

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

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Boustany, Carine M., Kalyani Bharadwaj, Alan Daugherty, David R. Brown, David C. Randall, and Lisa A. Cassis. Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. *Am J Physiol Regul Integr Comp Physiol* 287: R943–R949, 2004. First published June 10, 2004; 10.1152/ajpregu.00265.2004.—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. RAS was quantified in adipose tissue and compared with 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 (32% kcal as fat) diet for 11 wk. After 8 wk, rats fed the moderately high-fat 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 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 vs. OR and LF rats. 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 (angiotensin II: 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.

retroperitoneal adipose; mean arterial pressure; angiotensin II

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 (ANG) 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 ANG II (16). We previously demonstrated that rat brown and white adipose tissues exhibit a high level mRNA expression of angiotensinogen, the only known precursor to ANG II (6). Further studies demonstrated that rat and human adipose tissue possess all of the components necessary for production of ANG II, including angiotensinogen (7, 15, 19), renin-like activity (41), ANG-converting enzyme (ACE) (7, 34, 38), and the ANG type 1 (AT₁) 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 (MHF) diet 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 vs. 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 wk. The total duration of the study was 11 wk. 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 wk, rats on the MHF diet were segregated into OP ($n = 8$) and OR groups ($n = 8$) based on their body weight gain distribution as described previously (31). Briefly, a body weight gain histogram was constructed for

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

Gene	Primers	Product Size, bp
Angiotensinogen	5'CACGGACAGCACCTATTTT3' 5'GCTGTGTCCACCCAGAACT3'	103
18S rRNA	5'CGCGTTCTATTTTGTGGT3' 5'AGTCGGCATCGTTTATGGTC3'	219
Renin	5'TTCTCTCCAGAGGGTGCTA3' 5'CCCTCTCACACAACAAGGT3'	211
ACE	5'GAGCCATCCCTCCCTTTTTC3' 5'GGCTGCAGCTCCTGGTATAG3'	154
AT ₁ receptor	5'ACTCTTCCCTACCGCCTTC3' 5'TTAGCCCAAATGGTCTCTG3'	145

ACE, angiotensin-converting enzyme; AT₁, angiotensin type 1.

all rats, which resulted in a bimodal distribution of rats into OP and OR groups (upper and lower one-third of rats, respectively). Body weight gain of OP and OR rats were compared with the highest body weight gain of the control (LF) by χ^2 analysis. OP rats had a higher body weight gain compared with the highest body weight gain of the control (LF), whereas OR rats had a lower body weight gain compared with 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)]/(body weight - sum of fat pads)·100 (12). The efficiency of weight gain was calculated as total body weight gain (g)/total energy intake (MJ).

Measurement of mean arterial pressure. After 10 wk on the diets, tail artery catheters were implanted under pentobarbital sodium (50 mg/kg) anesthesia in 5 rats/group. Rats were allowed to recover for 1 day, and subsequently mean arterial pressure (MAP) was recorded for 30 min each day over 5 days between 8 and 10 AM 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 analog-to-digital converter. MAP, systolic pressure, and diastolic pressure 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 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 M) for separation of plasma. The concentration of five different ANG peptides in plasma (1 ml) was determined using high-performance liquid chromatography for separation of individual peptides, followed by quantification through radioimmunoassay with a chicken ANG II antibody exhibiting cross-reactivity to each ANG (5). Plasma angiotensinogen was determined indirectly by measurement of ANG I 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 ANG I using a commercially available kit (DiaSorin, Stillwater, MN). Total serum cholesterol was measured using an enzymatic assay kit (Wako Chemicals).

ANG receptor autoradiography. Tissues (kidney, adrenal, spleen) were frozen in isopentane. Four sets of adjacent sections (16 μ m) were prepared from each tissue. Analysis of ANG II receptor density was performed by incubating sections in a phosphate buffer with protease inhibitors containing 400 pM of ¹²⁵I-labeled [Sar¹, Ile⁸]ANG II (2,200 Ci/mmol, Peptide Radioiodination Center, Washington State University) for 2 h at 22°C. Adjacent sections were used to determine total binding (radioligand alone), nonspecific binding (addition of 10

μ M unlabeled ANG II), AT₁ receptors (addition of 10 μ M of the AT₂ receptor antagonist PD-123319), and AT₂ receptors (addition of 10 μ M of the AT₁ receptor antagonist losartan). At the end of the incubation, sections were washed extensively and exposed to film for image analysis (National Institutes of Health 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 (SC) fat] of all rats using the phenol-guanidine isothiocyanate method (Trizol kit, Invitrogen, Carlsbad, 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 μ g) was reverse transcribed for 1 h at 55°C with the following components: random decamers, 10 \times reverse transcription buffer, deoxynucleotide triphosphate (dNTP) mix, ribonuclease inhibitor, and reverse transcriptase (RETROscript, Ambion, Austin, TX). Relative quantification of gene expression was performed with an iCycler (Bio-Rad) using a standard curve method. SYBR Green PCR core reagents (Applied Biosystems) were used at the following concentration (total volume of 50 μ l): 1 \times SYBR Green mix, 3 mM MgCl₂, 1.25 mM dNTP mix, 0.01 μ M fluorescein, 0.5 μ M primers, 2.5 U AmpliTaq gold. 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 94°C, 40 cycles with 1 min at 94°C, 1 min at 64°C, 1 min at 72°C, and a final elongation step for 10 min at 72°C.

Statistical analysis. All results are means \pm SE. Body weight and energy intake were analyzed by two-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 (ANG peptides, 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 did not differ significantly between groups, by day 10 OP rats had significantly greater body weights compared with OR rats (Fig. 1). In contrast, OP rats did not differ from LF rats until day 30 on the diet. On the final

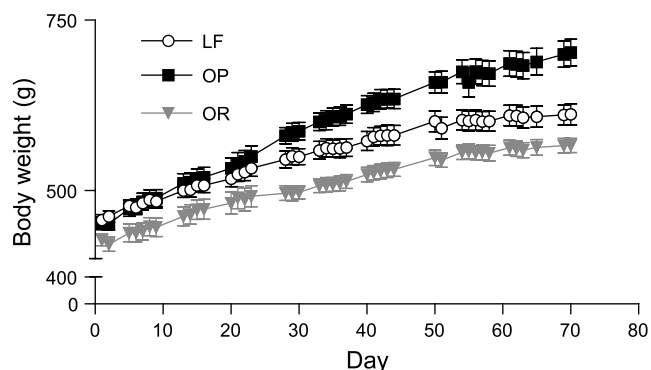


Fig. 1. Effect of a moderately high-fat (MHF) diet on body weight. Rats were fed either a low-fat (LF) or MHF diet (32% kcal as fat) for 11 wk. Body weights of obesity-prone (OP) rats were significantly increased compared with obesity-resistant (OR) rats beginning on day 10 of the diet. Data are means \pm SE of $n = 8$ rats/group.

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 vs. OR and LF (Table 2). In addition, OP rats exhibited an elevation in serum cholesterol concentrations compared with OR and LF rats (Table 2).

Food intake was increased in OP rats vs. OR and LF rats. 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 vs. 4.4 kcal/g for MHF diet) (Fig. 2). 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 with OR and LF rats (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 wk on the MHF diet, MAP was increased in OP rats vs. OR and LF rats (Table 3). Systolic blood pressure was increased in OP rats vs. OR and LF rats, 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 with OR and LF rats (Table 3). Blood pressure power at 0.4 Hz was increased in OP rats compared with OR and LF rats (Table 3).

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 gram of tissue weight, which was ~100-fold greater in liver, kidney, and lung compared with EF, RPF, and SC fat (data not shown). Moreover, for each tissue, this index was similar between LF, OR, and OP rats (data not shown). However, the sum of the weight of fat pads (Table 2) was twofold greater in OP rats, indicating that the total RNA yield from adipose tissue was greater in OP rats compared with OR and LF rats. Angiotensinogen mRNA expression was selectively increased (2-fold) in the RPF of OP rats vs. OR and LF rats (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 ± 0.09 ; OR: 0.45 ± 0.09 ; OP: 0.58 ± 0.01).

Elevations in angiotensinogen mRNA expression in RPF (2-fold; Fig. 3A) were reflected by a similar increase (2-fold) in

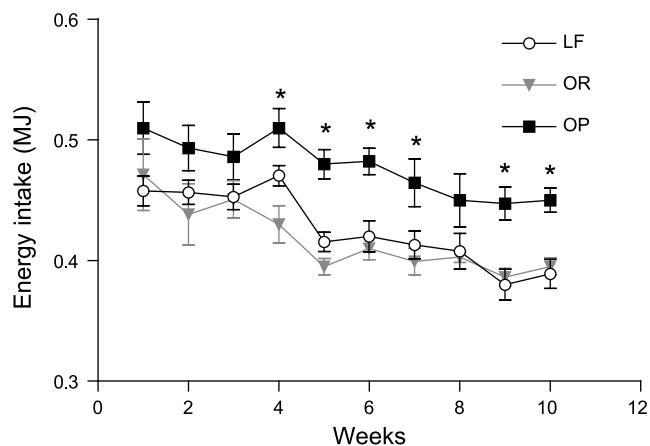


Fig. 2. Effect of the MHF diet on weekly energy intake. Rats were fed either a LF or MHF diet for 11 wk. Energy intake was calculated by multiplying food intake (g) by the amount of energy provided by the diets (4.4 kcal/g for MHF diet, and 3.9 kcal/g for LF diet). Energy intake was increased in OP rats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

plasma angiotensinogen concentration in OP rats compared with OR and LF rats (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⁻¹·h⁻¹). A marked increase in the plasma concentrations of ANG II was determined in OP rats compared with OR and LF rats (Fig. 5). Moreover, elevations in plasma concentrations of ANG III and ANG 5–8 were detected in OP rats, giving rise to a threefold increase in the sum of circulating ANG peptides in OP rats (Fig. 5). Plasma ANG II concentrations correlated positively ($r^2 = 0.45$; $P = 0.009$) to MAP in all rats from this study. In addition, plasma ANG II concentration correlated positively with plasma angiotensinogen ($r^2 = 0.52$; $P < 0.05$) and with RPF angiotensinogen mRNA expression ($r^2 = 0.81$; $P < 0.05$).

ANG receptor density was decreased in the kidney of OP rats compared with OR and LF rats (LF: 4.3 ± 0.2 ; OR: 3.2 ± 0.3 ; OP: 2.9 ± 0.2 nCi; $P < 0.05$) (Fig. 6). Reductions in ANG II receptor density in OP rats were most pronounced in the kidney medulla. However, residual binding in the presence of losartan (estimate of AT₂ receptor binding) or PD-12319 (estimate of AT₁ receptor binding) was not different between

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

Variable	LF Rats	OR Rats	OP Rats
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*

Values are means ± SE. LF, low fat; OR, obesity resistant; OP, obesity prone. *Significantly different from OR and LF rats ($P < 0.05$).

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

Variable	LF Rats	OR Rats	OP Rats
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*

Values are means ± SE. BP, blood pressure; SBP, systolic BP; DBP, diastolic BP; MAP, mean arterial BP. *Significantly different from OR and LF rats ($P < 0.05$).

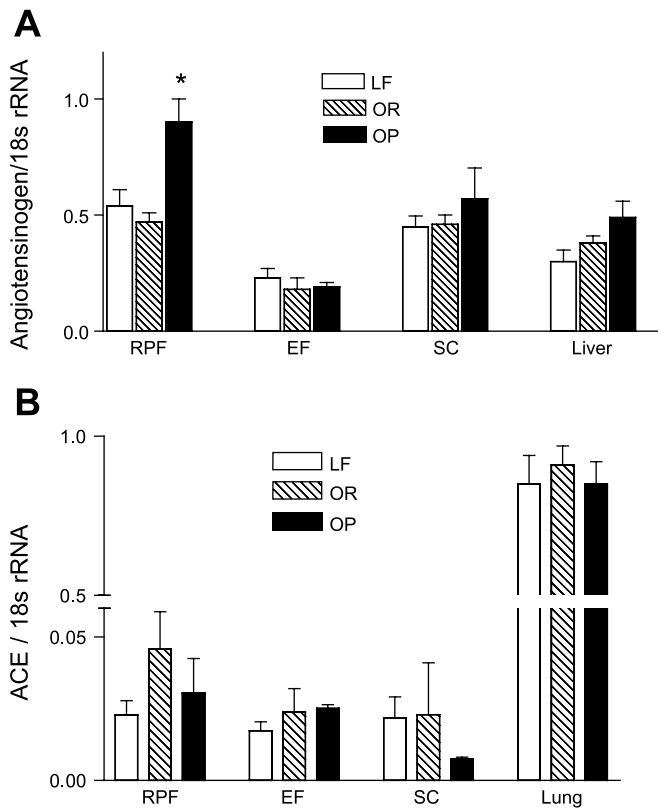


Fig. 3. *A*: quantification of angiotensinogen mRNA expression by real-time PCR in retroperitoneal fat (RPF), epididymal fat (EF), subcutaneous (SC) fat, and liver of OP, OR, and LF rats. Rats were fed either a LF or MHF diet (32% kcal as fat) for 11 wk. Tissues were processed for real-time PCR as described 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 with OR and LF rats. *B*: quantification of ACE mRNA by real-time PCR in the RPF, EF, SC fat and lung of LF, OR, and OP rats. Data are means \pm SE of $n = 8$ rats/group. *Significantly different from LF and OR rats ($P < 0.05$).

OP, OR, and LF rats (%AT₁: LF: 62 ± 6 ; OR: 50 ± 8 ; OP: 58 ± 6 ; and %AT₂: LF: 38 ± 6 ; OR: 50 ± 8 ; OP: 42 ± 6). In the adrenal and spleen, there were no differences in AT₁ or AT₂ receptor density between groups (data not shown).

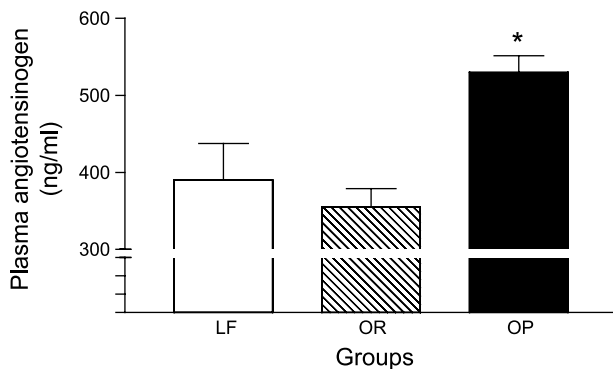


Fig. 4. Effect of a MHF diet on plasma angiotensinogen concentrations. Plasma angiotensinogen was increased in OP rats compared with OR and LF rats. Data are means \pm SE of $n = 8$ rats/group. *Significantly different from OR and LF rats ($P < 0.05$).

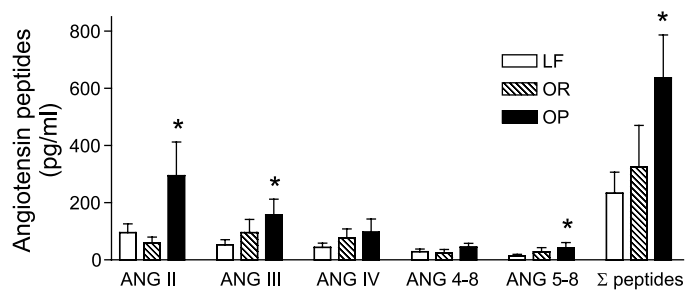


Fig. 5. Effect of a MHF diet on the plasma concentrations of angiotensin (ANG) peptides. Plasma ANG peptides were measured as described in METHODS. The concentrations of ANG II, ANG III, and ANG 5–8 were increased in OP rats compared with OR and LF rats. Σ peptides is the sum concentration of all ANG peptides in plasma, and was increased 3-fold in OP rats. Data are means \pm SE of $n = 8$ rats/group. *Significantly different from OR and LF rats ($P < 0.05$).

DISCUSSION

An important new finding in this study is that circulating concentrations of ANG peptides were markedly increased in rats with diet-induced obesity. Moreover, plasma concentrations of ANG II correlated positively to MAP, 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 with control rats. 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 et al. (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 et al. (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 preexisting 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 wk 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-h 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 0.4 Hz was examined as an index of

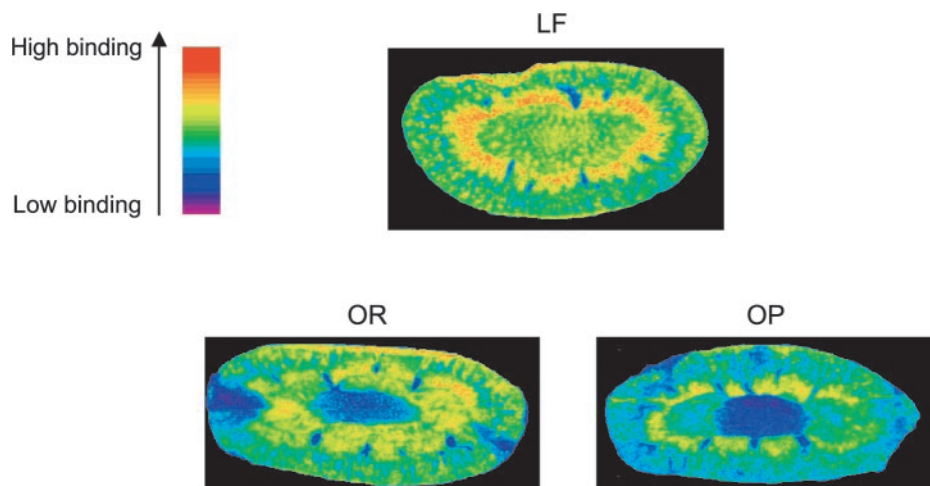


Fig. 6. Receptor autoradiography for ^{125}I -labeled $[\text{Sar}^1, \text{Ile}^8]\text{ANG II}$ binding in representative tissue sections from the kidney of OP, OR, and LF rats. ANG II receptor density was decreased in the kidney of OP rats compared with OR and LF rats.

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, because 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 (44, 45). Angiotensinogen gene expression in SC and visceral adipose tissue correlated positively with body mass index. Conversely, studies in obese patients with hypertension revealed a slight decrease or no change in SC angiotensinogen gene expression (17, 20). Due to obvious limitations, previous studies in humans have examined angiotensinogen gene expression in SC 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 (32), overexpression of angiotensinogen in mouse adipose tissue was paralleled by an increase in plasma angiotensinogen concentration and blood pressure. These results suggest that adipose-derived angiotensinogen can contribute to the circulating pool of angiotensinogen. Although the total RNA yield per gram 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 ANG II concentrations accompanying obesity hypertension. Furthermore, plasma ANG II concentration correlated positively with plasma angiotensinogen. Under most physiological conditions, the rate-limiting step for ANG II generation is the cleavage of ANG I 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 ANG production (14, 25, 33). In support, administration of angiotensinogen to rats at physiological concentrations dose-dependently increased ANG I 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 ANG II generation and thus blood pressure. In this study, plasma ANG II concentration correlated positively with MAP. These results are consistent with a strong link between abdominal obesity and cardiovascular disease, namely hypertension (46).

To determine whether other components of the RAS were influenced by diet-induced obesity, we examined mRNA expression of renin and ACE in adipose and nonadipose sources. Surprisingly, we did not observe alterations in gene expression of these components of the RAS in any tissues examined from obese rats. Dobrian and colleagues (11, 12) reported a twofold elevation in PRA 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 PRA and ANG peptide concentrations were measured in the same plasma sample from individual rats. We report a marked increase in plasma ANG II concentrations in OP rats that correlates positively to elevations in blood pressure. Given this marked

increase in circulating ANG II concentration, kidney renin mRNA expression and PRA would be anticipated to decrease from negative feedback (39). Thus even a normal level of PRA is surprising given the marked elevation in systemic ANG II. A possible explanation for this abnormality is the heightened sympathetic nerve activity observed in OP rats. ANG II-induced negative feedback on renin may be counterbalanced by catecholamine-mediated stimulation of renin synthesis and release. Moreover, the observed downregulation of renal ANG receptors suggests an inability of ANG II to effectively inhibit renin synthesis and release in the kidney of OP rats. In this study, PRA was measured at the end of the study in rats with established hypertension. It is possible that PRA was elevated during the initial increase in blood pressure in obese rats.

The marked elevations in systemic ANG peptides in obese rats could mediate hypertension through many different mechanisms. As suggested by our results, ANG II 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 ANG II, given the ability of ANG II to increase reactive oxygen species (42). Finally, the arterial hypertrophy previously observed in the kidney of OP rats may have resulted from elevations in ANG II (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 ANG II 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.

GRANTS

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