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Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension

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Running head: Angiotensin in Obesity-Induced Hypertension

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ABSTRACT

In obesity-related hypertension, activation of the renin-angiotensin system (RAS) has been reported despite marked fluid volume expansion. Adipose tissue expresses components of the RAS, and is markedly expanded in obesity. This study evaluated changes in components of the adipose and systemic RAS in diet-induced obese, hypertensive rats. The RAS was quantified in adipose tissue, and compared to primary sources for the circulating RAS. Male, Sprague Dawley rats were fed either a low fat (LF, 11% Kcal as fat) or moderately high fat (MHF, 32% kcal as fat) diet for 11 weeks. After 8 weeks, rats fed the MHF diet segregated into obesity-prone (OP) and obesity-resistant (OR) groups based on their body weight gain (Body weight; OR: 566 ± 10, OP: 702 ± 20 g; $P<0.05$). Mean arterial blood pressure (MAP) was increased in OP rats (LF: 97 ± 2, OR: 97 ± 2, OP: 105 ± 1 mmHg; $P<0.05$). Quantification of mRNA expression by real-time PCR demonstrated a selective increase (2-fold) in angiotensinogen gene expression in retroperitoneal adipose tissue from OP rats versus OR and LF. Similarly, plasma angiotensinogen concentration was increased in OP rats (LF: 390 ± 48, OR: 355 ± 24, OP: 530 ± 22 ng/ml; $P<0.05$). In contrast, other components of the RAS were not altered in OP rats. Marked increases in the plasma concentrations of angiotensin peptides were observed in OP rats (AngII; LF: 95 ± 31, OR: 59 ± 20, OP: 295 ± 118 pg/ml; $P<0.05$). These results demonstrate increased activity of the adipose and systemic RAS in obesity-related hypertension.

Keywords: retroperitoneal adipose, mean arterial pressure, angiotensin II

Introduction

The prevalence of obesity is markedly rising in Western societies (18), and is of considerable concern given the strong association between obesity and cardiovascular diseases such as hypertension (37). Retrospective analysis of results from the Framingham study revealed an attributable risk for hypertension as high as 30% in obese men and women (46). Experimental evidence demonstrates that excess weight gain increases blood pressure (23); conversely, loss of weight in hypertensive patients lowers blood pressure (36). Despite this clinically established association, mechanisms linking obesity to hypertension are not fully understood.

The renin-angiotensin system (RAS) is important in blood pressure control, and results from experimental animals and humans suggest activation of the RAS with obesity and hypertension (21). A variety of evidence supports the existence of tissue RAS, important in the local production of angiotensin II (AngII) (16). We previously demonstrated that rat brown and white adipose tissues exhibit a high level mRNA expression of angiotensinogen, the only known precursor to AngII (6). Further studies demonstrated that rat and human adipose tissue possess all of the components necessary for production of AngII, including angiotensinogen (7;15;19), renin-like activity (41), angiotensin converting enzyme (ACE) (7;34;38) and the angiotensin type 1 (AT_1) receptor (4;9;10). Importantly, nutritional regulation of the angiotensinogen gene in adipose tissue has been demonstrated, with elevations in obesity and reductions with starvation (19;43). Recent studies demonstrate that overexpression of the angiotensinogen gene selectively in

adipose tissue of mice results in elevated plasma angiotensinogen and modest hypertension (32). Thus, alterations in adipose-derived angiotensinogen have the ability to impact the systemic RAS and influence blood pressure.

Previous studies demonstrate that feeding rats a moderately high fat diet (MHF) results in obesity-induced hypertension (11;31). In the present study, we tested the hypothesis that the adipose and systemic RAS are activated in rats with diet-induced obesity and hypertension. The diet-induced obesity model used in these studies closely mimics the neurohumoral and hemodynamic changes observed in obese humans, including elevations in cholesterol, triglycerides and insulin (11;30). Moreover, an attractive feature of this model is that rats fed the MHF diet segregate into two groups, obesity prone (OP) and obesity resistant (OR), allowing for discrimination of the effects from obesity versus those from the diet, respectively.

Materials and Methods

Animals

All procedures involving animals were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. Male Sprague Dawley rats (450 g, Charles River) were randomly assigned to receive either a MHF diet (D12266B, 32 kcal% as fat, Research Diets) (n = 24) or a low fat (LF) diet (D12489B, 10.6 kcal % as fat, Research Diets) (n = 8) for 11 weeks. The total duration of the study was 11 weeks. Throughout the study, rats were housed individually for assessment of body weight and daily food intake.

Food and water were provided ad libitum. After 8 weeks, rats on the MHF diet were segregated into OP (n = 8) and OR (n = 8) based on their body weight gain distribution as described previously (31). Briefly, a body weight gain histogram was constructed for all rats which resulted in a bimodal distribution of rats into OP and OR groups (upper and lower 1/3rd of rats, respectively). Body weight gain of OP and OR rats were compared to the highest body weight gain of the control (LF) by Chi^2 analysis. OP rats had a higher body weight gain compared to the highest body weight gain of the control (LF), whereas OR rats had a lower body weight gain compared to the highest body weight gain of LF rats. Body weight gain and body weight of OP rats were significantly different (t-test) from OR. The adiposity index was calculated from the sum of the individual fat pad weights [epididymal fat (EF), retroperitoneal fat (RPF)]/(BW-sum of fat pads)*100 (12). The efficiency of weight gain was calculated as total energy intake (MJ)/ total BW gain (g).

Measurement of Mean Arterial Pressure

After 10 weeks on the diets, tail artery catheters were implanted under pentobarbital (50 mg/kg) anesthesia in 5 rats/group. Rats were allowed to recover for one day and subsequently mean arterial pressure (MAP) was recorded for 30 min each day over 5 days, between 8 and 10AM on all rats to avoid diurnal variations. The arterial pressure signal from a Cobe transducer attached to the tail artery catheter was amplified and displayed on a Grass model 7 polygraph. Data were digitally sampled at 500 Hz using a National Instruments E-series A/D converter. MAP, systolic and diastolic pressures were

resolved from data recordings. Power spectral analysis was performed using Welch's method and was used as an index of sympathetic nerve activity (2). The blood pressure (BP) power was normalized to the maximal value across all groups. On the final day, catheters were implanted through the femoral artery under pentobarbital anesthesia in all rats and a final end point MAP was recorded.

Analytical Procedures

Blood was collected from all rats by aortic puncture into tubes containing EDTA (0.38 mol/L) for separation of plasma. The concentration of five different angiotensin peptides in plasma (1 ml) was determined using HPLC for separation of individual peptides, followed by quantification through radioimmunoassay with a chicken AngII antibody exhibiting cross-reactivity to each angiotensin (5). Plasma angiotensinogen was determined indirectly by measurement of AngI generated in the presence of an excess of porcine renin (0.001 units, Sigma, St. Louis, MO). Plasma renin activity (PRA) was determined indirectly by measurement of AngI using a commercially available kit (DiaSorin Inc, MN). Total serum cholesterol was measured using an enzymatic assay kit (Wako chemicals USA, VA).

Angiotensin receptor autoradiography

Tissues (kidney, adrenal, spleen) were frozen in isopentane. Four sets of adjacent sections (16 µm) were prepared from each tissue. Analysis of AngII receptor density was performed by incubating sections in a phosphate buffer with protease inhibitors containing 400 pM of [I^{125}]Sar^1,Ile^8AngII (2200 Ci/mmol, Peptide Radioiodination Center, Washington

State University) for 2 hours at 22EC. Adjacent sections were used to determine total binding (radioligand alone), non-specific binding (addition of 10 µM unlabelled AngII), AT_1 receptors (addition of 10 µM of the AT_2 receptor antagonist, PD123319), and AT_2 receptors (addition of 10 µM of the AT_1 receptor antagonist, losartan). At the end of the incubation, sections were washed extensively and exposed to film for image analysis (NIH Image 5.2 software program) and determination of relative receptor density using arbitrary densitometry.

RNA isolation and quantification of gene expression

Total RNA was extracted from tissues [liver, kidney, lung, EF, RPF and subcutaneous fat (SC)] of all rats using the phenol guanidine-isothiocyanate method (Trizol kit, Invitrogen, CA) according to the manufacturer's instructions. An index of total RNA yield in proportion to tissue weight was calculated by normalizing the amount of total RNA to the weight of tissue utilized for extraction. Total RNA (0.4 µg) was reverse transcribed for 1 hr at 55EC with the following components: Random decamers, 10X reverse transcription buffer, deoxynucleotide triphosphate mix, ribonuclease inhibitor and reverse transcriptase [RETROscript (Ambion, TX)]. Relative quantification of gene expression was performed with an iCycler (BioRad, CA) using a standard curve method. SYBR Green PCR core reagents (Applied Biosystems, CA) were used at the following concentration (total volume of 50 µl): SYBR Green mix (1X), $MgCl_2$ (3 mM), dNTP mix (1.25 mM), Fluorescein (0.01 µM), primers (0.5 µM), AmpliTaq gold (2.5 u). 18s rRNA was used as an endogenous control gene. Primers were designed using Primer 3 software and sequences are

displayed in Table 1. The real-time PCR conditions for all genes were: 5 min at 94EC, 40 cycles with 1 min at 94EC, 1 min at 64EC, 1 min at 72 EC, and a final elongation step for 10 min at 72EC.

Statistical analysis

All results are mean ± SEM. Body weight and energy intake were analyzed by 2-way ANOVA with time as a repeated measure and treatment as a between group factor. Real-time PCR measurements of gene expression and plasma components (angiotensins, angiotensinogen, PRA and cholesterol) were analyzed by ANOVA to compare between group effects. Tukey's test for between group differences was used when appropriate. Correlation analysis was performed on various measured parameters using linear regression to calculate the goodness of fit and correlation coefficient (r^2). Statistical significance was accepted at a value of $P < 0.05$.

Results

Metabolic Characteristics of OP, OR and LF rats

While initial body weights (BW) did not differ significantly between groups, by day 10 OP rats had significantly greater body weights compared to OR (Fig. 1A). In contrast, OP rats did not differ from LF until day 30 on the diet. On the final day, body weights differed between OP and OR rats by > 130 g (72% increase from baseline) (Fig. 1), and were reflected as an increase in total body weight gain (Table 2). The adiposity index was significantly increased in OP versus OR and LF (Table 2). In addition, OP rats exhibited

an elevation in serum cholesterol concentrations compared to OR and LF (Table 2).

Food intake was increased in OP rats versus OR and LF (Fig. 2). Food intake was converted to energy intake to account for differences in total energy provided by the diets (3.9 Kcal/g for LF diet versus 4.4 Kcal/g for MHF diet). Energy intake was similar between OR and LF, but increased in OP rats. Importantly, the efficiency of weight gain was increased in OP rats compared to OR and LF (LF: 5.4 ± 0.3, OR: 5.1 ± 0.2, OP: 7.5 ± 0.5 g/Mj; $P<0.05$).

Hemodynamic Differences Between OP, OR and LF Rats

After 10 weeks on the MHF diet, MAP was increased in OP rats versus OR and LF (Table 3). Systolic blood pressure was increased in OP rats versus OR and LF, whereas diastolic blood pressure was not different between groups (Table 3). Measurement of MAP in anesthetized rats confirmed elevations in MAP in OP rats compared to OR and LF (Table 3). Blood pressure power at 0.4 Hz was increased in OP rats compared to OR and LF (Table 3).

The Adipose and Systemic RAS in OP, OR and LF Rats

To compare the synthetic properties of the different tissues examined, we calculated the yield of total RNA per gm tissue weight, which was approximately 100-fold greater in liver, kidney and lung compared to EF, RPF and SC fat (data not shown). Moreover, for each tissue, this index was similar between LF, OR and OP (data not

shown). However, the sum of the weight of fat pads (Table 2) was 2-fold greater in OP rats, indicating that the total RNA yield from adipose tissue was greater in OP rats compared to OR and LF. Angiotensinogen mRNA expression was selectively increased (2-fold) in the RPF of OP rats versus OR and LF (Fig. 3A). In contrast, in liver, EF and SC, angiotensinogen mRNA expression was not significantly different between groups (Fig. 3A). In the lung, a tissue with high level expression of ACE, there were no differences in ACE mRNA expression between groups (Fig. 3B). Similarly, in EF, RPF and SC, ACE mRNA levels did not differ between groups (Fig. 3B). Renin mRNA expression was at the lower limits of detection in adipose tissue, and was not different between groups (data not shown). In addition, kidney renin mRNA expression was not significantly different between groups (LF: 0.32 $\pm$ 0.09, OR: 0.45 $\pm$ 0.09, OP: 0.58 $\pm$0.01).

Elevations in angiotensinogen mRNA expression in RPF (2-fold; Fig. 3A) were reflected by a similar increase (2-fold) in plasma angiotensinogen concentration in OP rats compared to OR and LF (Fig. 4). Linear regression analysis demonstrated a strong correlation ($r^2 = 0.77$; $P < 0.05$) between RPF angiotensinogen mRNA expression and plasma angiotensinogen concentrations. In contrast, PRA was not different between groups (LF: 4.3 ± 0.8, OR: 4.6 ± 0.4, OP: 4.8 ± 0.5 ng/ml/hr). A marked increase in the plasma concentrations of AngII was determined in OP rats compared to OR and LF (Fig. 5). Moreover, elevations in plasma concentrations of AngIII and Ang5-8 were detected in OP rats, giving rise to a 3-fold increase in the sum of circulating angiotensins in OP rats (Fig. 5). Plasma AngII concentrations correlated positively ($r^2 = 0.45$, $P = 0.009$) to MAP in

all rats from this study. In addition, plasma AngII concentration correlated positively with plasma angiotensinogen ($r^2 = 0.52$; $P < 0.05$) and with RPF angiotensinogen mRNA expression ($r^2 = 0.81$; $P < 0.05$).

Angiotensin receptor density was decreased in the kidney of OP rats compared to OR and LF (LF:4.3 ± 0.2 , OR: 3.2 ± 0.3, OP: 2.9 ± 0.2 nCi ; $P < 0.05$) (Fig. 6). Reductions in AngII receptor density in OP rats were most pronounced in the kidney medulla. However, residual binding in the presence of losartan (estimate of AT_2 receptor binding) or PD 123319 (estimate of AT_1 receptor binding) was not different between OP, OR and LF (%AT_1; LF: 62 ± 6, OR: 50 ± 8, OP: 58 ± 6 and %AT_2; LF: 38 ± 6, OR: 50 ± 8, OP: 42 ± 6). In the adrenal and spleen, there were no differences in AT_1 or AT_2 receptor density between groups (data not shown).

Discussion

An important new finding in this study is that circulating concentrations of angiotensin peptides were markedly increased in rats with diet-induced obesity. Moreover, plasma concentrations of AngII correlated positively to mean arterial pressure, demonstrating a strong link between the RAS and obesity-induced hypertension. In addition, elevations in angiotensinogen mRNA expression were observed in selective adipose depots, but not in the liver, of OP rats. Adipose angiotensinogen mRNA expression was mirrored by a similar-magnitude increase in the circulating angiotensinogen concentration. In contrast, other components of the RAS were not altered

in OP rats compared to control. These observations support a role for the RAS in hypertension from obesity, and suggest that adipose tissue contributes to heightened levels of the systemic RAS.

The obesity aspect of the diet-induced obesity model initially created by Levin (31) has been previously described (27-29) Using a diet containing a moderate increase in fat (32% Kcal as fat), similar to the Western Diet (40), we characterized the obesity development in OP rats by monitoring food intake and body weight throughout the duration on the diet. As originally described by Levin (31), rats fed the MHF diet segregate into OP and OR rats with considerable differences in body weight gain and adiposity. Similar to previous findings in this model, energy intake was increased in OP rats (11;30). However, the efficiency of weight gain (total energy intake/body weight gain) was increased in OP rats, suggesting that elevations in food intake are not the sole mechanism for increased body weights in OP rats (26). These results concur with the hypothesis of pre-existing metabolic differences between OP and OR Sprague Dawley rats (27).

As described recently by Dobrian et al. (11), blood pressure was increased in OP rats after 10 weeks on the MHF diet. In addition, results from this study extend previous findings by demonstrating an elevation in MAP in conscious rats in the absence of restraining procedures. Elevations in systolic, rather than diastolic pressure appear to primarily mediate the increase in MAP in OP rats. Recent studies have demonstrated that rats selectively bred to develop diet-induced obesity exhibit elevations in urinary 24 hour

norepinephrine levels (27). Moreover, obesity-related hypertension in humans is associated with elevations in systemic catecholamines (8;22). In this study, the blood pressure power at 0.4Hz was examined as an index of the sympathetic nervous system, and suggests an overall high sympathetic tone in OP rats. Reciprocal interactions between the RAS and the sympathetic nervous system may collectively contribute to obesity related hypertension.

In this study, angiotensinogen mRNA expression was increased selectively in the retroperitoneal adipose tissue of OP rats. These findings are in agreement with recent results demonstrating a selective increase in intra-abdominal adipose angiotensinogen mRNA expression in mice fed very high fat diets (45%) (35). It is unlikely that increased angiotensinogen mRNA expression in adipose tissue results directly from the high fat diet, since in this study OR rats exhibiting normal adiposity in response to the MHF diet did not exhibit these changes. Rather, these results suggest that elevations in angiotensinogen mRNA expression are associated with obesity. Regional differences in angiotensinogen mRNA expression in adipose tissue have been previously described (1; 45). However, mechanisms for site-selective modulation of angiotensinogen mRNA expression in adipose tissue are unknown.

Previous investigators have examined angiotensinogen gene expression in human adipose tissue from obese patients. Angiotensinogen gene expression in subcutaneous and visceral adipose tissue correlated positively with body mass index (44;45).

Conversely, studies in obese patients with hypertension revealed a slight decrease or no change in subcutaneous angiotensinogen gene expression (17;20). Due to obvious limitations, previous studies in humans have examined angiotensinogen gene expression in subcutaneous adipose tissue. Our results demonstrate regional variations in angiotensinogen gene expression with greater expression in an intra-abdominal depot from obese hypertensive rats. Future studies examining regional expression of angiotensinogen in visceral and intra-abdominal adipose depots in humans with obesity hypertension are warranted.

Importantly, in this study a strong positive correlation was observed between angiotensinogen mRNA expression in the RPF and the plasma angiotensinogen concentration. In recent studies, overexpression of angiotensinogen in mouse adipose tissue was paralleled by an increase in plasma angiotensinogen concentration and blood pressure (32). These results suggest that adipose-derived angiotensinogen can contribute to the circulating pool of angiotensinogen. While the total RNA yield/gm of adipose tissue was not different between OP and control rats, the absolute amount of adipose mass (including retroperitoneal adipose with greater angiotensinogen mRNA expression) was increased in obese rats. Thus, the contribution of adipose tissue to the total circulating angiotensinogen concentration would be anticipated to be greater in obese rats. We suggest that elevations in adipose angiotensinogen mRNA expression contributed to the increased circulating pool in obese hypertensive rats.

A major finding in this study is the elevation in plasma AngII concentrations accompanying obesity-hypertension. Furthermore, plasma AngII concentration correlated positively with plasma angiotensinogen. Under most physiological conditions, the rate limiting step for AngII generation is the cleavage of AngI from angiotensinogen by the aspartyl protease renin. This reaction is tonically regulated by renin release from the juxtaglomerular cells of the kidney. However, in most species the concentration of angiotensinogen in the blood approximates the Michaelis-Menten constant for renin. Thus, small changes in plasma angiotensinogen concentration can affect plasma angiotensin production (14;25;33) . In support, administration of angiotensinogen to rats at physiological concentrations dose-dependently increased AngI generation and blood pressure (25). In addition, gene dosing of angiotensinogen expression in mice results in a dose-dependent increase in plasma angiotensinogen concentration and blood pressure (24). Taken-together these results imply that changes in the plasma angiotensinogen concentration can affect AngII generation, and thus blood pressure. In this study, plasma AngII concentration correlated positively with MAP. These results are consistent with a strong link between abdominal obesity and cardiovascular disease, namely hypertension (46).

To determine if other components of the RAS were influenced by diet-induced obesity, we examined mRNA expression of renin and ACE in adipose and non-adipose sources. Surprisingly, we did not observe alterations in gene expression of these components of the RAS in any tissues examined from obese rats. Dobrian et al. (11;12) reported a 2-fold elevation in plasma renin activity in OP rats derived from a similar MHF

diet; however, kidney renin gene expression or other components of the RAS were not examined. In this study, both plasma renin activity and angiotensin peptide concentrations were measured in the same plasma sample from individual rats. We report a marked increase in plasma AngII concentrations in OP rats, that correlates positively to elevations in blood pressure. Given this marked increase in circulating AngII concentration, kidney renin mRNA expression and plasma renin activity would be anticipated to decrease from negative feedback (39). Thus, even a normal level of plasma renin activity is surprising given the marked elevation in systemic AngII. A possible explanation for this abnormality is the heightened sympathetic nerve activity observed in OP rats. AngII-induced negative feedback on renin may be counterbalanced by catecholamine-mediated stimulation of renin synthesis and release. Moreover, the observed down-regulation of renal angiotensin receptors suggests an inability of AngII to effectively inhibit renin synthesis and release in the kidney of OP rats. In this study plasma renin activity was measured at the end of the study in rats with established hypertension. It is possible that plasma renin activity was elevated during the initial increase in blood pressure in obese rats.

The marked elevations in systemic angiotensin peptides in obese rats could mediate hypertension through many different mechanisms. As suggested by our results, AngII may increase blood pressure in OP rats by stimulating the sympathetic nervous system with release of norepinephrine in the brain and periphery (3;13). Furthermore, Dobrian et al (12) demonstrated increased oxidative stress in OP rats, and highlighted a possible role for AngII, given the ability of AngII to increase reactive oxygen species (42). Finally, the arterial

hypertrophy previously observed in the kidney of OP rats may have resulted from elevations in AngII (11).

In summary, our findings demonstrate elevations in expression of angiotensinogen in intra-abdominal adipose tissue of rats with diet-induced obesity and hypertension. Importantly, these changes were accompanied with an activation of the systemic RAS, highlighting a role for AngII in obesity-related hypertension. Future studies, employing adipose-specific deficiency of angiotensinogen, are warranted to further define the role of adipose-derived angiotensinogen in obesity-related hypertension.

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Figure Legends

Figure 1. Effect of a MHF diet on body weight. Rats were fed either a LF or MHF diet (32% kcal as fat) for 11 weeks. Body weights of OP rats were significantly increased compared to OR beginning on day 10 of the diet. Data are mean ± SEM of n = 8/group.

Figure 2. Effect of the MHF diet on weekly energy intake. Rats were fed either a LF or MHF diet for 11 weeks. Energy intake was calculated by multiplying food intake (g) by the amount of energy provided by the diets (4.4 kcal/g for MHF diet, and 3.9 Kcal/g for LF diet). Energy intake was increased in OP rats compared to OR and LF beginning at week 4 on the diet. Data are mean + SEM of n=8/group. *, significantly different from LF, OR; $P < 0.05$.

Figure 3. A, Quantification of angiotensinogen mRNA expression by real-time PCR in RPF, EF, SC and liver of OP, OR and LF rats. Rats were fed either a LF or MHF diet (32% kcal as fat) for 11 weeks. Tissues were processed for real-time PCR as described in METHODS. To correct for unequal loading of RNA, a ratio of angiotensinogen to 18s rRNA was constructed. Angiotensinogen mRNA expression was increased in the RPF of OP rats compared to OR and LF. B, Quantification of ACE mRNA by real-time PCR in the RPF, EF, SC and lung of LF, OR and OP rats. Data are mean ± SEM of n = 8/group. *, significantly different from LF, OR; $P < 0.05$.

Figure 4. Effect of a MHF diet on plasma angiotensinogen concentrations. Plasma angiotensinogen was increased in OP rats compared to OR and LF. Data are mean $\pm$ SEM of n = 8/group. *, significantly different from OR and LF; $P < 0.05$.

Figure 5. Effect of a MHF diet on the plasma concentrations of angiotensin peptides. Plasma angiotensin peptides were measured as described in METHODS. The concentrations of AngII, AngIII, and Ang5-8 were increased in OP rats compared to OR and LF. 3 peptides is the sum concentration of all angiotensin peptides in plasma, and was increased 3-fold in OP rats. Data are mean $\pm$ SEM of n = 8/group. *, significantly different from OR and LF; $P < 0.05$.

Figure 6. Receptor autoradiography for [I^{125}]Sar^1,Ile^8AngII binding in representative tissue sections from the kidney of OP, OR and LF rats. AngII receptor density was decreased in the kidney of OP rats compared to OR and LF.

Table 1. Primers sequences used for quantification of gene expression by real-time PCR and the resulting product size.

Gene	Primers	Product size*
Angiotensinogen	5’CACGGACAGCACCCTATTTT3’ 5’GCTGTTGTCCACCCAGAACT3’	103
18s rRNA	5’CGCGGTTCTATTTTGTTGGT3’ 5’AGTCGGCATCGTTTATGGTC3’	219
Renin	5’TTCTCTCCCAGAGGGTGCTA3’ 5’CCCTCCTCACACAACAAGGT3’	211
ACE	5’GAGCCATCCTTCCCTTTTTC3’ 5’GGCTGCAGCTCCTGGTATAG3’	154
AT1	5’ACTCTTTCCTACCGCCCTTC3’ 5’TTAGCCCAAATGGTCCTCTG3’	145

*, base pair

Table 2. Comparison of weight gain, obesity index and total serum cholesterol between OP, OR and LF rats.

Variable	LF	OR	OP
Initial body weight (g)	457±8	428±8	452±10
Final body weight (g)	612±15	566±10	702±20*
Total weight gain (g)	155±10	138±5	236±18*
Sum of fat pads (g)	23±4	24±2	43±4*
Obesity index, %	4.2±0.3	4.3±0.2	6.5±0.2*
Serum cholesterol (mg/dl)	84±19	76±33	149±23*

*, significantly different from OR and LF.

Table 3. Comparison of SBP, DBP, MAP in conscious rats and MAP under anesthesia between OP, OR and LF

Variable	LF	OR	OP
SBP (mmHg)	122 ± 2	121 ± 1	136 ± 3*
DBP (mmHg)	84 ± 3	87 ± 3	89 ± 1
MAP (mmHg)	97 ± 2	97 ± 2	105 ± 1*
MAP under anesthesia (mmHg)	91 ± 7	84 ± 5	123 ± 9*
BP Power at 0.4 Hz	0.38 ± 0.03	0.42 ± 0.03	0.71 ± 0.17*

*, significantly different from OR and LF

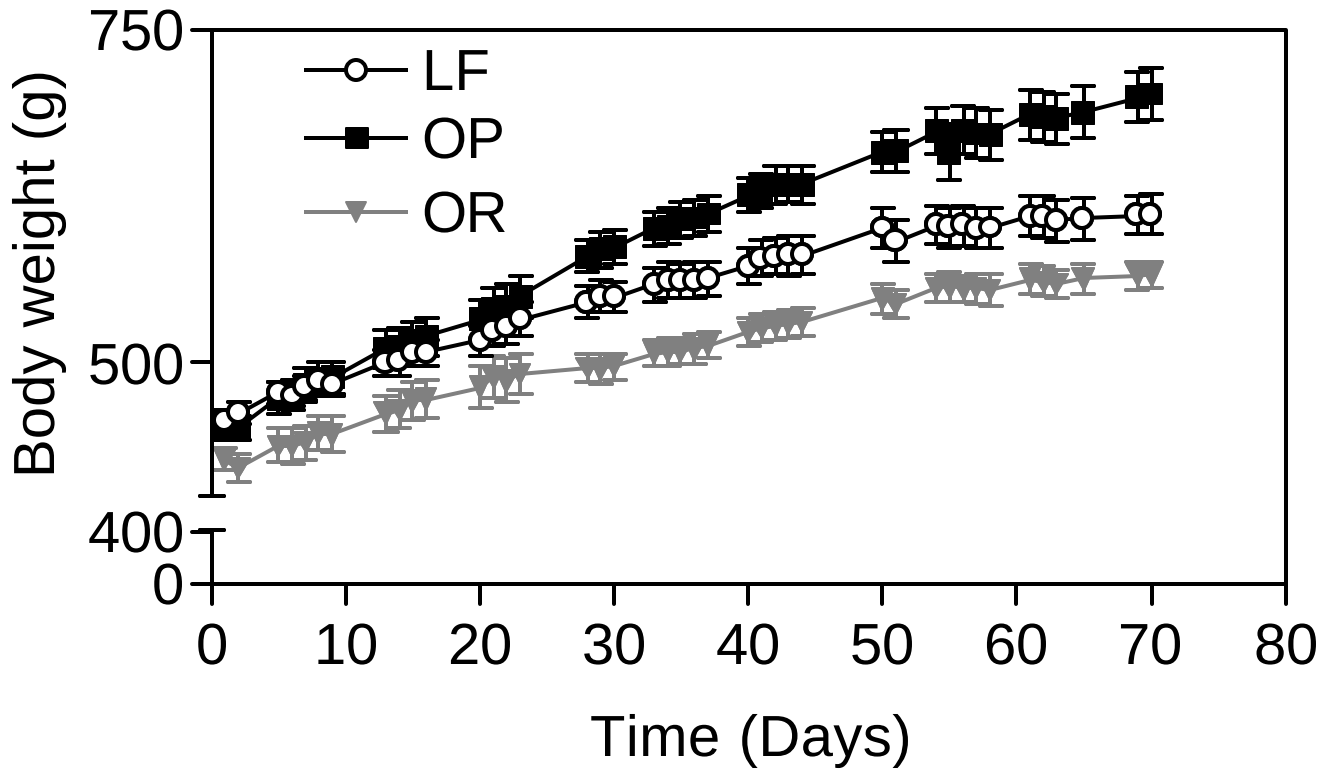

750
500
400
0
Body weight (g)
LF
OP
OR
0
10
20
30
40
50
60
70
80
Time (Days)

Figure 1

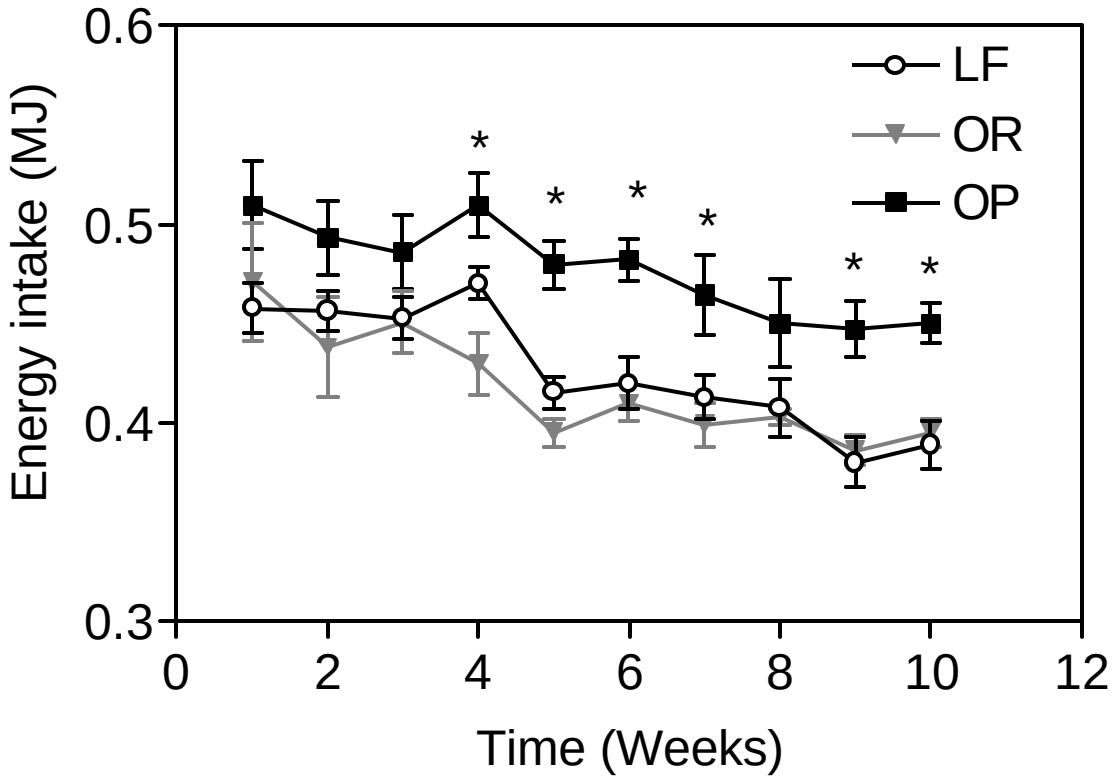
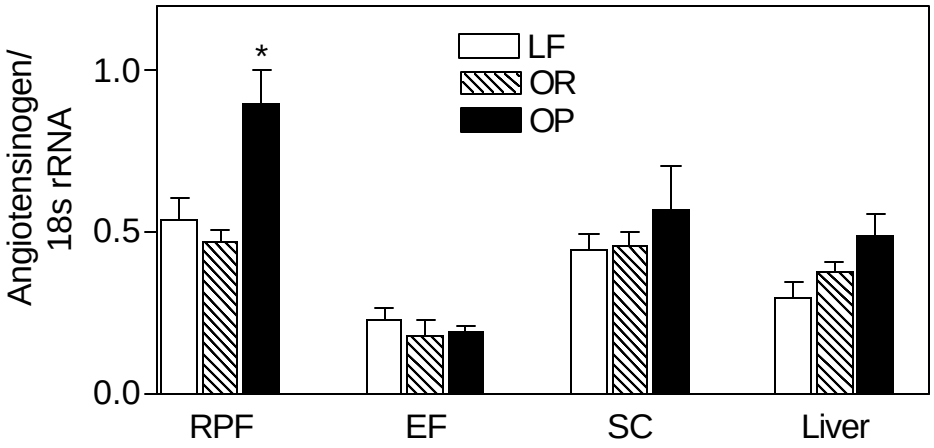
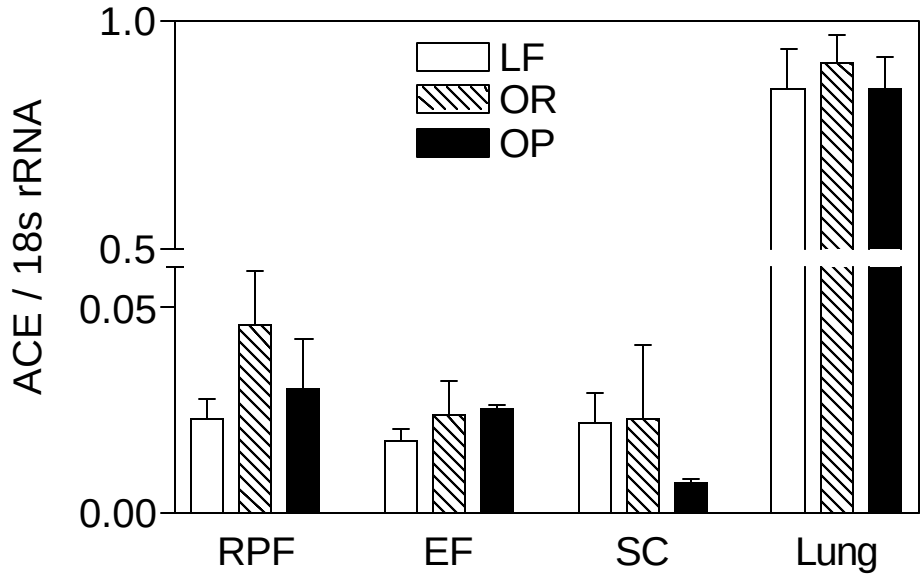


Figure 2


Angiotensinogen/
18s rRNA
1.0
0.5
0.0
*
LF
OR
OP
RPF
EF
SC
Liver

A


ACE / 18s rRNA
1.0
0.5
0.05
0.00
LF
OR
OP
RPF
EF
SC
Lung

B

Figure 3

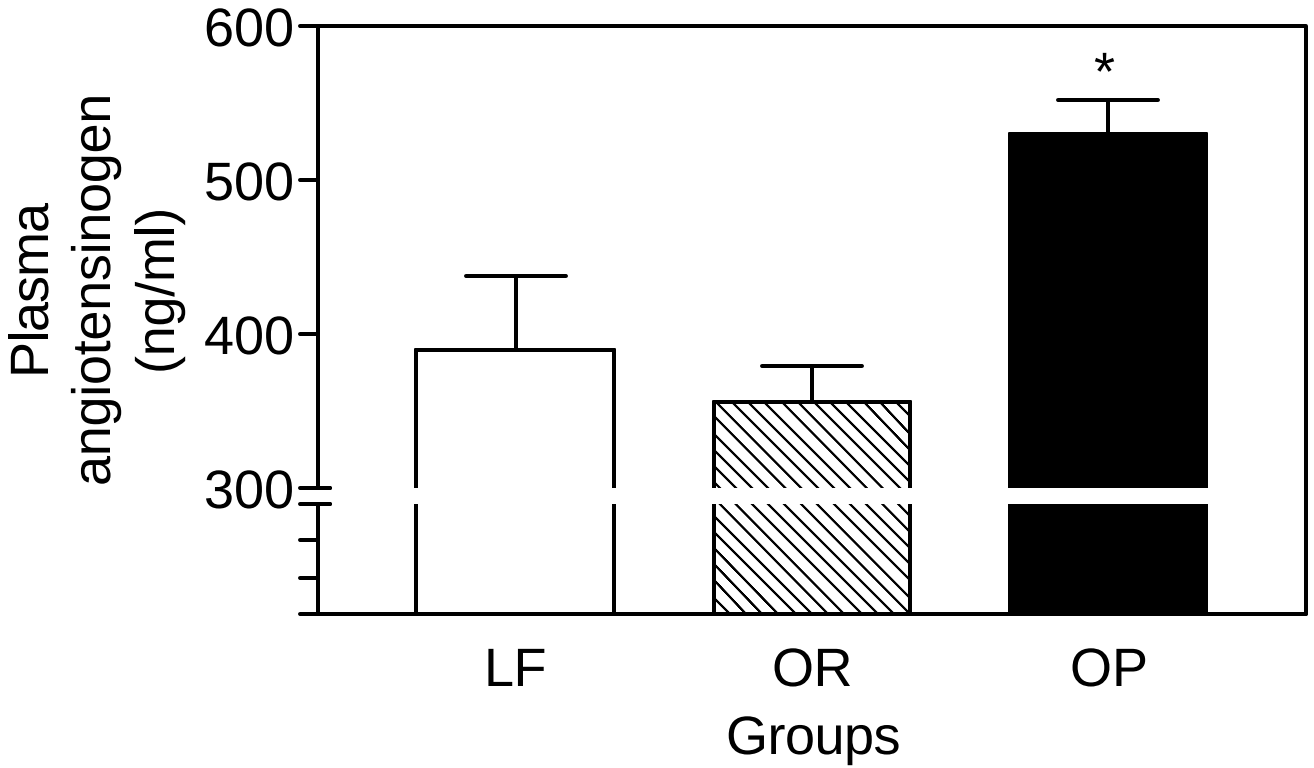


Figure 4

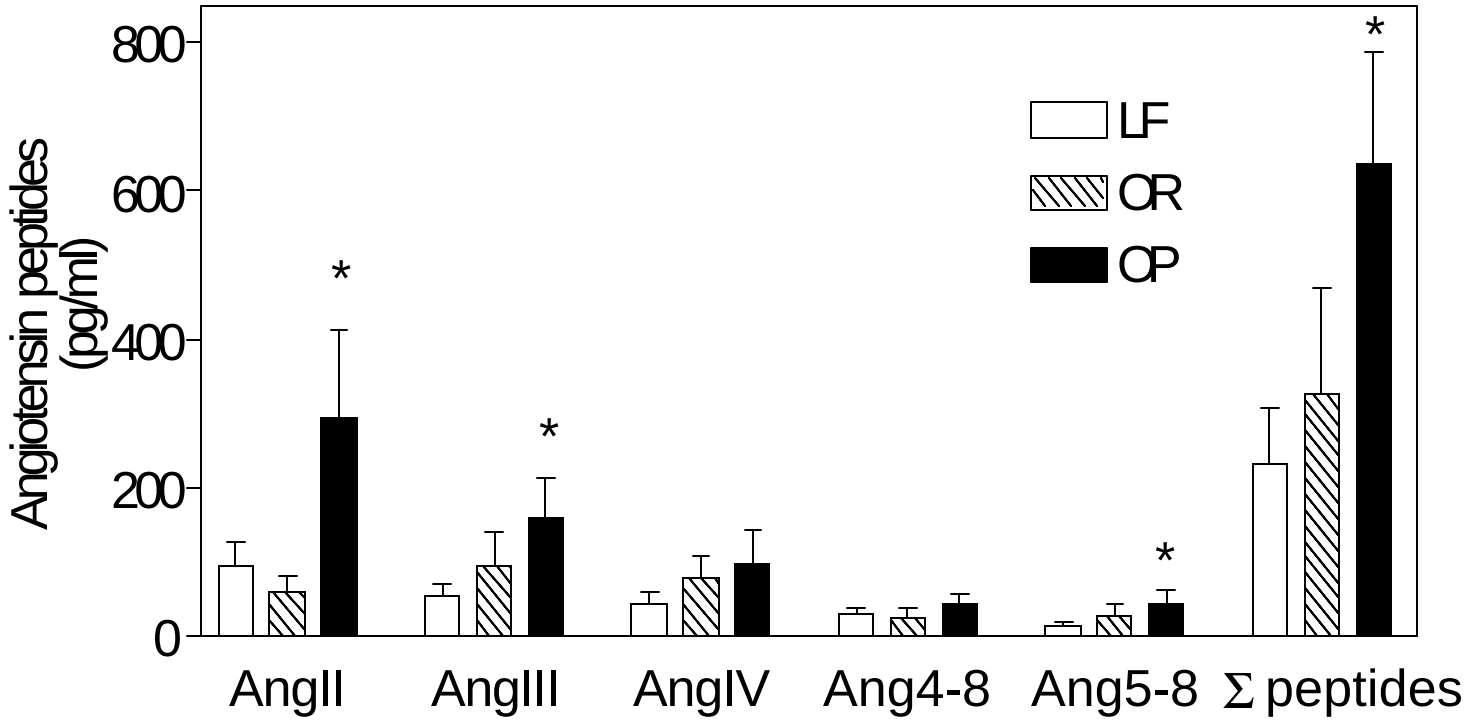

Angiotensin peptides (pg/ml)
800
600
400
200
0
LF
CR
CP
*
*
*
*
AngII
AngIII
AngIV
Ang4-8
Ang5-8
Σ peptides

Figure 5

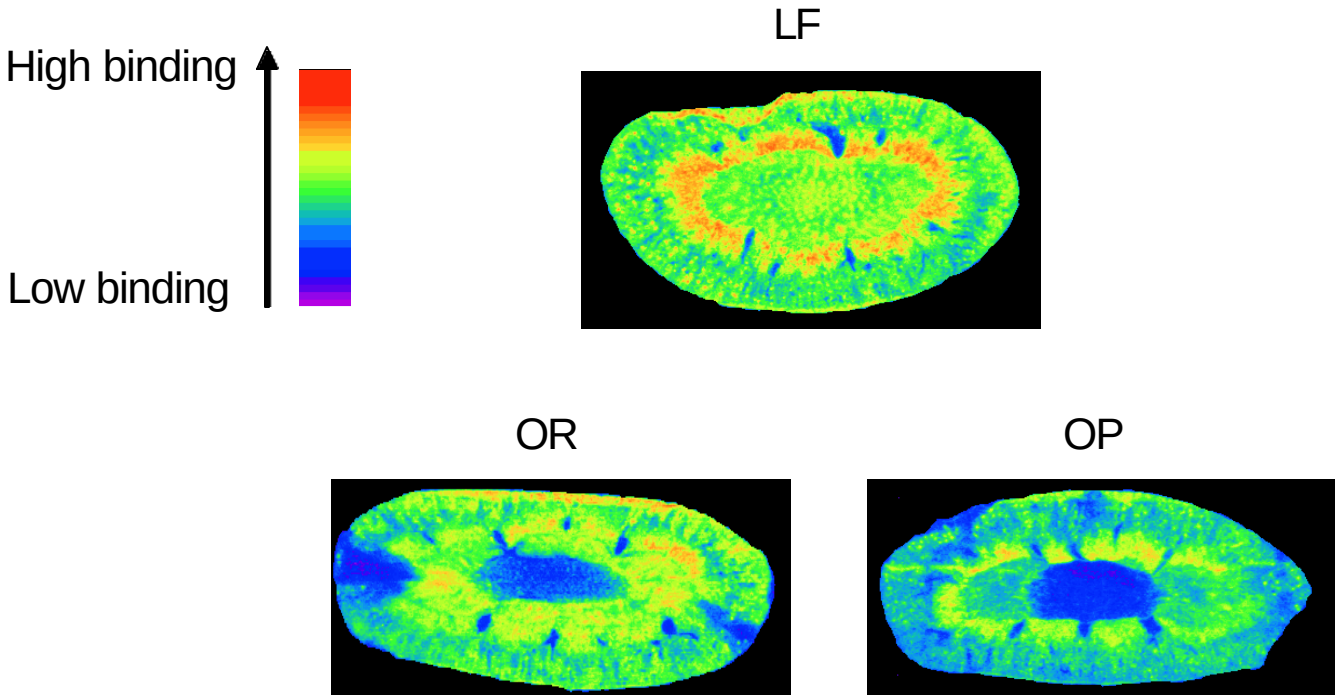


Figure 6