

# Investigation of the relationship between serum adropin levels, oxidative stress biomarkers, and blood pressure in DOCA-salt hypertensive rats

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## Ethics Committee Approval

Ethics Committee approval was taken from the  
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Committee of Animal Care and Usage, Kütahya,  
Turkey (Protocol no: 2019.01.09).

All procedures in this study involving human  
participants were performed in accordance with  
the 1964 Helsinki Declaration and its later  
amendments.

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## Conflict of Interest

No conflict of interest was declared by the  
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## Abstract

**Background/Aim:** Adropin is involved in the pathophysiology and development of cardiovascular diseases, such as hypertension. The aim of this study was to investigate the effects of adropin in serum, potential use as a biochemical biomarker of oxidative stress, and effects on blood pressure in deoxycorticosterone acetate (DOCA) salt hypertensive rats.

**Methods:** Eighteen male Sprague-Dawley rats were divided into two groups: (1) Control (C) and (2) Hypertensive (H). Systolic and diastolic blood pressures (SBP and DBP, respectively), and mean blood pressure (MBP) were measured using the tail-cuff method. At the end of the study, serum endothelin-1 (ET-1), adropin, nitric oxide (NO), total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were also analyzed.

**Results:** Significant increases in SBP, DBP, MBP, cardiac hypertrophy index (CHI), and left ventricular hypertrophy index (LVCI) in the H group compared with the C group were found. Serum levels of ET-1, TOS, and OSI were significantly higher in the H group and serum levels of NO, adropin, and TAS were lower than in the C group. A negative correlation between serum adropin levels and the variables SBP, DBP, MBP, TOS, OSI, CHI, and LVHI was found. Adropin levels were positively correlated positively with serum NO levels in both groups.

**Conclusion:** Serum adropin levels decreased in hypertensive DOCA-salt rats. Lower serum adropin levels were found to be significantly associated with hypertension and may play a role in this disease. However, further comprehensive and diverse studies are needed.

**Keywords:** Adropin, Oxidative stress, DOCA-salt hypertension, Blood pressure

## Introduction

Adropin has been detected in a variety of tissues and organs, including the pancreas, liver, brain, kidneys, endocardium, myocardium, and epicardium [1]. Adropin is capable of controlling lipid metabolism, reducing insulin resistance, and improving vascular endothelial cell function, and has preventive effects on the pathogenesis and progression of cardiovascular disease. It also has anti-inflammatory effects [2]. In the study by Aydın et al., it was shown that the level of adropin is a prognostic marker rather than a treatment [3].

Hypertension is one of the most common progressive diseases that has significant public health implications. It is an independent and important risk factor for several cardiovascular diseases characterized by elevated blood pressure, such as arterial aneurysms, strokes, heart failure, and atherosclerosis [4]. Deoxycorticosterone acetate (DOCA), an inactive acetate, is experimentally used as a mineralocorticoid to produce hypertension and cardiovascular pathology [5]. The DOCA-salt-induced hypertensive rat is a popular experimental model for use in studying antihypertensive effects. DOCA in combination with saline treatment leads to an increase in inflammation and overactive sympathetic tone [6].

Therefore, I aimed to investigate whether serum adropin levels are effective in the development of DOCA-salt-induced hypertension. I examined the relationship between hemodynamic, and hypertrophic parameters, serum adropin levels, and markers of oxidative stress.

## Materials and methods

### Animals and experimental protocol

Adult male Sprague-Dawley rats weighing 250–350 g were fed standard rat chow and water ad libitum and maintained under well-controlled conditions, namely, a temperature of 22 ± 2 °C, humidity of 55–65% and a 12-hour light/dark cycle. The rats were randomly divided into two groups of nine rats each: (1) C-control and (2) H-hypertensive). The protocol was approved by the Ethics Committee of Kutahya Health Sciences University of Animal Care and Usage, Kütahya, Turkey (Protocol numbered: 2019.01.09).

DOCA (25 mg/kg subcutaneously, twice weekly, dissolved in 0.4 ml of dimethylformamide) and salt solution were administered to rats for four weeks to induce hypertension [7]. DOCA-treated animals received 1% NaCl solutions instead of drinking water, while the control group received normal drinking water.

### Blood pressure measurements and hemodynamic parameters

On the day before DOCA treatment and on the 28<sup>th</sup> day of DOCA treatment, SBP, DBP, and MBP levels were measured between 9 am and 12 pm. An indirect-tail-cuff method, monitored with a Biopac Student Lab PRO 3.7 software (Model No. MP36, AD Instruments Co.) and a pneumatic pulse generator (MAY NIBP200-A, Ankara, Turkey), was used. The rat was fixed in a plastic holder (Kursunluoglu Metal Co., Denizli, Turkey), and a tail cuff was attached to the rat's tail to obtain blood pressure measurements. Each measurement was taken three times in a quiet room at room temperature without

anesthesia, and the average was taken as the value at each time point.

### Blood sample collection

All animals were anesthetized with Ketamine/Xylazine HCl (10–90 mg/kg intraperitoneally) at the end of the study. Serum was collected by centrifuging blood samples from the aorta for 10 min at 4,000rpm. This serum was then stored at –20 °C until further biochemical examination.

### Hypertrophic parameters

Hearts were removed, washed with saline, and weighed. The left ventricles, including the interventricular septum, were divided and the weight determined. The cardiac hypertrophy index (CHI) was calculated as the ratio of heart weight (HW, mg) to body weight (BW, g), and the ratio of the left ventricular weight (LVW, mg) to BW (g) represented the left ventricular hypertrophy index (LVHI).

### Biochemical parameters

Serum concentrations of endothelin 1(ET-1), nitric oxide (NO), and adropin were determined using rat enzyme-linked immunosorbent assay (ELISA) kits (Cusabio, China). Serum concentrations of total antioxidant status and total oxidant statuses (TAS and TOS, respectively) which are oxidative stress parameters, were measured using a commercial Rel Assay kit (Mega Tip, Gaziantep, Turkey) according to the manufacturer's protocol [8, 9]. Changes in absorbance of serum samples were determined using an enzyme microplate reader (Thermo Multiscan GO, 1510, Thermo Fisher Scientific Inc., Finland). The oxidative status index (OSI) in arbitrary units was calculated as shown: OSI (arbitrary unit) = ([TOS, mmol/L]/ [TAS, mmol Trolox equivalent/L])/100).

### Statistical analysis

All values were expressed as mean (standard error of the mean (SEM)) Statistical analysis was performed using the Mann–Whitney U test and the SPSS program 16.0 (SPSS Inc., Chicago, USA). The Spearman test was used for correlation analysis. A significance level of  $P \leq 0.05$  was considered statistically significant.

## Results

Before DOCA administration, there was no statistically significant difference in SBP, DBP, or MBP of the rats in any of the groups studied. After 4 weeks, the H group had significantly higher SBP, DBP, and MBP than the C group due to DOCA-salt treatment (Table 1).

Table 1: Hemodynamic parameters in the control and hypertension groups

	Before DOCA treatment			After DOCA treatment		
	Control (n=7)	Hypertension (n=7)	P-value	Control (n=7)	Hypertension (n=7)	P-value
SBP	121.3 (2.06)	121.7 (1.08)	0.730	122.0 (1.23)	167.4 (0.80)	<0.001
DBP	79.1 (1.87)	81.8 (0.79)	0.161	80.9 (1.49)	95.9 (0.64)	<0.001
MBP	101.6 (1.76)	101.1 (1.17)	0.931	102.1 (0.99)	122.9 (1.18)	<0.001

Continuous data are expressed as the mean (SEM), categorical variables are expressed as a percentage, and values of  $P \leq 0.05$  are considered statistically significant. SBP: systolic blood pressure, DBP: diastolic blood pressure, and MBP: mean blood pressure.

Serum ET-1 levels were significantly higher in the H group than in the C group ( $P = 0.014$ ), but serum adropin and NO levels were significantly lower ( $P = 0.003$  and  $P < 0.001$ , respectively) as shown in Table 2. The serum TAS level of the H group was statistically lower than that of the C group ( $P = 0.04$ ). The serum levels of TOS and OSI in the H group were statistically higher than those of the C group ( $P = 0.001$  and  $P <$

0.001, respectively). In addition, after DOCA injection, the CHI and LVHI values of the H group increased more than those of the C group ( $P < 0.001$ ) as shown in Table 2.

Table 3 shows a negative correlation between serum adropin levels and SBP ( $r = -0.77$ ;  $P < 0.001$ ), DBP ( $r = -0.88$ ;  $P < 0.001$ ), MKB ( $r = -0.82$ ;  $P < 0.001$ ), CHI ( $r = -0.86$ ;  $P < 0.001$ ), LVHI ( $r = -0.77$ ;  $P < 0.001$ ), ET-1 ( $r = -0.48$ ;  $P = 0.04$ ), TOS ( $r = -0.54$ ;  $P = 0.01$ ), and OSI ( $r = -0.68$ ;  $P = 0.002$ ). In addition, a positive correlation between serum levels of adropin and NO in all groups was found ( $r = 0.65$ ;  $P = 0.003$ ).

Table 2: Biochemical and hypertrophic parameters in the control and hypertension groups

Variable	Control	Hypertension	P-value
ET-1	3.40 (1.00)	13.3 (2.99)	0.014
NO	2.67 (0.74)	0.88 (0.18)	0.003
Adropin	40.0 (0.99)	24.9 (1.15)	<0.001
TAS	0.63 (0.12)	0.33 (0.08)	0.040
TOS	13.8 (0.83)	25.0 (3.49)	0.001
OSI	2.84 (0.52)	11.0 (2.05)	<0.001
CHI	2.27 (0.24)	3.26 (0.14)	<0.001
LVHI	1.02 (0.13)	1.90 (0.08)	<0.001

Continuous data are expressed as the mean (SEM), and values of  $P \leq 0.05$  are considered statistically significant. ET-1: Endothelin-1, NO: Nitric oxide, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, CHI: Cardiac hypertrophy index (HW/BW: heart weight/body weight), LVHI: Left ventricular hypertrophic index (LVW/BW: left ventricular weight/body weight).

Table 3: Spearman correlation between adropin and other clinical characteristics

	All groups (n = 18)		Control (n = 9)		Hypertension (n = 9)	
	r	P-value	r	P-value	r	P-value
ET-1	-0.48	0.040	0.09	0.803	0.16	0.668
NO	0.65	0.003	-0.06	0.871	0.88	0.452
TAS	0.40	0.090	-0.08	0.830	-0.13	0.732
TOS	-0.54	0.010	0.07	0.847	0.43	0.244
OSI	-0.68	0.002	-0.07	0.847	0.11	0.765
SKB	-0.77	<0.001	-0.12	0.744	-0.05	0.881
DKB	-0.88	<0.001	-0.87	0.002	-0.22	0.569
MKB	-0.82	<0.001	-0.39	0.288	-0.15	0.695
CHI	-0.86	<0.001	-0.11	0.762	-0.76	0.010
LVHI	-0.77	<0.001	-0.04	0.915	-0.26	0.488

$P \leq 0.05$  was considered to be statistically significant. ET-1: Endothelin-1, NO: Nitric oxide, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MBP: Mean blood pressure, CHI: Cardiac hypertrophy index (HW/BW: heart weight/body weight), LVHI: Left ventricular hypertrophic index (LVW/BW: Left ventricular weight/body weight).

The serum levels of adropin and DBP in the C group presented a significant negative correlation ( $r = -0.87$ ;  $P = 0.002$ ). In the H group, a negative correlation between CHI and serum adropin levels ( $r = -0.76$ ;  $P = 0.01$ ) was found. In the H group, no correlation between serum adropin and blood pressure variables was noted (Table 3).

## Discussion

Arterial blood pressure has been shown to increase dramatically when rats are injected subcutaneously with DOCA and NaCl is administered in their drinking water [10]. In this study, SBP, DBP, MBP, and serum level ET-1 increased in hypertensive DOCA-salt rats when compared with controls as shown in our previous studies [11, 12]. These findings may serve as precursors for the development of hypertension in rats. In this study, a decrease in serum NO levels was observed in hypertensive DOCA-salt rats when compared with the controls. One reason for the decrease in serum levels NO could be the higher levels of ET-1 in H group. Schiffrin et al. [13] demonstrated that renin secretion from the kidney decreases when ET-1 levels increase. The DOCA-salt is a model for hypertension with low renin levels [14]. Cheng et al. [15] reported that production of NO causes a reduction in hypertension by activating the angiotensin II type 1/phosphoinositide 3 kinase/protein kinase B/endothelial nitric oxide synthase (AT1/PI3K/Akt/eNOS) pathway after renin administration. Thus, another reason for the decrease in serum

NO levels could be the decrease in renin in the H group. In my previous study, I showed that serum renin levels were low in rats with DOCA-salt hypertension [11].

Sato et al. [16] showed that activation of eNOS via the PI3K/Akt pathway was impaired in DOCA-salt hypertensive rats. Lovren et al. [17] observed that adropin promotes eNOS expression via upstream activation of vascular endothelial growth factor receptor 2 (VEGFR2), which in turn activates the PI3K/Akt and ERK1/2 pathways. The reduced NO production in parallel with eNOS formation may be due to the lower adropin levels in DOCA-salt hypertensive rats. In this study, it was not possible to investigate the activation of eNOS. Allcock et al. [18] showed that the production of NO by the kidney was increased in DOCA-salt hypertensive rats. Han et al. [19] observed that plasma levels of NO were not altered by DOCA-salt hypertension. The discrepancies between these studies could be due to the temporal and spatial specificity of eNOS expressions and other upstream signaling pathways.

In this study, serum concentrations of adropin were found to decrease in hypertensive DOCA-salt rats. Topuz et al. [2] discovered low adropin concentrations in patients with endothelial dysfunction. Gulen et al. [20] demonstrated that adropin levels were significantly lower in hypertensives when compared with the normotensive group. Bolayir et al. [21] discovered that patients with non-dipping hypertension had lower serum adropin levels than hypertensives and normotensives, indicating that SBP at night was strongly negatively related to adropin. Gu et al. [22] showed that lower plasma adropin levels were related to higher blood pressure in hypertension, and ET-1 and adropin were negatively correlated. In another study, it was shown that lower adropin concentration was measured in obese children, and no correlation was found between adropin concentration and hypertension was found [23]. In this study, no correlation between serum adropin level, ET-1 level, and SBP in the H group was found. Lower serum adropin levels are associated with higher blood pressure and may not be protective against endothelial damage in DOCA-salt hypertensive rats.

It has been shown that CHI and LVHI are higher in spontaneously hypertensive rats [24]. In this study, treatment with DOCA caused a significant increase in CHI and LVHI in the H group when compared with the C group. In the H group a negative correlation between serum adropin levels and CHI was detected. No study in the literature comparing serum adropin and CHI with the results of this study has been published.

In this study, TAS decreased in the H group when compared with the C group, whereas TOS and OSI increased significantly. These findings were consistent with those from previous studies [25, 26]. Accordingly, the decrease in serum TAS and increase in TOS and OSI could lead to an increase in blood pressure in hypertensive rats. Moreover, the serum levels of TAS negatively correlated with the levels of OSI, and no significant correlations between blood pressure, adropin, TAS, and TOS in the H group were found. No study in the literature comparing adropin, TAS, and TOS in hypertension that can be compared with the findings of this study is available.

## Conclusion

Although results are contradictory, studies on the association between adropin and hypertension have been published. This study is the first one in the literature to examine the levels of serum adropin in DOCA-salt hypertensive rats. As a result, serum adropin levels are reduced in DOCA-salt hypertensive rats. Lower adropin may directly affect eNOS, cause a reduction in NO production, and be associated with hypertension. These data suggest that adropin is an endogenous hypertensive factor that plays a role in hypertension onset and development.

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