



ISSN 0975-413X  
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(4):32-49  
(<http://derpharmachemica.com/archive.html>)

## Interaction between Renin Angiotensin System and Apelin/APJ System in Hypertensive Rats

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### ABSTRACT

White adipose tissue (WAT) is now recognized as the largest endocrine organ of the body. WAT secretes a number of bioactive peptides and proteins, collectively termed "adipokines". Adipokines have different biological effects, including blood pressure control. Dysregulated production and release of specific adipokines, namely renin angiotensin system (RAS) peptides & apelin, from WAT in the setting of obesity may contribute to hypertension. Based on previous concepts, the present study aimed to clarify role of RAS and Apelin/APJ system in obesity-induced hypertension through modulation of Angiotensin type 1 receptor (AT1R), Angiotensin 1-7/Mas receptor and Apelin/APJ receptor expression and to elucidate the possible interaction between the two systems. 48 rats were used in this study divided into 6 groups, group 1 (control group) given standard rat chow, group 2 (control + captopril group): given standard rat chow + captopril 40mg/kg, group 3 (Control + L-NAME group): given standard rat chow + L-NAME 20mg/kg, group 4 (control hypertensive group): fed high fat high sucrose diet (HF-HS) for 10 weeks, Group 5: (hypertensive + captopril group): Fed HF-HS diet & received captopril (40mg/kg) for 10 weeks, Group 6: (hypertensive + L-NAME group): Fed HF-HS diet & received L-NAME (20mg/kg) for 10 weeks. At the end of experiment, body weight, fat weight and systolic blood pressure were measured. Visceral adipose tissue (epididymal fat) was collected for gene expression of AT1R, Ang1-7 R and apelin/APJ. Results of this study demonstrated activation of the adipose (RAS) and Apelin/APJ system in rats with diet-induced hypertension, consistent with a strong link between visceral obesity and hypertension. Obesity induced hypertension was associated with marked upregulation in AT1R, apelin/APJ R expression and down regulation of Ang-(1-7)/Mas R in adipose tissue. Captopril treatment showed significant decrease in body weight, fat weight and systolic blood pressure associated with significant increase in adipose tissue gene expression of Apelin/APJ receptor and Ang 1-7/Mas receptor. Whereas L-NAME significantly increased blood pressure and decreased body weight, fat weight with significant increased AT1R expression. Obesity induced hypertension was associated with increased AT1R & apelin/APJ receptor expression and decreased Ang-(1-7) R expression which provides evidence that apelin/APJ upregulation by HFHS diet could not antagonize hypertensive effect of AngII/AT1R. This may be explained by apelin resistance or high expression of Ang II. ACE inhibition decreased blood pressure significantly in obese hypertensive rats through increased Ang-(1-7) R & apelin/APJ receptor expression. L-NAME raised blood pressure significantly in obese rats through increased AT1R expression and possible inhibition of nitric oxide (NO)- mediated vasodilator action of Ang-(1-7) & apelin. These results suggest an interaction between Apelin and RAS peptides to regulate ABP in obese subjects.

**Key words:** RAS, Apelin, Obesity induced hypertension.

## INTRODUCTION

Hypertension is a major risk factor of high prevalence worldwide for cardiovascular diseases (CVD) [1]. Clinical and experimental studies have confirmed a strong association between obesity and hypertension. However, the exact mechanism whereby obesity causes hypertension remains unknown [2].

Currently, one of the main mechanisms that could explain the development of high arterial pressure in obesity includes activation of the renin-angiotensin system (RAS). Adipose tissue possesses a local RAS, with more significant local paracrine as well as systemic effects than the subcutaneous fat; this contributes to the cardiometabolic derangements including sodium retention and volume expansion, progressive renal disease and elevated blood pressure [3].

Angiotensin II (Ang II), the physiologically-active product of the RAS, has recently been found to be one of the adipokines secreted by adipocytes. Ang II is known to exert its effects via Ang II receptor type 1 (AT1R) & Ang II receptor type 2 (AT2R). AT1Rs are more abundantly expressed than AT2R, and most of the functions of RAS in adipose tissue appear to be mediated through activation of AT1R [4].

The renin-angiotensin system (RAS) plays a key role in blood pressure regulation. The effects are not only achieved through the vasoconstrictor Ang II via AT1R, but also through its metabolite angiotensin-(1-7). Ang-(1-7) exerts actions opposite to those of Ang II, which are mediated by its receptor (Mas R) [5].

Apelin is an adipokine isolated as a selective endogenous ligand of orphan receptor, APJ, genetically identified to have closest identity to the AT1R. In spite of the high homology between APJ receptor and AT1R, the Apelin/APJ system has been found to exert opposing actions to (Ang II)-AT1R in regulation of cardiovascular function.

Moreover, apelin peptides have been shown to be specific substrates of Angiotensin converting enzyme ACE2, a carboxypeptidase enzyme cleaving the C-terminal phenylalanine residue of either Ang-I or Ang-II to form Ang-(1-7), a functional antagonist of Ang -II that acts predominantly as a vasodilator [6].

Initial experiments in animal models indicate that the apelin-APJ pathway has opposing actions to the RAS pathway in a number of physiologic and pathophysiologic settings. While Ang II increases vascular tone and raises blood pressure, apelin is a vasodilator and lowers blood pressure [7].

The apparent involvement of apelin peptides in the enzymatic cascades of the RAS suggests the possible interaction between ACE/Ang II/AT1R, ACE2/Ang-(1-7)/Mas R and the apelin/APJ receptor which makes it an interesting area for research into the mechanisms of obesity associated hypertension as well as opening up new targets for treatment.

### **The aim of this study is to investigate:**

1. The role of renin-angiotensin system in obesity-induced hypertension through modulation of Angiotensin 1-7 receptors (Mas receptors) and AT1 receptors expression in adipose tissue.
2. The role of Apelin/APJ system in obesity-induced hypertension through modulation of Apelin APJ receptor expression.
3. The possible interaction between (RAS) system and apelin/APJ system.
4. The role of NO as a possible mechanism of action for both Apelin and Ang 1-7.

## MATERIALS AND METHODS

### **Experimental Animals:**

A total of 48 male albino rats (150-180 gm, 10-12 weeks old) were used in this study. The experimental protocol and procedures were approved by the animal house of Research Institute of Ophthalmology, Ministry of health, Cairo, Egypt according to guidelines for the care and use of laboratory animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Animals were housed in wire mesh cages at room temperature with normal light-dark cycle and had free access to water and their respective diets for the whole experimental period (10 weeks).

Rats were randomly divided into the following groups (each containing 8 rats):

**Group 1: (control group):** Fed the commercial rat chow diet (12% calories as fat) and received daily vehicle of 1 ml distilled water by oral gavage for the whole time of the experiment (10 weeks).

**Group 2 :( control+ captopril group):** Fed the commercial rat chow diet received captopril (40mg/kg) given daily by oral gavage for 10 weeks.

**Group 3 :( control+ L-NAME group):** Fed the commercial rat chow & received L-NAME (20mg/kg) given daily by oral gavage for 10 weeks.

**Group4: (control hypertensive group):** Fed high fat-high sucrose (HF-HS) diet & received daily vehicle of 1ml distilled water by oral gavage for 10 weeks.

**Group5: (hypertensive + captopril group):** Fed HF-HS diet & received captopril (40mg/kg) given daily by oral gavage for 10 weeks.

**Group6: (hypertensive + L-NAME group):** Fed HF-HS diet & received L-NAME (20mg/kg) given daily by oral gavage for 10 weeks.

#### **Experimental protocol:**

##### **Induction of hypertension:**

To induce a rat model of hypertension in our study, rats were fed high fat high sucrose (HF-HS) diet for 10 weeks according to Dobrian *et al.* and Angela *et al.* [8 - 9].

##### **Composition of HF-HS diet:**

Its composition was 31.2% fat, 49.1% carbohydrate (34.1% sucrose plus 15% corn starch), 17.3% protein, 5.0% fiber, 3.5% minerals, 0.4% CaCO<sub>3</sub>, 1% vitamin mix, 0.004% antioxidants, and 0.2% cholesterol. Fats provided 42% of the calories, and the diet yielded (450 kcal per 100 g) [10 - 11].

##### **Measurement of systolic blood pressure**

Systolic blood pressure (SBP) was assessed using Power Lab Data Acquisition and Analysis Systems: noninvasive blood pressure monitor (ML 125 NIBP, AD Instruments, Australia). SBP was measured from the tail of conscious rats by the tail-cuff technique for which all animals were pre-trained until blood pressure was steadily recorded with minimal stress and restraint [12]. In the tail-cuff technique, animals were warmed for 30 min at 28°C in a thermostatically controlled heating cabinet (UgoBasille, Italy) for better detection of tail artery pulse; the tail was passed through a miniaturized cuff and a tail-cuff sensor that was connected to an amplifier. The amplified pulse was recorded during automatic inflation and deflation of the cuff. Systolic BP (cuff deflation pressure) was defined as the point at which the cuff pressure corresponds to the restoration of the first caudal artery pulse. The average of at least three measurements was taken at each occasion. Criteria for inclusion of measurements from individual mice were 5 out of 10 successful measurements with a standard deviation <50. [13]

##### **At the end of experiment:**

\* Body weight was measured.

\* Rats were sacrificed under anesthesia by i.p. injection of sodium thiopental (10 mg/100 g body weight), epididymal & perirenal fat (visceral fat) was removed, weighed, and stored at -70 °C in lysis buffer (composed of 0.2 N Sodium hydroxide (NaOH) 1% (wt/vol) sodium dodecyl sulfate) until gene expression by polymerase chain reaction (PCR) was done for assessment of:

1-Angiotensin 1-7 receptors (Mas receptors)

2-Angiotensin II type 1 receptor (AT1 R)

3-Apelin APJ receptors

**Semi-Quantitation of *Ang1-7receptor (Mas receptor), AT1R and Apelin/APJ receptor* gene expression by reverse transcriptase polymerase chain reaction (RT-PCR) (Pfaffl *et al.*, 2001).**

##### **Extraction of RNA from the adipose tissue:**

Total RNA was extracted from adipose tissue using SV Total RNA Isolation system (*Promega, Madison, WI, USA*).

**Homogenization of adipose tissue:**

1. About 30mg of adipose tissues were homogenized in 175  $\mu$ l previous mentioned lyses buffer for 10 min and then tissue centrifuged for 20 min at 15,000 rpm.
2. 350  $\mu$ l of SV RNA Dilution Buffer was added to 175  $\mu$ l of tissue homogenate; it was mixed by inverting the tube 3-4 times. The mixture was placed in a water bath at 70°C for 3 minutes.
3. The mixture was centrifuged at 12,000-14,000 rpm for 10 minutes at 20-25°C.

**RNA purification:**

1. The cleared lysate solution was transferred to a fresh microcentrifuge tube by pipetting.
2. 200 $\mu$ l of 95% ethanol were added to the cleared lysate, and were mixed by pipetting 3-4 times. This mixture was transferred to the Spin Column Assembly and was centrifuged at 12,000-14,000 rpm for one minute.
3. The Spin Basket was taken from the Spin Column Assembly, and the liquid was discarded in the collection tube. The spin basket was put back into the collection tube. 600  $\mu$ l of SV RNA wash solution were added to the spin column assembly. Centrifugation at 12,000-14,000 rpm for one minute was done.
4. The DNase incubation mix was prepared by combining 40 $\mu$ l yellow core buffer, 5  $\mu$ l 0,09M MnCl<sub>2</sub> and 5  $\mu$ l of DNase I enzyme per sample in a sterile tube.
5. 50  $\mu$ l of the freshly prepared DNase incubation mix were applied directly to the membrane inside the spin basket.
6. The mixture was incubated for 15 minutes at 20-25°C then 200 $\mu$ l of DNase stop solution were added to the spin basket, and were centrifuged at 12,000-14,000 rpm for one minute.
7. 600  $\mu$ l of SV RNA wash solution with ethanol added (100ml of 95% ethanol to a bottle containing 58, 8ml concentrated SV RNA wash solution) were added then were centrifuged at 12,000-14,000 rpm for one minute.
8. The collection tube was emptied and 250  $\mu$ l of SV RNA wash solution with ethanol were added and centrifuged at 14000 rpm for two minutes.
9. The spin basket was transferred from the collection tube to the elution tube, and 100  $\mu$ l of nuclease-free water were added to the membrane.
10. Centrifugation at 12,000-14,000 rpm for one minute was done. The spin basket was discarded and the elution tube containing the purified RNA was stored at -70°C.

**Determination of RNA yield and quality:**

The yield of total RNA obtained was determined spectrophotometrically at 260 nm.

**Reverse transcription into cDNA:**

The extracted RNA was reverse transcribed into cDNA using RT-PCR kit. (*Stratagene, USA*).

**Procedure:**

Three  $\mu$ l of random primers were added to the 10  $\mu$ l of RNA which was denatured for 5 minutes at 65°C in the thermal cycler.

- a) The RNA primer mixture was cooled to 4°C.
- b) The cDNA master mix was prepared according to the kit as follows and was added (for each sample):

Component	Volume
First strand buffer	5 $\mu$ l
10 mM dNTPs	2 $\mu$ l
RNase inhibitor (40 U/ $\mu$ l)	1 $\mu$ l
MMLV-RT enzyme (50 U/ $\mu$ l)	1 $\mu$ l
DEPC-treated water	10 $\mu$ l

- c) Total volume of the master mix was 19  $\mu$ l for each sample. This was added to the 13  $\mu$ l RNA-primer mixture resulting in 32  $\mu$ l of cDNA.
- d) The last mixture was incubated in the programmed thermal cycler one hour at 37°C followed by inactivation of enzymes at 95°C for 10 minutes, and finally cooled at 4°C. Then RNA was changed into cDNA.

**QPCR (quantitative real time PCR):**

1. The gene-specific forward and reverse primer pair was normalized. Each primer (forward and reverse) concentration in the mixture was 5 pmol/ $\mu$ l.

	Sequence of the primers used for real-time PC
ANG 1-7receptor	5'-GGAACAGGACGGAGGTTACA-3' (sense) 5'-AGTCAGGAGGTGGAGAGCAA-3' (anti-sense)
Apelin receptor	APJ Sens: GCCCTTGCTTTCTGAAAATCA, Reverse: GGACAGTTAAAGGATGTGCATAGGA
AT1 receptor	5'-caagtcgactcaagcctgtc-3' 5'-tcactccacctcagaacaagacg-3'

2. The experiment and the following PCR program was set up:
  - 50°C 2 min., 1 cycle.
  - 95°C 10 min., 1 cycle.
  - 95°C 15 sec. → 60°C 30 sec. → 72°C 30 sec., 40 cycles.
  - 72°C 10 min., 1 cycle.
3. A real time- PCR reaction mixture was 50µl.

The following mixture was prepared in each optical tube:

- 25 µl SYBR Green Mix (2x).
  - 0.5 µl kidney cDNA.
  - 2 µl primer pair mix (5 pmol/ µl each primer).
  - 22.5 µl H<sub>2</sub>O.
4. After PCR is finished, the tubes from the machine were removed.
  5. The real time- PCR result was analyzed with the step one applied biosystem software.

#### Statistical analysis of data:

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 16 (SPSS Inc, USA). For quantitative data, the mean and standard deviation were calculated. ANOVA (Analysis of variance) was used to test the difference about mean values of measured parameters among groups, multiple comparison between pairs of groups were performed using LSD. To determine the relation between variables, Pearson Correlation was done.

For interpretation of results of tests of significance; significance was adopted at  $P < 0.05$ .

## RESULTS

Effect of induction of hypertension by HF-HS diet for 10 weeks on the studied parameters:

**Table (1): Effect of HF-HS diet for 10 weeks (control hypertensive group 4) on body weight (BW), fat weight and systolic blood pressure (BP) compared to control group (group 1)**

Parameters	group 1	group 4
Body weight (gm)	232.71±15.98	385.43± 29.46*
Fat weight (gm)	1.57± .28	4.00± .13*
BP (mmHg)	93.14± 3.67	135.43± 4.54*

Values are presented as mean±SD \*: statistically significant compared to corresponding value in group 1 ( $P < 0.05$ )

Table (1) shows a significant increase in fat weight (gm), body weight (gm) and systolic blood pressure (mmHg) in hypertensive group 4 compared to control group 1.

**Table (2): Effect of HF-HS diet for 10 weeks on adipose tissue gene expression of Apelin APJ receptor, AT1R and Ang-(1-7) R compared to control group (group 1).**

Parameters	group 1	group 4
Apelin R	.12±.03	1.81±.59*
AT1 R	1.40±.35	12.14±1.29*
Ang-(1-7) R	10.80±1.35	1.82±.61*

Values are presented as mean±SD \*: statistically significant compared to corresponding value in group 1 ( $P < 0.05$ )

Table (2) shows a significant increase in adipose tissue gene expression of Apelin receptor and AT1R. However, there is significant decrease in adipose tissue gene expression of Ang 1-7 receptor in hypertensive group 4 compared to control group 1.

**Effect of ACE inhibition by Captopril treatment on different parameters among studied groups:**

**Table (3): Effect of captopril (40mg/kg) on body weight, fat weight and arterial blood pressure in control group 1 and captopril treated control group 2**

Parameters	group 1	group 2
Body weight (gm)	232.71±15.98	229.29±15.92
Fat weight (gm)	1.57±.28	1.46±.26
BP(mmHg)	93.14±3.67	93.71±4.82

*Values are presented as mean±SD*

*\*: statistically significant compared to corresponding value in group 1 (P<0.05)*

As shown in table (3), no significant change appeared in body weight, fat weight (gm) or systolic blood pressure between control group 1 and captopril treated control group 2.

**Table (4): Effect of Captopril (40mg/kg) on adipose tissue gene expression of Apelin receptor, AT1R, and Ang 1-7 receptor in control group 1 and captopril treated control group 2**

Parameters	group 1	group 2
Apelin R	.12±.03	.23±.09
AT1 R	1.40±.35	2.70±.55
Ang-(1-7) R	10.80±1.35	8.76±1.49

*Values are presented as mean±SD*

*\*: statistically significant compared to corresponding value in group 1 (P<0.05)*

As shown in table (4), there was no significant difference in adipose tissue gene expression of Apelin receptor, AT1R or Ang 1-7 receptor between captopril treated control group 2 compared to control group 1.

**Table (5): Effect of captopril (40mg/kg) on body weight, body fat weight and arterial blood pressure in captopril treated hypertensive group 5 compared to hypertensive control group 4.**

Parameters	group 4	group 5
Body weight (gm)	385.43± 29.46	320.29± 13.70*
Fat weight (gm)	4.00± .13	2.84± .15*
BP(mmHg)	135.43± 4.54	116.43± 9.78*

*Values are presented as mean±SD*

*\*: statistically significant compared to corresponding value in group 4 (P<0.05)*

As shown in Table (5), captopril treated hypertensive rats (group 5) showed a significant decrease in body weight (gm), fat weight (gm) and systolic blood pressure (mmHg) when compared to hypertensive control rats (group 4).

**Table (6): Effect of captopril (40mg/kg) on adipose tissue gene expression of Apelin receptor, AT1R and Ang-(1-7) R in captopril treated hypertensive group 5 compared to hypertensive control group 4.**

Parameters	group 4	group 5
Apelin R	1.81± .59	3.97± .92*
AT1 R	12.14± 1.29	13.79± 1.24*
Ang-(1-7) R	1.82± .61	3.93± .84*

*Values are presented as mean±SD*

*\*: statistically significant compared to corresponding value in group 4 (P<0.05)*

Table (6) shows a significant increase in adipose tissue gene expression of Apelin receptor  $3.97 \pm 0.92$ , AT1R  $13.79 \pm 1.24$  and Ang 1-7 receptor  $3.93 \pm 0.84$  in captopril treated hypertensive group 5 compared to control hypertensive group 4.

**Effect of NO blockade by L-NAME on different parameters among studied groups:**

**Table (7): Effect of L-NAME (20mg/kg) on body weight, fat weight and arterial blood pressure in control group 1 and L-NAME treated control group 3**

Parameters	Group 1	Group 3
Bodyweight (gm)	232.7±15.9	267.8±6.3*
Fat weight (gm)	1.5±.27	1.4±.28
BP(mmHg)	92.2±2.2	125.4±7.9*

Values are presented as mean±SD  
\*: Significant difference from group1 (P<0.05)

**Table (8): Effect of L-NAME (20mg/kg) on adipose tissue gene expression of Apelin receptor, AT1R and Ang-(1-7) R in control group 1 and L-NAME treated control group 3**

Parameter	Group 1	Group 3
Apelin R	0.116±.026	0.360±.16*
AT1R	1.401±.34	4.27±1.65*
Ang-(1-7) R	10.80±1.34	14.4±3.03*

Values are presented as mean±SD  
\*: Significant difference from group1 (P<0.05)

As observed in table (7), L-NAME given to control group significantly(p<0.05) increased body weight, fat weight & blood pressure in group(3) with mean values 267.8±6.3, 1.47±.28 & 125.4±7.9 respectively compared to group(1), this was associated with significant increased AT1receptor expression in adipose tissue in group(3) as observed in table (8).

**Table (9): Effect of L-NAME (20mg/kg) on body weight, fat weight and arterial blood pressure in L-NAME treated hypertensive group 6 compared to hypertensive control group 4**

Parameters	Group 4	Group 6
Body weight (gm)	385.4±29.4	346.71±13.3*
Fat weight (gm)	4.00±.13	3.84±.09
BP(mmHg)	135.4±4.5	144.29±9.2*

Values are presented as mean ±SD  
\* Significant difference from group4 (P<0.05)

**Table (10): Effect of L-NAME (20mg/kg) on adipose tissue gene expression of Apelin R, AT1R and Ang-(1-7) R in L-NAME treated hypertensive group 6 compared to hypertensive control group 4**

Parameter	Group 4	Group 6
Apelin R	1.8±.58	2.02±.34
AT1R	12.1±1.29	15.44±.26*
Ang-(1-7) R	1.8±.61	2.28±.15

Values are presented as mean ±SD  
\* Significant difference from group4 (P<0.05)

As observed in table (9), L-NAME given to hypertensive group for 10 weeks significantly (p<0.05) increased blood pressure & decreased body weight, fat weight, in group (6) with mean values 144.29±9.2, 346.71±13.3& 3.84±.09 respectively compared to group (4), this was associated with significant increased AT1R expression in adipose tissue in group (6) compared to group (4) as observed in table (10).

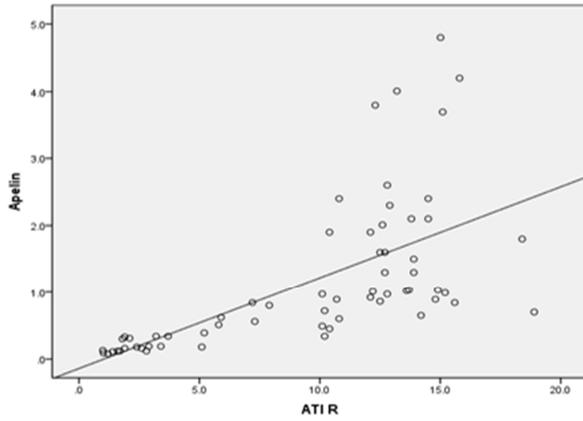


Fig [1]:significant positive correlation between apelin R & AT1R

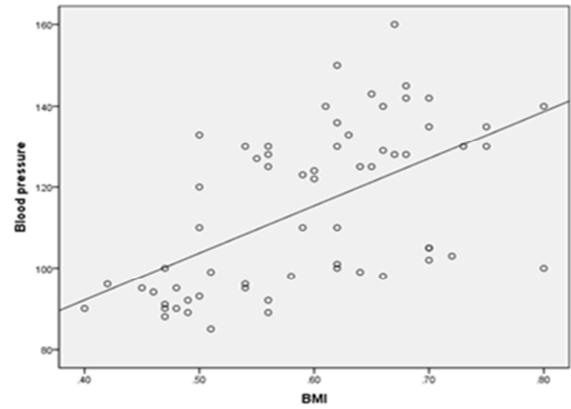


Fig [2]: Significant positive correlation between body weight& blood pressure

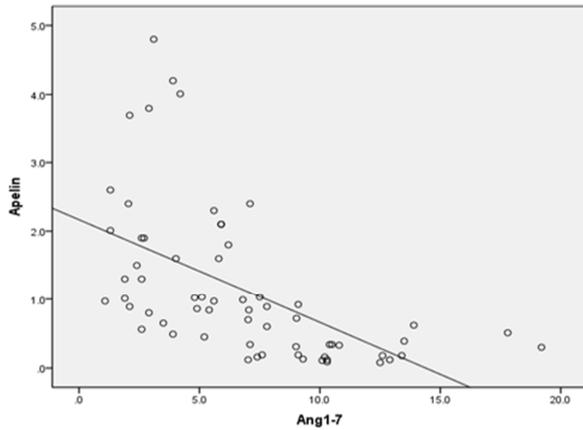


Fig [3]:significant negative correlation between apelin R & Ang-(1-7) R

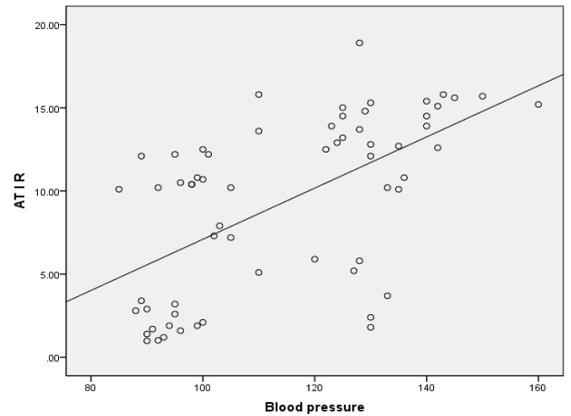


Fig [4]: significant positive correlation between AT1R& blood pressure

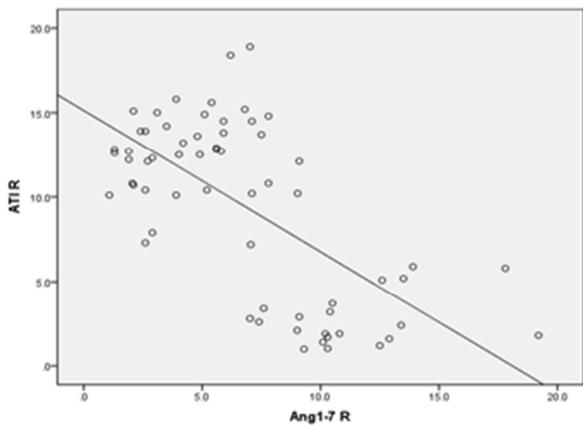


Fig [5]:significant negative correlation between AT1 R&Ang-(1-7) R

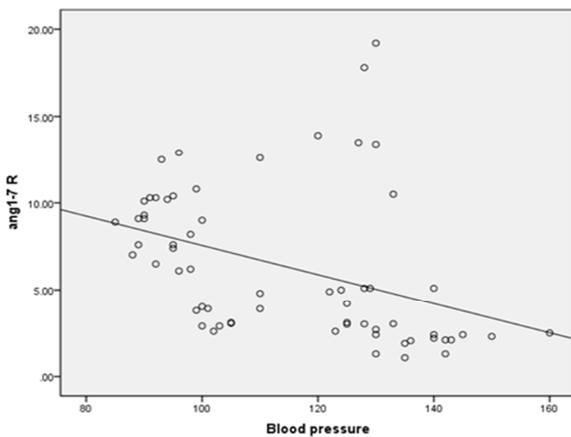
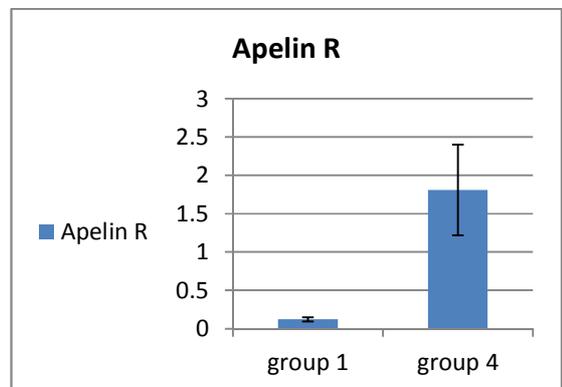
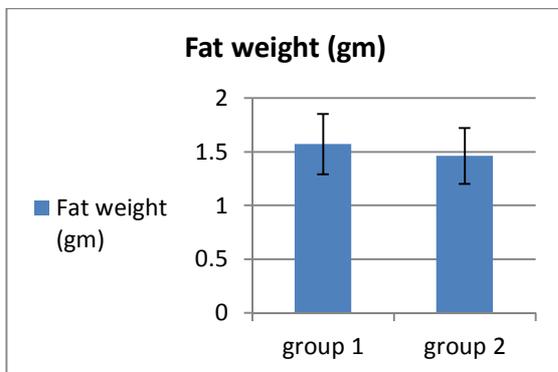
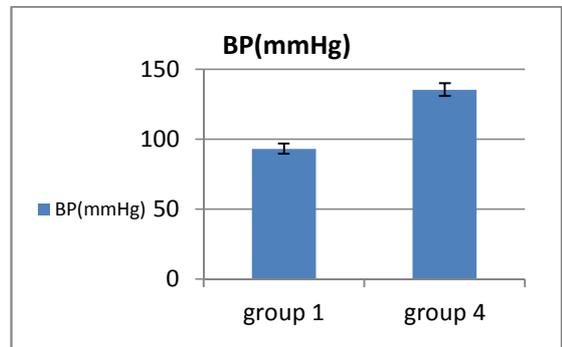
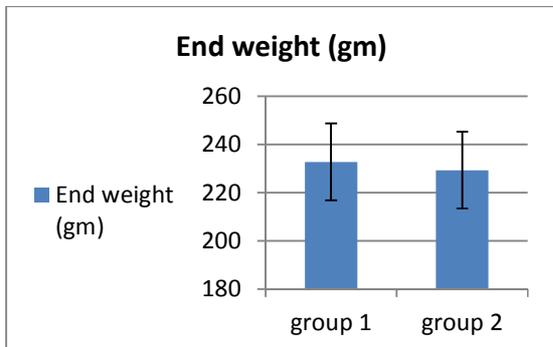
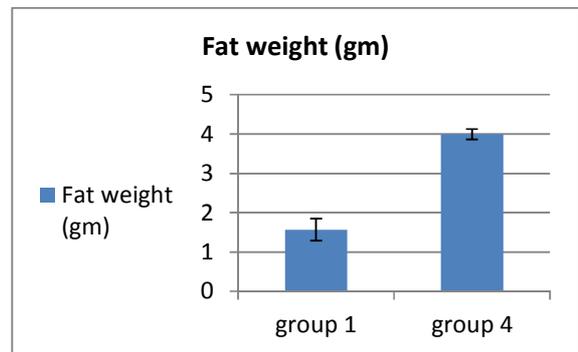
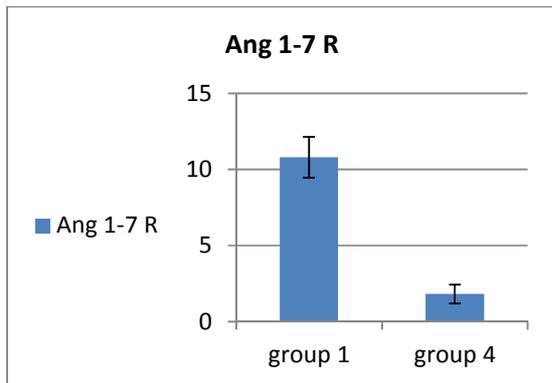
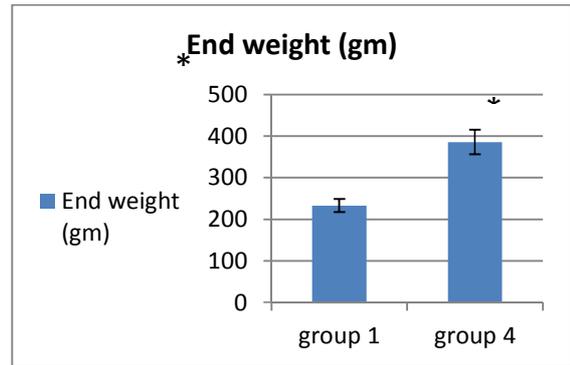
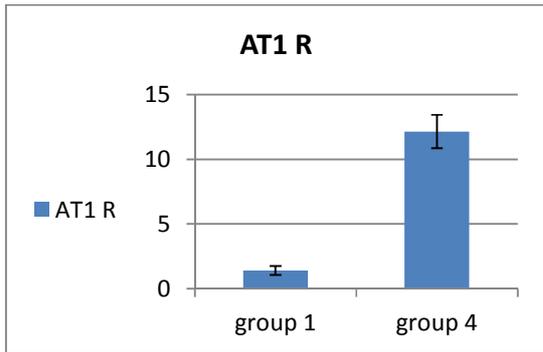
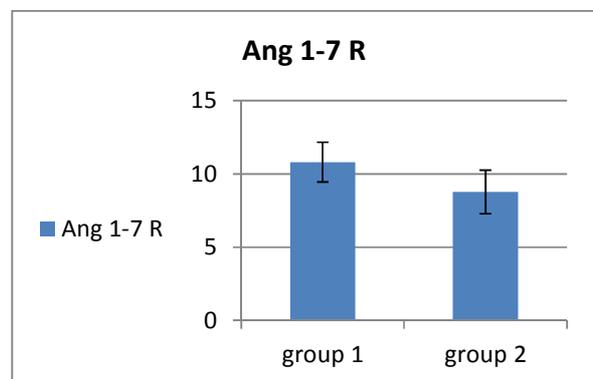
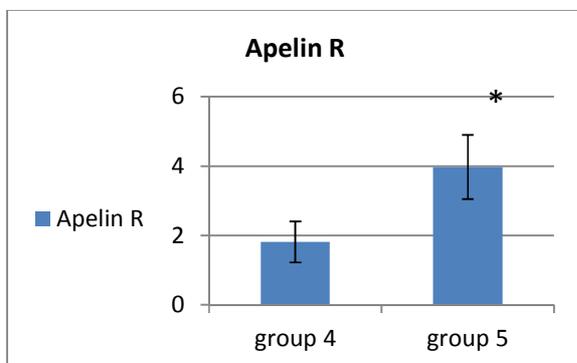
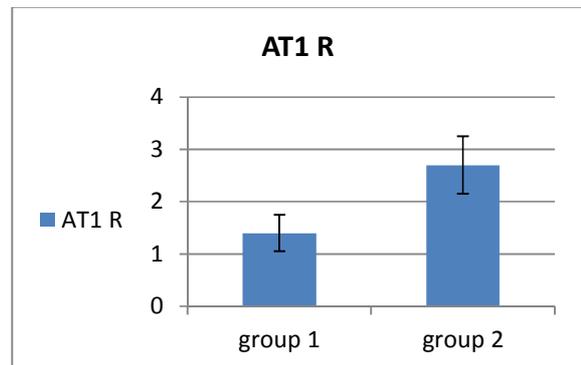
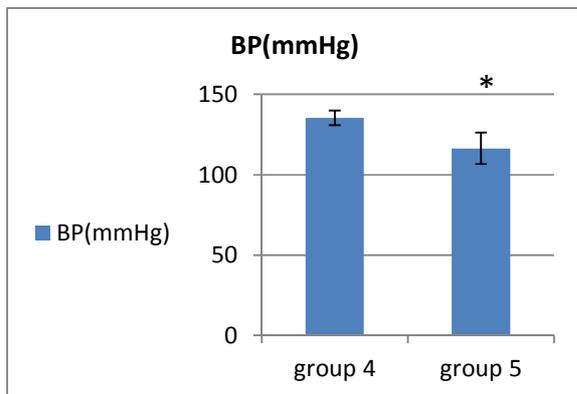
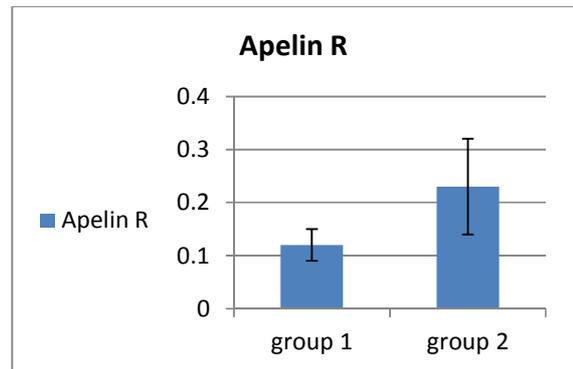
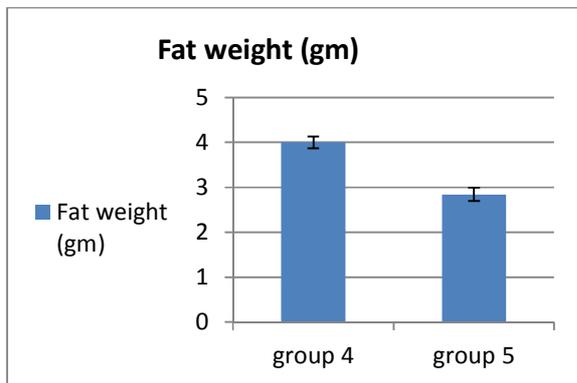
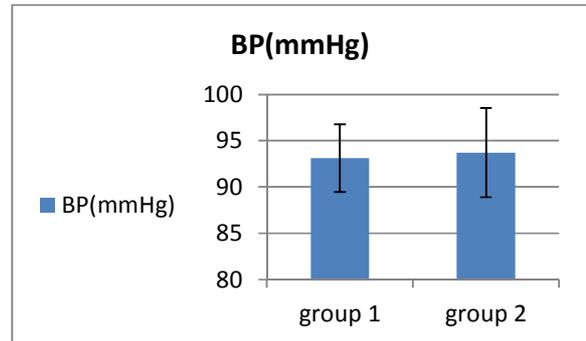
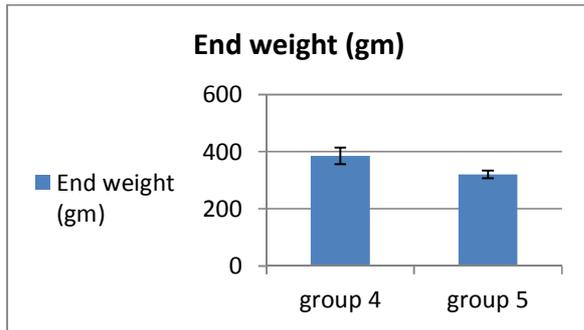
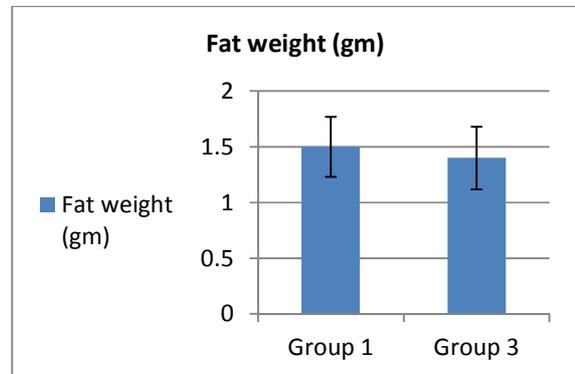
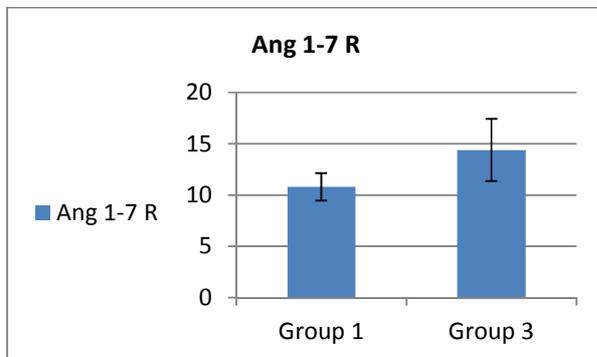
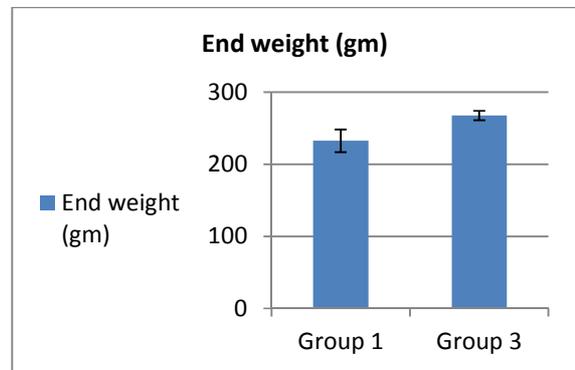
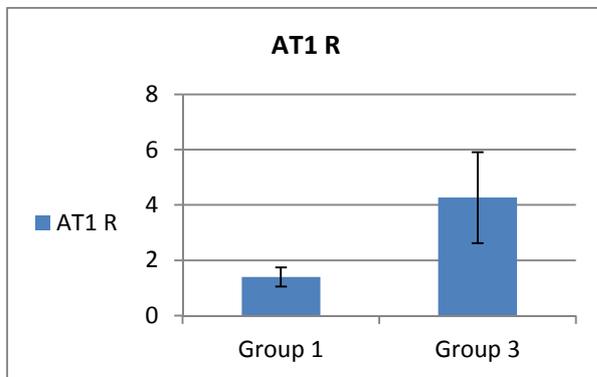
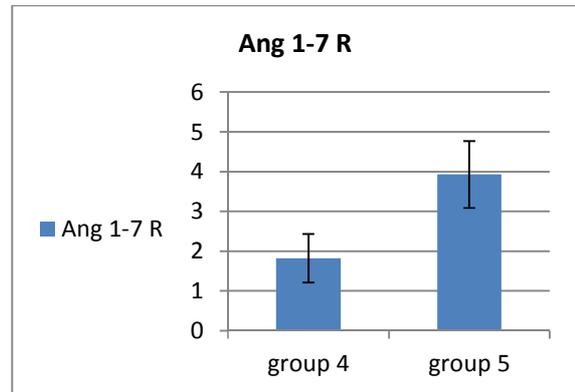
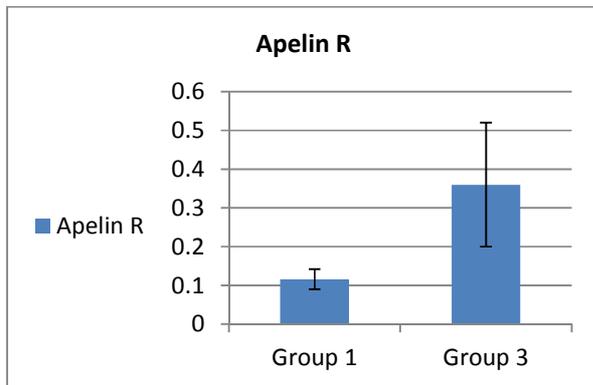
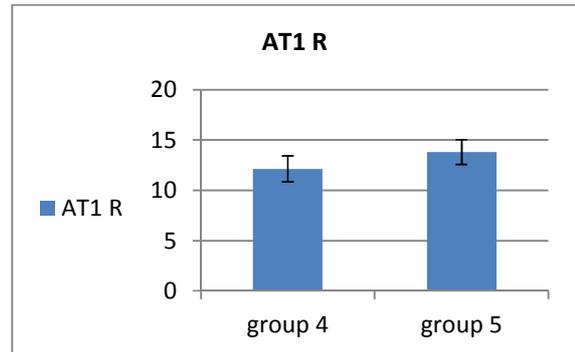
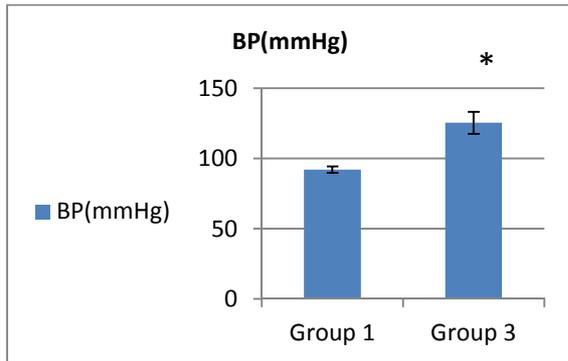
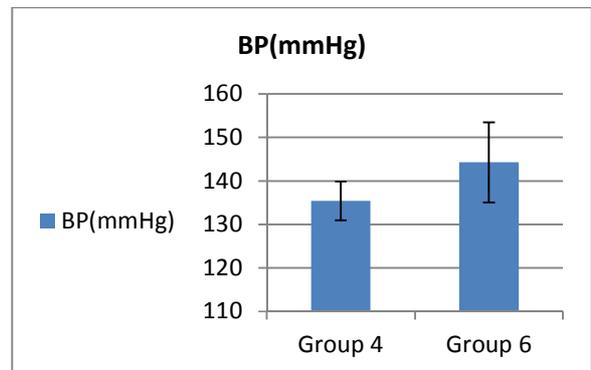
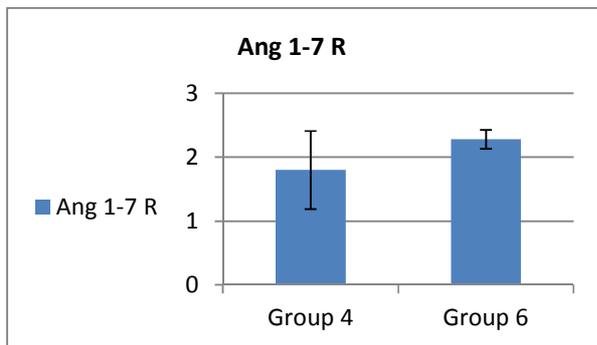
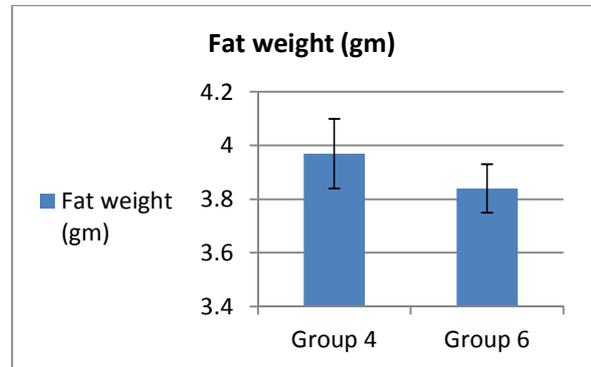
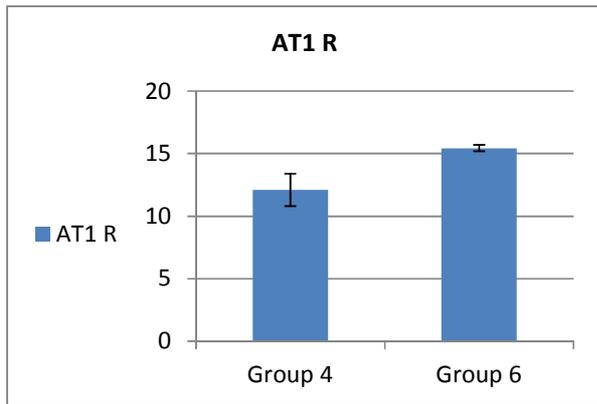
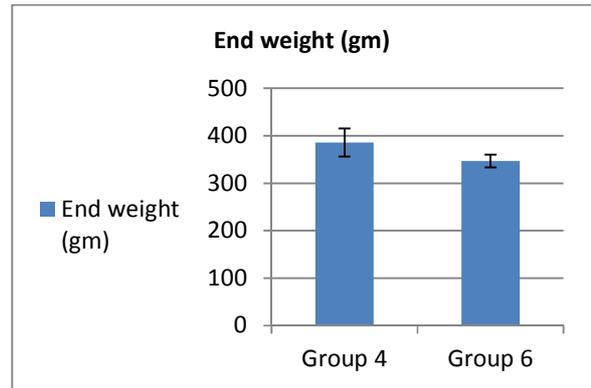
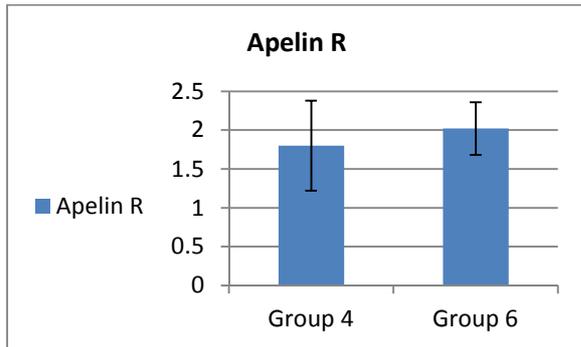


Fig [6]: significant negative correlation between Ang-(1-7) R & blood pressure









**DISCUSSION**

Visceral adipose tissue hypertrophy is a stimulus for increased production of adipocytokines that are thought to mediate and/or control obesity-induced hypertension including components of renin angiotensin system (RAS) and Apelin/APJ system [14].

One of the key emerging features of the apelin/APJ system is its interaction with the respective receptors of RAS which may have implications for understanding the pathophysiology of several major cardiovascular diseases including hypertension.

The present work was designed to clarify the possible interaction between RAS and Apelin/APJ system in the development of obesity associated hypertension, and whether this interaction may be the answer to this question:

**Why does hypertension develop in obese individuals?**

To accomplish this, the present work attempted to study gene expression of AT1R, Ang-(1-7) Mas receptors and Apelin/APJ receptors in visceral adipose tissue of obese hypertensive rats.

The diet-induced obesity model used in the current study closely mimics the neurohumoral and hemodynamic changes observed in obese humans. The diet chosen was a HF-HS diet composed of 31.2% fat, 49.1% carbohydrate and 17.3% protein, for 10 weeks to induce hypertension [11, 15]

As expected, after 10 weeks of HF-HS consumption, our results showed a significant rise in body weight, visceral fat weight and systolic arterial blood pressure compared to control group which confirms development of obesity induced hypertension. Moreover, a positive correlation was observed between body weight, visceral fat weight & systolic blood pressure, which further confirm the link between obesity & hypertension.

The above results are in agreement with who found that, feeding animals a HF-HS diet for 7 weeks resulted in a significantly increased body weight, which was accompanied by heavier adipose tissues in all anatomical locations analyzed. Moreover, [16] that, rat models of diet-induced obesity (32% kcal as fat) develops hypertension, having advantages over other similar animal models in their close similarity to human obesity hypertension, namely activation of RAS, dyslipidemia, increased oxidative stress, and associated vascular and renal pathology. Despite this well established association, mechanisms linking obesity to hypertension are not fully understood.

Constantly emerging experimental and clinical evidence continues to support a key role of the local adipose tissue RAS in the pathogenesis of hypertension. In the last decade our understanding of the convoluted RAS has expanded onto the existence of novel angiotensins which counteract the hypertensive, growth-promoting, and proliferative effects of Ang II/AT1R. Indeed, Ang-(1-7) and its forming enzyme ACE2, and receptor Mas have been a topic of interest in hypertension research.

Indeed, results of the present study demonstrated that ingestion of HF-HS for 10 weeks resulted in a significant increase in expression of AT1R, with significant decrease in expression of Ang1-7/Mas receptor in visceral fat compared to control group. In addition, our results demonstrated a strong positive correlation between AT1R expression & blood pressure and a strong negative correlation between expression of Ang1-7/Mas receptor & blood pressure. This supports evidence that the protective arm of RAS (ACE2/ANG1-7) is downregulated while the harmful arm of RAS (ACE1/ANGII) is upregulated in visceral adipose tissue of obese hypertensive rats.

In accordance with our results, [17] stated that with chronic high fat (HF) feeding for 4 months, body weight and adipose mass were markedly increased compared with low fat (LF) feeding. Moreover, they exhibited higher blood pressure compared to LF-fed controls, expression of angiotensinogen increased in both adipose tissue and liver, plasma concentrations of angiotensin peptides were markedly elevated, plasma concentrations of AngII, Ang IV, and Ang I were greater in HF compared with LF mice. Plasma ACE2 activity increased. Elevations in ACE2 mRNA expression did not result in an increase in enzymatic activity or protein abundance in adipose tissue from 4-mo HF-fed mice. These results demonstrate that adipocytes express ACE2 and that ACE2 is nutritionally regulated by HF feeding.

*Hung et al.* stated that, during adipogenesis, the receptor of AngII and AT1R are up-regulated acting as a dampening feedback. Local accumulation of AngII stimulates the expression of ACE2 and production of Ang-(1-7) by ACE2 mediated by Mas receptor.[18]

Experimental studies have described apelin as a new adipokine produced and secreted by human and mouse mature adipocytes and plays an important role in obesity-related metabolic and cardiovascular alterations. Apelin/APJ receptor shares a close identity to AT1R and named as APJ (putative receptor protein related to AT1), but does not bind AngII. Emerging data on a potential interaction between the two pathways suggest that ACE2, hydrolyzes apelin with similar potency to angiotensin II and, therefore, is responsible for the degradation of both peptides.

One of the most important issues in the present study was measuring Apelin/APJ receptor expression in visceral adipose tissue of obese hypertensive rats with a special focus on its crosstalk with Ang 1-7/Mas receptor signaling and AT1R expression.

Results of the current study showed that prolonged ingestion of HF-HS for 10 weeks, resulted in significant increase in expression of apelin/APJ receptor in visceral fat compared to control group and the positive correlation between apelin/APJ & both body weight and systolic blood pressure. These results suggest that apelin/APJ system has been implicated in obesity-related hypertension and propose the involvement of apelin in the pathophysiology of hypertension.

Intriguingly, findings in our research showed that Apelin/APJ expression positively correlated with AT1R and negatively correlated with Ang 1-7 receptor expression in obese hypertensive rats. Because the apelin/APJ system exerts potent vasodilation, it has been proposed that diet-induced obesity hypertension (OH) may be due to the impairment of systemic and adipose tissue apelin/APJ-mediated hypotensive action. [20]

Although apelin has been viewed as a beneficial vasodilator adipokine up-regulated in obesity, it remains to establish whether the increased levels of apelin observed in obesity are an attempt to overcome either insulin resistance or obesity-related cardiovascular diseases or maybe another metabolic defect such as apelin resistance. Moreover, upregulation of apelin/APJ receptor expression in visceral adipose tissue observed in our obese hypertensive rats may be a compensatory reaction to the vasoconstrictor effects of elevated AT1R expression.

In contrast to our results, apelin/APJ system showed completely different results in cases of essential hypertension which are not related to obesity. *Zhang et al.* reported that circulating apelin levels were decreased in patients with essential hypertension. Plasma apelin levels were negatively correlated with body weight and SBP, and positively correlated with the apelin mRNA levels of perirenal fat tissue.[20] Moreover, Lower levels of plasma apelin or levels of apelin/APJ mRNAs and protein have been observed in cardiovascular tissues in spontaneous hypertensive rats [21] and essential hypertensive patients [22] compared to normotensive controls.

*Wu and coworkers*, reported that that apelin/APJ system involves in decreasing the blood pressure and have a close relationship with hypertension, presumably, pathophysiology of hypertension as well. Such as, apelin/APJ system may be concerned in hyperfunction of the sympathetic nervous system, renin-angiotensin-aldosterone system, endothelial injury, excessive endothelin, sodium retention, vascular remodeling, insulin resistance elicit hypertension, as well as in hypertension-induced organ damaged. [23]

Also, they infer that apelin/APJ system would be a promising therapeutic target for hypertension and other cardiovascular disease in the future, this is based on the variation of apelin level in hypertension therapeutic process and the recent researches on APJ agonist and antagonist.

The presence of RAS in adipose tissue as previously documented suggests a paracrine and/or autocrine function. The intake of RAS inhibitors causes reduction in blood pressure and they are widely used as antihypertensive medication. Recently, AT1R antagonists and ACE inhibitors were reported to increase ACE2 thereby affecting Ang 1-7/ Mas receptors as well as Apelin/APJ receptors proposing them as new players of the RAS.

Therefore, the current research studied the effect of ACE inhibitor captopril (40mg/kg) on the different studied parameters and find that whereas captopril had no effect on any of the parameters in control group, it caused significant changes on all parameters of the hypertensive group which confirms activation of RAS in adipose tissue of obese hypertensive rats.

In addition to the significant decrease in body weight, fat weight and blood pressure, captopril also caused significant increase in Ang1-7/Mas receptor & apelin/APJ receptor gene expression in visceral fat in treated hypertensive rats (group 5) compared to their untreated controls (group 4).

The above mentioned results suggest that, the beneficial effects of captopril (decreasing systolic blood pressure, body weight and visceral fat weight) may be partly explained by up regulation of adipose tissue expression of Ang-(1-7)/Mas R to & apelin/APJ.

The previous results are in accordance with *Young-Bin et al.* who found that, treatment of rats with captopril for 4 weeks caused weight loss by 15% and a decreased epididymal fat mass. Plasma Ang-(1-7) level was increased by captopril treatment. In addition, the levels of Mas receptor expression and HSL (Hormone-sensitive lipase) were increased in adipocytes of captopril-treated rats by 380% and 260%, respectively. These effects were markedly

attenuated by infusion with Mas receptor antagonist. Similarly, the levels of Mas receptor expression and HSL were increased in adipocytes with single treatment of Ang-(1-7). Taken together, an inhibition of ACE with captopril may increase Ang-(1-7) level, resulting in body weight loss via Mas receptor expression and HSL.[24]

*Iwanaga et al.* demonstrated that, apelin/APJ pair is intrinsically related to the AngII/AT1R pair in regulating the pathophysiology of cardiovascular function. Knock-out mice for APJ receptor present increased vasopressive response to Ang II. However, they present normal blood pressure levels. In a major study involving HF rats (with reduced expression of apelin and APJ receptor), treatment with telmisartan – an antagonist of AT1R – made apelin/APJ levels reverse back to normal, thus suggesting that the efficacy of AT1 receptors inhibition in HF may also result from restoring normal values of apelin, a powerful positive inotropic endogenous agent. Therefore, direct regulation of the apelin/APJ pair by AngII/AT1R is left as a suggestion. [25]

*Jayasooriya et al.* found that mouse lacking the ACE gene presented increased energy expenditure with reduced fat mass associated with increased Ang-(1-7) plasma levels. It was demonstrated that mice with the deletion of the Ang-(1-7) Mas receptor presented increased body fat with metabolic disturbance. These observations suggest a vital role of the RAS in fat mass control. [26]

*Young-Bin et al.* also found that, Ang-(1-7) stimulates lipolysis and stimulatory effect of Ang-(1-7) on lipolysis was blocked by Mas receptor antagonist and Inhibitor for PI3K(phosphoinositol 3-kinase)/Akt or eNOS. Captopril intake or Ang-(1-7)-infusion for 4 weeks decreased body weight gain and fat mass with increased Mas receptor expression in adipocytes. The effect of captopril was attenuated by pretreatment with Mas receptor antagonist. These results suggest that Ang-(1-7) stimulates lipolysis via Mas receptor/ PI3K/eNOS signaling pathway thereby losing body weight in captopril-intake rats. However, Ang-(1-7)-induced lipolysis was not blocked by the Akt inhibitor. This observation indicates that Ang-(1-7) may regulate lipolysis via Akt-independent signaling pathway.[24]

*Kim et al.* found that Ang-(1-7) regulates lipolysis, treatment of primary adipocytes with Ang- (1-7) significantly increased lipolysis by 40%. It has been reported that  $\beta$ adrenergic agonist is a powerful ligand to activate lipolysis through a cAMP production in vitro and in vivo. The group also found that Ang-(1-7)-induced lipolysis was relatively weak as compared to isoproterenol-induced lipolysis, there was an additive effect of Ang-(1-7) with isoproterenol. Therefore, these results suggest a paracrine and/or autocrine function of Ang-(1-7) in adipose tissue. [27]

*Young-Bin et al. and Park et al.* found that increased circulating Ang-(1-7) stimulates adiponectin release from adipocytes followed by an increased fatty acid metabolism through AMPK activation. In addition, increased eNOS activation by Ang-(1-7) may cause HSL to facilitate lipolysis. However, there are several factors involving in captopril-induced weight loss, such as a reduced Ang II level and an increased Ang-(1-9). The reduction of Ang II may stimulate lipolysis and inhibit preadipocyte differentiation. The RAS including Mas receptor is present in adipose tissue. Mas receptor deficient mice are heavier in adipose tissue weight, whereas Ang-(1-7) transgenic rats are smaller in abdominal fat mass than in wild-type animals.[24,28]

Apelin/APJ and Ang (1-7)/Mas upregulation by captopril counteract the hypertensive & harmful effects of angiotensin II, which provide further evidence of cross interaction between RAS and Apelin/APJ system in obesity-associated hypertension and also suggest that this interaction might explain the strong association between obesity and hypertension.

An important finding in the current research also was the negative correlation between AT1R & Ang1-7/Mas receptor expression which confirms that, they run in opposite direction in obesity associated hypertension.

The previous results agreed with *Hongxian et al.* who stated that Perindopril (ACE inhibitor) increased apelin expression in adipose tissue. The expression of Apelin/APJ system appears to be suppressed by Ang II-AT1R system through AT1R in adipocytes, by interfering Akt phosphorylation.

*Chun et al.* demonstrated that ACEI, AT1R blocker, and AT2R blocker not only reduce the deleterious effects of Ang II on adipocytes but also increase the production of Apelin through which they achieve their protective effect. [29]

*Hung et al.* (stated that the mRNA expressions of angiotensinogen (AGT), renin, ACE1, and AT2R were up-regulated while AT1R mRNA was down-regulated during adipogenesis. Apelin expression increased during adipogenesis, and this increase was further augmented by blocking RAS using captopril, perindopril & losartan. RAS blockers also prevented excessive lipid accumulation and the generation of ROS (reactive oxygen species) in differentiating adipocytes. Their study suggests that RAS blockers achieve their beneficial effects by their enhancement of adipocyte secretion of apelin.[18].

*Hung et al.* reported that blockade of the RAS ameliorates apelin expression and secretion in 3T3-L1 adipocytes. Thus, through increased apelin production, RAS blockers could prevent excessive lipid accumulation and the generation of ROS in differentiating adipocytes. Inhibition of ROS production by apelin has also been shown in adipocytes.[19]

*Wu et al.* investigated the crosstalk between Ang II, AT1R signaling and apelin/APJ expression in Sprague-Dawley rats fed a high-fat. First, the levels of plasma apelin and apelin/APJ mRNAs of perirenal adipose tissue were reduced in obesity-related hypertensive rats fed a high-fat diet, and these changes were restored by AngII inhibition with ACE inhibitor treatment with perindopril. Second, long-term treatment with AngII down-regulates Apelin and APJ expressions through the inhibition of the p38/ERK signaling pathway in cultured 3T3-L1 cells, and this effect was also restored by AT1R antagonism with olmesartan.[23]

Accumulating evidence have proposed that both apelin and Ang-1-7 were shown to exert a potent vasodilator effect and lower blood pressure (BP) via a nitric oxide (NO)-dependent mechanism [30,31]

To assess the role of NO in obesity-induced hypertension, L-NAME was used in the present study in a low dose (20mg/kg) in an attempt to block apelin and Ang 1-7 action.

The effect of L-NAME on control group (group 3) showed significantly higher body weight & systolic blood pressure compared to group (1). L-NAME was also associated with significant increase in AT1R expression in group (3).

Concerning the effect of L-NAME on hypertensive groups, the results of current study demonstrated significantly higher blood pressure in hypertensive group (group 6) compared to their control group but with significant decrease in body weight and fat weight. This is associated with significant increase in expression of AT1R together with significant increase in the expression of Apelin and Ang 1-7 receptors compared to their control group (group 4).

The above results are in agreement with *Figuroa-Guillén et al.* who found that Ang II evoked blood pressure increase in rats in a dose-related manner. In L-NAME-treated rats a greater maximal effect was observed, indicating that L-NAME promotes Ang II hypersensitivity. In L-NAME-treated rats, Ang II response was blocked by losartan (1 and 3 mg/kg), a selective AT1 receptor antagonist, indicating that AT1R receptor influence L-NAME hypertensive mechanism. These results suggest that Ang II hypersensitivity in L-NAME-induced hypertension can be due to increased AT1R expression or sensitivity changes. [32]

*Khandaker et al.* found that apelin protects against cardiac fibrosis and vascular remodeling through the synergistic inhibition of Ang II signaling and increased production of NO by apelin. Mice receiving apelin plus L-NAME, or apelin plus Ang II plus L-NMAE exhibited significant cardiovascular fibrosis. [33]

*Tesanovic et al.* found that long-term Ang (1-7) treatment caused vasoprotection, via improvement in endothelial function, which appears to be mediated by the restoration of nitric oxide bioavailability and involve a complex interaction of both Mas and AT2 receptors. [34]

*Bogdan et al.* found that AP13 (apelin 13) does not modify by itself isolated rat portal vein tone, but decreased the Ang II-induced contractions mainly by an NO mediated mechanism, AP13 could increase the activity of both constitutive and inducible NOS on either endothelium-intact or endothelium-denuded rat portal vein rings. [35]

### CONCLUSION

Results of the current study demonstrated activation of the adipose tissue RAS and Apelin/APJ system in rats with diet-induced obesity and hypertension, consistent with a strong link between visceral obesity and hypertension.

Obesity induced hypertension (after 10 weeks of HF-HS) was associated with increased AT1R & apelin/APJ receptor expression and decreased Ang 1-7/Mas receptor expression which provides evidence that apelin (vasodilator adipokine) could not antagonize hypertensive effect of AngII/AT1R. This may be explained by apelin resistance or high expression of Ang II.

The current results also confirmed that, blockers of the RAS are high-efficacy drugs in the treatment of obesity-induced hypertension as they provide benefits against several aspects of the metabolic syndrome. Captopril (ACEi) prevented the development of obesity induced hypertension as it caused significant decrease in blood pressure probably through increased Ang 1-7/Mas R and Apelin/APJ receptor expression.

Finally, L-NAME even in small dose, aggravated obesity induced hypertension, through antagonizing apelin/APJ and Ang 1-7/Mas vasodilator action and increasing AT1R expression.

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