gov/11384769/)].
- Cox CM, D'Agostino SL, Miller MK, Heimark RL, Krieg PA. Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. *Dev Biol.* 2006;**296**(1):177-89. doi: [10.1016/j.ydbio.2006.04.452](https://doi.org/10.1016/j.ydbio.2006.04.452). [PubMed: [16750822](https://pubmed.ncbi.nlm.nih.gov/16750822/)].
- Goidescu CM, Vida-Simiti LA. The Apelin-APJ System in the Evolution of Heart Failure. *Clujul Med.* 2015;**88**(1):3-8. doi: [10.15386/cjmed-380](https://doi.org/10.15386/cjmed-380). [PubMed: [26528040](https://pubmed.ncbi.nlm.nih.gov/26528040/)]. [PubMed Central: [PMC4508609](https://pubmed.ncbi.nlm.nih.gov/PMC4508609/)].
- Yu XH, Tang ZB, Liu LJ, Qian H, Tang SL, Zhang DW, et al. Apelin and its receptor APJ in cardiovascular diseases. *Clin Chim Acta.* 2014;**428**:1-8. doi: [10.1016/j.cca.2013.09.001](https://doi.org/10.1016/j.cca.2013.09.001). [PubMed: [24055369](https://pubmed.ncbi.nlm.nih.gov/24055369/)].
- Szokodi I, Tavi P, Foldes G, Voutilainen-Myllyla S, Ilves M, Tokola H, et al. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circ Res.* 2002;**91**(5):434-40. doi: [10.1161/01.res.0000033522.37861.69](https://doi.org/10.1161/01.res.0000033522.37861.69). [PubMed: [12215493](https://pubmed.ncbi.nlm.nih.gov/12215493/)].
- Wysocka MB, Pietraszek-Gremplewicz K, Nowak D. The Role of Apelin in Cardiovascular Diseases, Obesity and Cancer. *Front Physiol.* 2018;**9**:557. doi: [10.3389/fphys.2018.00557](https://doi.org/10.3389/fphys.2018.00557). [PubMed: [29875677](https://pubmed.ncbi.nlm.nih.gov/29875677/)]. [PubMed Central: [PMC5974534](https://pubmed.ncbi.nlm.nih.gov/PMC5974534/)].
- Katugampola SD, Maguire JJ, Matthewson SR, Davenport AP. [(125)I]-Pyr(1)Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. *Br J Pharmacol.* 2001;**132**(6):1255-60. doi: [10.1038/sj.bjp.0703939](https://doi.org/10.1038/sj.bjp.0703939). [PubMed: [11250876](https://pubmed.ncbi.nlm.nih.gov/11250876/)]. [PubMed Central: [PMC1572672](https://pubmed.ncbi.nlm.nih.gov/PMC1572672/)].
- Wu D, He L, Chen L. Apelin/APJ system: a promising therapy target for hypertension. *Mol Biol Rep.* 2014;**41**(10):6691-703. doi: [10.1007/s11033-014-3552-4](https://doi.org/10.1007/s11033-014-3552-4). [PubMed: [24990699](https://pubmed.ncbi.nlm.nih.gov/24990699/)].
- Kim DY, Cheon HG. Losartan induces browning via induction of apelin-APJ pathway. *Japan-Korea Joint Session of the 92nd Annual Meeting of the Japanese Pharmacological Society.* 2019. p. JK9-9.
- Fares H, DiNicolantonio JJ, O'Keefe JH, Lavie CJ. Amlodipine in hypertension: a first-line agent with efficacy for improving blood pressure and patient outcomes. *Open Heart.* 2016;**3**(2). e000473. doi: [10.1136/openhrt-2016-000473](https://doi.org/10.1136/openhrt-2016-000473). [PubMed: [27752334](https://pubmed.ncbi.nlm.nih.gov/27752334/)]. [PubMed Central: [PMC5051471](https://pubmed.ncbi.nlm.nih.gov/PMC5051471/)].
- Hung WW, Hsieh TJ, Lin T, Chou PC, Hsiao PJ, Lin KD, et al. Blockade of the renin-angiotensin system ameliorates apelin production in 3T3-L1 adipocytes. *Cardiovasc Drugs Ther.* 2011;**25**(1):3-12. doi: [10.1007/s10557-010-6274-4](https://doi.org/10.1007/s10557-010-6274-4). [PubMed: [21161354](https://pubmed.ncbi.nlm.nih.gov/21161354/)].
- Siddiquee K, Hampton J, Khan S, Zadory D, Gleaves L, Vaughan DE, et al. Apelin protects against angiotensin II-induced cardiovascular fibrosis and decreases plasminogen activator inhibitor type-1 production. *J Hypertens.* 2011;**29**(4):724-31. doi: [10.1097/HJH.0b013e32834347de](https://doi.org/10.1097/HJH.0b013e32834347de). [PubMed: [21358420](https://pubmed.ncbi.nlm.nih.gov/21358420/)]. [PubMed Central: [PMC3982221](https://pubmed.ncbi.nlm.nih.gov/PMC3982221/)].
- Akcilar R, Turgut S, Caner V, Akcilar A, Ayada C, Elmas L, et al. Apelin effects on blood pressure and RAS in DOCA-salt-induced hypertensive rats. *Clin Exp Hypertens.* 2013;**35**(7):550-7. doi: [10.3109/10641963.2013.764889](https://doi.org/10.3109/10641963.2013.764889). [PubMed: [23387534](https://pubmed.ncbi.nlm.nih.gov/23387534/)].

17. Wannamethee SG, Lowe GD, Rumley A, Cherry L, Whincup PH, Sattar N. Adipokines and risk of type 2 diabetes in older men. *Diabetes Care*. 2007;**30**(5):1200-5. doi: [10.2337/dc06-2416](https://doi.org/10.2337/dc06-2416). [PubMed: [17322479](https://pubmed.ncbi.nlm.nih.gov/17322479/)].
18. Azizi Y, Faghihi M, Imani A, Roghani M, Nazari A. Post-infarct treatment with [Pyr1]-apelin-13 reduces myocardial damage through reduction of oxidative injury and nitric oxide enhancement in the rat model of myocardial infarction. *Peptides*. 2013;**46**:76-82. doi: [10.1016/j.peptides.2013.05.006](https://doi.org/10.1016/j.peptides.2013.05.006). [PubMed: [23727032](https://pubmed.ncbi.nlm.nih.gov/23727032/)].
19. Jia J, Men C, Tang KT, Zhan YY. Apelin polymorphism predicts blood pressure response to losartan in older Chinese women with essential hypertension. *Genet Mol Res*. 2015;**14**(2):6561-8. doi: [10.4238/2015.June.12.10](https://doi.org/10.4238/2015.June.12.10). [PubMed: [26125862](https://pubmed.ncbi.nlm.nih.gov/26125862/)].
20. Przewlocka-Kosmala M, Kotwica T, Mysiak A, Kosmala W. Reduced circulating apelin in essential hypertension and its association with cardiac dysfunction. *J Hypertens*. 2011;**29**(5):971-9. doi: [10.1097/HJH.0b013e328344da76](https://doi.org/10.1097/HJH.0b013e328344da76). [PubMed: [21346619](https://pubmed.ncbi.nlm.nih.gov/21346619/)].
21. Andersen CU, Hilberg O, Mellekjaer S, Nielsen-Kudsk JE, Simonsen U. Apelin and pulmonary hypertension. *Pulm Circ*. 2011;**1**(3):334-46. doi: [10.4103/2045-8932.87299](https://doi.org/10.4103/2045-8932.87299). [PubMed: [22140623](https://pubmed.ncbi.nlm.nih.gov/22140623/)]. [PubMed Central: [PMC3224425](https://pubmed.ncbi.nlm.nih.gov/PMC3224425/)].
22. Schinzari F, Veneziani A, Mores N, Barini A, Di Daniele N, Cardillo C, et al. Beneficial Effects of Apelin on Vascular Function in Patients With Central Obesity. *Hypertension*. 2017;**69**(5):942-9. doi: [10.1161/HYPERTENSIONAHA.116.08916](https://doi.org/10.1161/HYPERTENSIONAHA.116.08916). [PubMed: [28289180](https://pubmed.ncbi.nlm.nih.gov/28289180/)].
23. Xie H, Luo G, Zheng Y, Hu D, Peng F, Xie L. Lowered circulating apelin is significantly associated with an increased risk for hypertension: A meta-analysis. *Clin Exp Hypertens*. 2017;**39**(5):435-40. doi: [10.1080/10641963.2016.1267199](https://doi.org/10.1080/10641963.2016.1267199). [PubMed: [28534648](https://pubmed.ncbi.nlm.nih.gov/28534648/)].
24. Zhong JC, Zhang ZZ, Wang W, McKinnie SMK, Vederas JC, Oudit GY. Targeting the apelin pathway as a novel therapeutic approach for cardiovascular diseases. *Biochim Biophys Acta Mol Basis Dis*. 2017;**1863**(8):1942-50. doi: [10.1016/j.bbadis.2016.11.007](https://doi.org/10.1016/j.bbadis.2016.11.007). [PubMed: [27825851](https://pubmed.ncbi.nlm.nih.gov/27825851/)].
25. Fan XF, Wang Q, Mao SZ, Hu LG, Hong L, Tian LX, et al. [Protective and therapeutic effect of apelin on chronic hypoxic pulmonary hypertension in rats]. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2010;**26**(1):9-12. Chinese. [PubMed: [20476553](https://pubmed.ncbi.nlm.nih.gov/20476553/)].